Third Israel-Italy Meeting

Advances in Autoimmunity and Rheumatology

When Friendship Meets Science

23–25 October 2013
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Hand-painted glass panel from “Studio Moretti-Caselli” in Perugia, Italy

Studio(Atelier) Moretti-Caselli, housed in a 15th century “palazzo” in the center of Perugia (Italy), has been producing hand-painted stained glass for cathedrals, monuments and private mansions around the world since its founding in 1860.

Anna and Maddalena Forenza, fifth-generation artists of the Moretti-Caselli family, are still active in restoring and producing hand-painted stained-glass windows from the initial sketches to the selecting, cutting and painting of the glass, to the baking process and, finally, assembling the pieces on their lead mountings.
EDITORIAL

Autoinflammation and Autoimmunity: Pathogenic, Clinical, and Therapeutic Aspects

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KEY WORDS: autoimmunity, autoantibodies, autoimmune/inflammatory syndrome induced by adjuvants (ASIA), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), helminths, psoriatic arthritis (PsA), multiple sclerosis (MA), Type 1 diabetes mellitus, anti-Saccharomyces cerevisiae antibodies (ASCA), ferritin

In this context, Di Gangi et al. [2] evaluate the potential usefulness of adalimumab, an anti-tumor necrosis factor (TNF) agent, in another autoinflammatory disease: hyper-immunglobulin D syndrome (HIDS). This is an autosomal recessive syndrome caused by specific mutations in the mevalonate kinase gene. Its main clinical feature is recurrent inflammatory attacks. Since unstimulated peripheral blood monocytes (PBMCs) from patients with inactive HIDS produce a complex network of inflammatory cytokines and undergo decreased apoptosis, the TNFα monoclonal antibodies, which indeed are able to induce apoptosis of lymphocytes, may be a valid therapeutic option. Further studies are warranted.

An interesting approach to treat autoinflammatory disorders is described by Bashi et al. [3]. Starting from the assumption that the aim of helminths is to survive in the host, thus inducing a tolerance scenario, and that their eradication is linked to the increase of autoimmune conditions in certain countries, helminth conjugates and ova may be a promising treatment for inflammatory bowel disease.

Cohen [4] proposes a shift in paradigm from a global suppression of the immune system, as we witness today, to a molecularly driven language of regulation. It was shown that the administration of a self-peptide to patients with new-onset Type 1 diabetes mellitus (T1D) could significantly arrest the autoimmune destruction of residual beta cells and enhance metabolic control of their diabetes. Cohen suggests that such peptides belong to the Immunological Homunculus; consequently, these self-antigens may function as immune modulators allowing such an apparently controversial result.

Galeazzi et al. [5] present another therapeutic strategy based on the selective delivery of an immunoregulatory cytokine to the sites of inflammatory disease. This newly developed treatment, Dekavil, an ‘armed antibody’, consists of the human F8 antibody (specific to the EDA domain of fibronectin, a marker of angiogenesis) fused to the anti-inflammatory cytokine interleukin-1. The interesting feature is its structure, which allows the delivery and accumulation of the IL-10 cytokine at the sites of disease. A phase Ib clinical trial with initial signs of therapeutic benefit is currently underway in patients with rheumatoid arthritis (RA).
In the search for a suitable treatment for psoriatic arthritis (PsA), a number of agents have been proposed so far. Sarzi-Puttini and colleagues [6] emphasize that despite the major role played by disease-modifying antirheumatic drugs (DMARDs) and anti-TNF, the clinical heterogeneity of PsA makes selecting the most appropriate treatment challenging. Studies on tocilizumab (anti-IL-6), rituximab (anti-CD20), abatacept (CTLA4/IgG), ustekinumab (anti-IL-12/23), IL-17 inhibitors and apremilast (phosphodiesterase 4 inhibitor) are ongoing with promising results.

The novel treatments for patients with autoimmune diseases not only seem to restore an immunological balance, but in some cases are so powerful as to repair the tissue damage. Aharoni and Arnon [7] report that glatiramer acetate can promote repair and remyelination in the inflamed CNS in multiple sclerosis (MS) and in its model experimental autoimmune encephalomyelitis (EAE). The authors suggest that glatiramer acetate affects myelination under inflammatory as well as non-inflammatory conditions, supporting the notion that repair process in the CNS can be up-regulated by therapy.

Among the drugs that have changed the therapeutic approach to autoimmune diseases belimumab. This B cell modulator was recently approved for the treatment of SLE. Thanks to Andreoli et al. [8], the first Italian data on real life experience are now available. Their results in over 18 patients show a significant reduction in disease activity (measured by SLEDAI-2k) at 9 months follow-up with no specific concerns regarding safety. Nonetheless, the administration of the drug was discontinued in 3 patients (16.7%): in 2 cases because of high disease activity and in the third because of recurrent infections. Better-characterized data, with a longer follow-up, are urgently awaited.

Not only new drugs but also improved usage and knowledge of already used agents may change the course of disease and in turn, the management of our patients. Nalli et al. [9] have consolidated expertise in the field of obstetric antiphospholipid syndrome (APS). In this condition, low dose aspirin in association or not with low molecular weight heparin is still the gold standard even though up to 25% of APS women may suffer from recurrent pregnancy loss. Low dose corticosteroids, hydroxychloroquine, intravenous immunoglobulins (IVIg) and plasma exchange, either alone or in combination, should be considered in selected cases as they can improve pregnancy outcome. The concept of autoimmune pregnancy has seen the arrival of the scene of new actors, as addressed by De Carolis et al. [10]. Besides antiphospholipid antibodies, natural killer (NK) cells and anti-thyroid antibodies are associated with impaired outcome of pregnancy, and the treatment should aim at restoring the immune system balance during gestation.

Furthermore, if IVIg are a classical therapeutic option for a number of autoinflammatory/autoimmune conditions, the use of subcutaneous immunoglobulins (SClgl) has now gained attention. Gelardi et al. [11] present their experience with the use of SClg in patients with refractory polymyositis/dermatomyositis. They suggest that patients with cancer-associated myositis, with recent cancer or with pre-neoplastic disease, patients in whom immunosuppressants or prolonged glucocorticoid treatment is contraindicated, and young women who wish to become pregnant, would benefit from first-line therapy with SClg.

The capacity of immunoglobulins to restore a normal balance in the immune system is evident in the case of patients with common variable immunodeficiency (CVID). The standard treatment of these patients comprises the lifelong replacement with IVIg, which reduces the frequency of infections and the progression of complications, including lung disease. Dolcino et al. [12] evaluate the gene-expression profiles of 10 CVID patients, showing that a number of genes involved in the innate and acquired immune responses are differentially expressed after IVIg treatment. Moreover, a marked decrease in CD8+T cells, and an increase in CD4+T cells and centocytes (CD23- CD27- IgM- IgD- B cells) were observed. Vadasz and Toubi [13] brilliantly depicted the key role played by B cells, focusing on B regulatory cells (Bregs). This subset of IL-10-producing B cells has been proved to prevent the induction of arthritis and to ameliorate established disease in syngeneic immunized mice with experimental arthritis. The authors showed a strict relationship between Bregs and semaphorin3A (a regulatory protein); however, the exact mechanisms by which Bregs play their role are still unclear.

Semaphorins, and especially semaphorin3A, are involved also in the pathogenesis of another condition mainly driven by an inflammatory process: systemic sclerosis (SSc). Vadasz and co-authors [14] evaluated the levels of semaphorin 3A in the serum and its expression on T regulatory cells, in SSc patients, healthy controls and SLE patients. They found that serum levels of semaphorin 3A were lower in SSc compared to healthy controls and similar to SLE, denoting an inefficient T regulatory activity in autoimmune diseases such as SSc.

Serpins (Serin protease inhibitors) represent another piece of the puzzle of autoimmunity. This is a wide group of structurally conserved molecules that have several functions in the homeostasis of living organisms, being also involved in regulation of cellular viability. Gatto [15] addresses SERPINB3 expression in SLE. Lymphocytes of affected patients have a reduced SERPINB3 expression that in turn may increase the autoantigen burden in lupus or B cell autoreactivity, suggesting that SERPINB3 can influence the SLE course, specifically lupus nephritis. This connection between Serpins and autoimmunity may link another autosomal dominant disorder with the spectrum of autoimmunity as well.

Hereditary angioedema (HAE) results from the congenital deficiency of the C1 Inhibitor (C1INH) component of the complement system. C1INH is itself a serine protease encoded by the C1INH gene, also called SERPING1, and low complement (due to complement activation) as well as an increased
association with “classic” autoimmune disorders have been described. Triggianese et al. [16] found enhanced production of autoantibodies in patients with HAE. Most probably, an increased activation of B cells in association with a high expression of TLR-9 is responsible of this phenomenon.

The increasingly close relationship between autoimmune and autoinflammatory disorders is no longer a surprise. Borella et al. [17] address the intricate pathways connecting the innate and the adaptive immunity. Evidence of common susceptibility genes, such as the nucleotide-binding oligomerization domain-like receptor protein 1 (NLRP1) associated with SLE; of shared environmental triggers, especially infectious agents; of the presence of similar symptoms; and of skin, musculoskeletal and gastrointestinal involvement suggest the use of similar therapies.

The recent identification of a new syndrome, namely Hyperferritinemic Syndrome, has opened a window in the understanding of autoimmune/autoinflammatory disorders. Rosario et al. [18] have indeed gathered under a single umbrella different conditions including sepsis, systemic inflammatory response syndrome (SIRS), multiorgan dysfunction syndrome (MODS), macrophage activation syndrome (MAS), adult-onset Still’s disease (AOSD), and the catastrophic variant of the antiphospholipid syndrome (CAPS) characterized by markedly elevated levels of ferritinemia. The novel idea suggests a pathogenic role for ferritin in these conditions, a matter recently examined by Colafrancesco et al. [19]. They found that sCD163, a molecule exclusively expressed on cells of monocytic origin, is overexpressed in different inflammatory conditions including MAS, sepsis and AOSD. Since ferritin production seems to be related mainly to macrophage activation, the observation of a positive correlation between ferritin serum levels and sCD163 in AOSD may be the link to the macrophagic origin of ferritin.

It is evident that new actors are on the scene, including Saccharomyces cerevisiae, commonly known as yeast. This was recently included among the possible pathogenic agents in autoimmunity. Rinaldi [20] suggests that the molecular structure of some antigens of yeast overlap with some of the most common autoantigens such as the autoantigen U2 snRNP. These data are strengthened by the evidence of an increased prevalence of anti-Saccharomyces cerevisiae antibodies (ASCA) in a number of autoimmune disorders including SLE. The role of ASCA should be re-evaluated in clinical practice together with dietary recommendations in selected groups of patients.

The seminal role of autoantibodies in the pathogenesis of autoimmune diseases is clear when considering RA. Indeed, anti-citrullinated protein/peptide antibodies (ACPA/anti-CCP) are a hallmark of the disease and are believed to play a role in disease initiation and pathogenesis. Citrullination is the post-translational conversion of arginine to citrulline catalyzed by peptidylarginine deiminase (PAD), an enzyme up-regulated under inflammatory conditions. Gertel et al. [21] added a juicy aspect, closing the gap between cigarette smoking, citrullination, lung disease and RA. Indeed, cigarette smoking – an inducer of citrullination – is the dominant risk factor for chronic obstructive pulmonary disease (COPD). COPD has an increased prevalence in patients diagnosed with RA and RA is associated with an increased risk of COPD independently of smoking. The authors speculate that ACPA in COPD patients might contribute to the induction of an autoimmune response and that treatment against the citrulline-specific immune response could putatively be applicable for COPD patients.

Not only the smoking habit, but another plague of industrialized countries, obesity, can be associated with the recent outbreak of autoimmune diseases. Versini et al. [22] focus on this issue and on the capacity of white adipose tissue (WAT) to secrete numerous soluble mediators called adipokines, involved in many processes including immunity and inflammation. In their systematic literature review, they found that obesity might be responsible for a dysregulation of Th17/Treg balance, and that obesity has a higher prevalence in ACPA-negative RA patients, in multiple sclerosis, in psoriasis and psoriatic arthritis, and in SLE. Furthermore, obesity may promote inflammatory bowel diseases (IBD), T1D and Hashimoto thyroiditis. Obese patients exhibit a more severe course of RA, SLE, IBD, psoriasis and PsA and a reduced therapeutic response in RA, IBD, psoriasis and PsA. Thus, in the future management of autoimmune patients, dietary and lifestyle recommendations should be strongly considered.

Infections may be another primum movens leading to the production of autoantibodies. In several circumstances, different infectious agents have been associated with the onset of autoimmune diseases. Perricone et al. [23] present the exceptional autoimmune condition in which the infectious agent is proven: rheumatic fever. In patients affected by this disease caused by the Streptococcus beta Haemolytic group A, several autoantibodies have been detected including anti-endothelial cell antibodies. These were demonstrated to be directed towards specific peptides of vimentin, cross-reacting with antigens of the pathogen. The infusion of these autoantibodies in Lewis rats appeared to provoke an experimental model of disease.

In another autoimmune condition, narcolepsy, the Streptococcus spp. as well as the H1N1 (both the infection and the vaccine) seem be the initial triggers. Arango et al. [24] underline the enormous role of genetic factors, namely, the allele HLA DQB1*06:02 in the disease pathogenesis.

Apart from the role as merely disease triggers, infections represent a major burden in patients with autoimmune diseases, such as SLE and RA. These patients have a higher morbidity and mortality linked to infections due to the imbalance in the immune system and the use of immunosuppressants. Barrett and collaborators [25] acquired data from the SEPSIS-ISR, an ongoing prospective study that collects data of all patients...
admitted to intensive care units of seven major hospitals in Israel with the diagnosis of sepsis during the decade 2002–2012. Unfortunately, the data on the prevalence of sepsis were not available at the time of writing this editorial.

As a consequence of the role of infections in autoimmunity, their prevention, especially through vaccinations, has become a fundamental issue in the fight. However, an association has been shown between vaccines and autoimmunity that has led to the description of a new syndrome, ASIA, the Autoimmune/Inflammatory syndrome Induced by Adjuvants. In this condition, autoimmune phenomena occur after exposure to an adjuvant, such as those contained in certain vaccines. Tomljenovic et al. [26] evaluate the association especially of hepatitis B virus vaccine and human papilloma virus vaccine with autoimmune diseases, in particular SLE. The adjuvants could be associated with specific tissue damage, acting as a Trojan horse, thus allowing the accumulation of toxic molecules in target organs. Despite the fact that the benefits from vaccination still largely overwhelm the risks, physicians should be aware of the possibility of an autoimmune reaction following vaccination.

Bar-On and co-workers [27] deal with the controversial topic of fibromyalgia (FM). This is a chronic debilitating disorder characterized by widespread pain, allodynia, hyperalgesia, fatigue, and unrefreshing sleep accompanied by mood and cognitive disturbances. The diagnosis is complex since there are specific tests, and the disease imposes a substantial financial burden. Another significant problem is the patients’ limited adherence to treatment. Addressing compliance and adherence in such patients might lead to reduced health care costs and enriched quality of care.

In improving the management of autoimmune diseases, progress in diagnostic tools is much awaited. The limitation of inappropriate test requests and the imbalance between available economic resources and increasing health needs are jeopardizing modern health care services. Melegari and Bonaguri [28] performed a study aimed at unifying the testing algorithms, focusing on antinuclear antibodies (ANA), extractable nuclear antigens (ENA) and double-strand DNA (dsDNA). They conclude that strict cooperation between clinicians, laboratory specialists and health care services is crucial for the diffusion of ANA reflex testing.

We believe that a Meeting such as this helps build the wall of knowledge on Autoimmune and Rheumatic diseases. The sharing of ideas, the cooperation and the friendship are the cement that strengthens this wall every year.

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long-Term Efficacy of Adalimumab in Hyper-immunoglobulin D and Periodic Fever Syndrome

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Hyperv-immunoglobulin D (HIDS) and periodic fever syndrome is an autosomal recessive autoinflammatory syndrome caused by specific mutations in the mevalonate kinase (MVK) gene. MVK is essential in the isoprenoid/cholesterol biosynthesis pathways, and MVK deficiency results in the accumulation of its substrate (mevalonic acid) and shortage of the end product. With regard to the pathogenesis of HIDS, it has been hypothesised that the autoinflammation is caused by a shortage of isoprenoids, which are compounds involved in the post-translational prenylation (farnesylation or geranyl-ation) of several important intracellular signaling molecules.

Decreased apoptosis of peripheral blood mononuclear cells (PBMC) in HIDS patients may be involved in the inability to curtail the immunologic response, thus leading to generalized inflammation even after a trivial stimulus. No conventional therapy exists to prevent or cure recurrent inflammatory attacks in HIDS. Nevertheless, experimental findings on the role of several pro-inflammatory cytokines – interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNFα) and IL-6 – in the pathogenesis of the disease [1-3] support the use of anti-cytokine treatment. In this regard, anakinra, an IL-1β-blocking agent, seems to be effective, and etanercept, a soluble p75 TNFα receptor-Fc fusion protein, has been used with variable success.

To the best of our knowledge, this is the first reported case of severe HIDS successfully treated with adalimumab, a fully human monoclonal TNFα antibody. We also discuss a potential mode of action of TNFα inhibitors in this syndrome.

KEY WORDS: hyper-immunoglobulin D periodic fever syndrome (HIDS), autoinflammatory syndrome, mevalonate kinase gene (MVK), tumor necrosis factor-alpha (TNFα) therapy, adalimumab

PATIENT DESCRIPTION

A 20 year old female reported a long history of recurrent fever attacks (39°–40°C) preceded by chills and accompanied by abdominal pain, diarrhea, headache, generalized malaise, arthromyalgia, sore throat, aphthous ulcers, and cervical lymphadenopathy. In the first few years of her clinical history, these symptoms were associated with maculopapular rash and hepatosplenomegaly. During the acute phase of the disease, she was in poor general condition but was asymptomatic between critical periods. The attacks had recurred monthly since the age of 8 months. They lasted 7–10 days and were associated with elevated acute-phase reactants. There was no consanguinity between her parents, and no other members of her family reported similar symptoms. Attacks recurred despite previous tonsillectomy, adenoidectomy and appendectomy. On admission to our clinic, she complained of the above symptoms related to fever attack. Additionally, she complained of inflammatory low back pain characterized by gradual onset over the past few months.

Laboratory data showed a significant increase in acute-phase reactants: erythrocyte sedimentation rate 35 mm/hour, C-reactive protein 205 mg/L (normal 0–5), serum amyloid A protein 120 μg/ml (normal 0–10), neutrophilic leukocytosis 14,000/µl, hemoglobin 10.2 g/dl, serum ferritin 242 ng/ml, transferrin 156 mg/dl, IgA 459 mg/dl (normal value 70–400) and total cholesterol 81 mg/dl (normal value 125–220). IgD serum levels were not tested. Proteinuria was absent, and the human leukocyte antigen (HLA) B27 test was negative.

Acute sacroiliitis on the iliac more than the sacral side of the right sacroiliac joint with no evidence of chronic changes was detected by magnetic resonance imaging (low signal intensity on T1 with enhancement after gadolinium administration, high signal intensity on short TI inversion recovery, and T2 fast short echo in the subchondral region). No vertebral involvement was detected.

A clinical response was obtained only after high dose prednisone (50 mg/day) was administered; previous treatment with low dose prednisone, non-steroidal anti-inflammatory drugs and colchicines had been ineffective.

A clinical response was obtained only after high dose prednisone (50 mg/day) was administered; previous treatment with low dose prednisone, non-steroidal anti-inflammatory drugs and colchicines had been ineffective.

Genetic analysis revealed a well-described missense mutation in exon 11 (V377I) and a novel missense mutation in exon 8 (c.683 C>T, p.P228L) of MVK. The patient was also found to have the MEFV (Mediterranean fever) mutation in exon 2 (c. 2177 C>T, p.V726A) in a heterozygous state.
HIDS diagnosis was confirmed by biochemical investigations of lymphocytes that revealed the markedly reduced activity of MVK (7 pmol/min/mg, control 142 pmol/min/mg).

Because of the patient’s clinical dependence on high dose steroids and the presence of sacroiliitis, she was treated with subcutaneous adalimumab (40 mg every 2 weeks). Treatment was immediately successful in aborting the inflammatory attacks and inflammatory low back pain. Clinical remission and normalization of acute-phase reactants were maintained until her last visit, one year after the start of treatment. One year follow-up of the sacroiliac joints revealed resolution of acute sacroiliitis on MRI. No side effects were observed with adalimumab therapy. Adalimumab was continued while steroid therapy was gradually tapered and eventually stopped. The patient’s quality of life improved markedly.

**COMMENT**

We report a sustained clinical remission in a young patient with HIDS treated with adalimumab. Several features of the present case report are noteworthy.

The patient was a compound heterozygote for two MVK mutations (common V377I mutation and a novel P228L substitution) with distinctly diminished MK activity in combination with the common V726A MEFV variant. It is not known if the concomitant heterozygosity of the MEFV mutation results in a more severe HIDS phenotype. With the notable exception of sacroiliitis in our patient, there were no other clinical differences between the phenotypic presentation of her disease and what is generally observed in the usual variant of the HIDS with no MEFV mutation.

The presence of sacroiliitis in HIDS patients has not been previously reported in the medical literature. A few case reports describe the coexistence of familial Mediterranean fever (FMF) and ankylosing spondylitis [4,5] without pointing to a clear ethiopathogenetic relationship. Alternatively, other authors have described seronegative spondyloarthropathy, with no vertebral involvement and negativity of HLA B-27, as a rheumatologic manifestation of FMF [6,7]. In our patient vertebral involvement was absent and the HLA B-27 test was negative. We also report an excellent response to adalimumab in aborting laboratory and clinical HIDS features and the resolution of bone edema in the sacroiliac joint.

Various studies highlight the activation of the cytokine network (IL-1β, IL-6 and TNFα) during inflammatory attacks in HIDS [1,2]. Unstimulated peripheral blood mononuclear cells from patients with inactive HIDS produce a complex network of cytokines capable of inducing the synthesis of pentraxine and serum amyloid A [3]. Cytokine activation may represent the endpoint of an inflammatory process, where the triggering events are still not fully understood. Cytokine activation suggests the use of anti-cytokines as a therapeutic option in HIDS patients. IL-1 receptor antagonists (e.g., anakinra) have been used with success in vaccine-induced models of HIDS and in other HIDS patients. In our patient, anakinra was not used since it has not been reported to be effective in patients with sacroiliitis.

Three anti-TNFα agents are effective in rheumatoid arthritis; two of them (infliximab and adalimumab, both anti-TNF monoclonal antibodies) are also effective in Crohn’s disease, while etanercept, which is derived from a soluble form of TNF receptor type II, is not. A possible explanation may reside in their different biologic activities on transmembrane TNFα, which is a precursor form of TNFα expressed as a 26 kD cell surface type II polypeptide on activated macrophages and lymphocytes. In a recent study [8] on Jurkat T cells that were stably expressing an uncleavable form of transmembrane TNFα, infliximab and adalimumab exerted almost equal complement-dependent cytotoxicity while etanercept showed considerably lower activity. Adalimumab and infliximab also induced apoptosis and cell cycle arrest in Jurkat T cells, reflecting an outside-to-inside signal transduction through transmembrane TNFα. A recent study [9] showed decreased apoptosis after stimulation with anisomycin in lymphocytes from fever-free patients with HIDS, but not in lymphocytes derived from TRAPS (TNF receptor-associated periodic syndrome) or FMF patients. The authors hypothesize that the defective regulation of apoptosis and the attendant increased lifespan of activated lymphocytes may be the cause of the exaggerated inflammatory response in HIDS patients.

Thus, TNFα monoclonal antibodies, which are able to induce apoptosis of lymphocytes, may be a more valid therapeutic option than soluble TNFα receptor. Notably, etanercept has been the anti-TNFα more frequently used in HIDS patients, but it has been found to be either ineffective or only partially effective. A single case report is available where infliximab dramatically improved nummular keratopathy; however, its effect on the frequency of HIDS flares was not well defined [10].

To the best of our knowledge, this is the first report of a patient with a long-term remission (1 year) of severe HIDS after adalimumab therapy, suggesting that adalimumab could be a therapeutic option in refractory HIDS patients. Further investigation on the use of TNFα monoclonal antibodies in HIDS therapy is warranted.

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**Capsule**

**How to boost cancer immunotherapy**

Why does our immune system protect us so well against infection but not against cancer? In part, this is because cancer cells use clever ways to escape immune responses designed to destroy them. A therapeutic strategy called “immune checkpoint blockade” thwarts these escape tactics and renders cancer cells vulnerable to immune attack. Although remarkably effective, only a subset of patients respond to it. Seeking possible explanations for this limited response, Kim et al. identified a specific immune cell population that interferes with the therapy in mouse tumor models. When the authors co-administered drugs that reduced the levels of these cells (called myeloid-derived suppressor cells), the efficacy of immune checkpoint blockade therapy improved considerably.

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Eitan Israeli

**Capsule**

**When genetic diversity hurts the kids**

Although we think of the genome as fixed, errors in DNA replication and recombination can cause changes. As the organism develops, individual nucleotides may mutate, or genetic material may duplicate or be deleted. Such “somatic mosaicism” means that different cells and tissues in the body may have different genomes. To determine whether this affects human disease, Campbell et al. took blood samples from 100 families with children who have genetic disorders. They found that approximately 4% of the parents (who were all healthy) exhibited somatic mosaicism, which suggests that the affected children inherited the mutation from a mosaic parent. These results suggest that somatic mosaicism is probably more common than previously thought and affects human health.

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Eitan Israeli

**Capsule**

**A breathtaking tale of sticky mucus in cystic fibrosis**

Patients with cystic fibrosis have difficulty breathing because their airways are clogged with thick mucus. Does this mucus accumulate because there is a defect in the way it is produced? Or does it accumulate because of other disease features, such as dehydration or airway wall remodeling? Distinguishing between these possibilities is important for future drug development. In a study of piglets with cystic fibrosis, Hoegger and fellow-researchers identified mucus production as the primary defect. The airway glands of the piglets synthesized strands of mucus normally, but the strands were never released and stayed tethered to the gland ducts.

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Eitan Israeli
The Body against Self: Autoinflammation and Autoimmunity

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**KEY WORDS:** autoinflammatory (AIF) disease, autoimmune (AIM) disease, adaptive immunity, innate immunity, inflammasome, interleukin-1 (IL-1)

**EFFECTOR MECHANISMS**

The sensors of innate immunity are named pattern recognition receptors (PRRs) and they bind pathogen-associated molecular patterns (PAMPs) or damaged cells (damage-associated molecular patterns, DAMPs). The effector cells are macrophages, dendritic cells, and other antigen-presenting cells. PRRs include three classes of receptors: TLRs, nucleotide-binding oligomerization domain-like receptors (NLRs), and retinoic acid-inducible gene-I-like receptors [5].

Activation of NLRP, i.e., NLRP1, NLRP3 (also known as NALP3 or cryopyrin) and NLR family CARD domain-containing protein 4 (NLRC4), leads to the formation of large protein complexes termed inflammasomes, which are critical for the defense against pathogens. Indeed, they mediate the activation of procaspase-1 and, consequently, the cleavage of the proforms of interleukin (IL)-1β and IL-18 to the active forms [5] [Figure 1].

Adaptive immunity acts through highly specific antigen receptors, the most important of which are T and B cell receptors. Patients affected with AIM diseases have autoreactive antigen-specific T cells and produce autoantibodies [1].

**GENETIC BACKGROUND**

Genetic association studies highlighted the presence of gene mutations that predispose to the development of both autoinflammatory and autoimmune diseases [2,3]. Genes associated with AIF diseases encode for proteins of the inflammasome [2]. Mutations in genes coding for regulatory molecules of T and B cell signaling and in genes involved in Toll-like receptor (TLR)-interferon (IFN) and in tumor necrosis factor (TNF)-nuclear factor kappa B (NFκB) signaling pathways have been observed in patients affected with AIM diseases [3]. Recently, an association was reported between single-nucleotide polymorphisms (SNPs) localized in the inflammasome gene nucleotide-binding oligomerization domain-like receptor protein 1 (NLRP1) and systemic lupus erythematosus (SLE) [4].

**ENVIRONMENTAL TRIGGERS**

In both autoinflammatory and autoimmune diseases, environmental as well as endogenous factors can elicit disease onset and flares. Infective agents may induce both AIF and AIM diseases, i.e., by interacting with TLR, as well as by molecular mimicry, bystander activation (expansion of previously activated T cells), and activation of T cells by microbial superantigens. In addition, AIF and AIM diseases can be triggered by physical agents and exposure to drugs and other chemical agents which can alter immune system homeostasis.

**CLINICAL FEATURES**

Constitutional symptoms, such as recurrent fever, fatigue, flu-like symptoms, weight loss, myalgia, malaise, lymphadenopathy and splenomegaly, are frequent in both diseases. Skin, musculoskeletal and gastrointestinal symptoms are the most common in AIM diseases, while involvement of other organs, such as lung, eye, ear, hematopoietic and nervous system, is less common.

Notably, while signs and symptoms are often similar in AIF and AIM diseases, biological and bioptic investigations may reveal more specific features [1]. For instance, AIF and AIM patients complain very often of myalgia and asthenia. However, muscle weakness and fatigue are associated with increased creatinine phosphokinase (CK) serum levels in AIM diseases, especially in polymyositis/dermatomyositis (PM/DM), whereas CK serum levels are usually within the normal range in AIF diseases.

AIF and AIM patients have a higher morbidity and mortality risk due to disease complications. In particular, AA amyloidosis develops in patients with long-lasting inflam-
matory conditions, whereas several autoimmune diseases are associated with premature atherosclerosis.

BIOMARKERS

Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) usually increase during a period of active disease. High ferritin serum levels typically arise in Still’s disease. High immunoglobulin serum levels can be found in SLE, primary Sjögren syndrome, hyper-immunoglobulin D syndrome (HIDS), and familial Mediterranean fever (FMF). Serum amyloid A protein (SAA) is often increased in patients with AIF diseases, while low complement component 3 and 4 serum levels are more frequently observed in SLE.

Autoantibodies are diagnostic biomarkers in AIM diseases, some of them displaying a pathogenic role, i.e., anti-P ribosomal protein in neuropsychiatric SLE [6], others displaying a protective role, such as IgG anti-pentraxin 3 antibodies, again in SLE [7].

THERAPY

Since the dysregulation of the immune system is the common pathogenic background of both autoinflammatory and autoimmune diseases, drugs such as NSAIDs (non-steroid anti-inflammatory drugs) and corticosteroids are used in both. Colchicine is useful for the majority of AIF diseases. Additionally, it is used to prevent the development of amyloidosis in most cases, ameliorating the disease in 95% of patients. Immunosuppressants are effectively used for the majority of severe manifestations in autoimmune patients, while their role in AIF diseases is less clear.

In recent years, specific anti-cytokine drugs have been developed. For instance, IL-1 antagonists are used in AIF diseases, anti-TNFα and anti-IL6 are used in rheumatoid arthritis as well as in spondyloarthropathies and irritable bowel syndrome, and anti-BLYS (B lymphocyte stimulator) is used in SLE.

AUTOINFLAMMATION IN AUTOIMMUNE DISEASES

Over the past years it has become clear that AIF processes play a pathogenic role even in AIM diseases. For instance, it was recently demonstrated that neutrophil extracellular traps (NETs), an important defensive mechanism against microorganisms, are involved in the pathogenesis of some autoimmune and autoinflammatory diseases. Defects in clearance of NETs expose immunostimulatory molecules to plasmacytoid dendritic cells, thus stimulating the activation of inflammasomes and the release of IL-1β and IL-18. IL-18, in turn, stimulates NETosis in human neutrophils, thus enhancing the formation of NETs, which results in a feed-forward inflammatory loop that potentially leads to disease flares and/or organ damage [8].

A recent study in a southern Brazilian population supports the association between specific SNPs localized in the inflammasome gene NLRP1 and SLE. Indeed, these SNPs were associated not only with an increased risk of developing SLE but also with the manifestation of specific pathologic features, such as rash and arthritis which are typically observed in AIF patients [6]. Thus, it might be that specific SNPs of inflammasomes affect the clinical and immunologic features of AIM diseases, favoring the development of some AIF-like manifestations and leading to an increase of some.
enhance the development of autoimmune diseases in genetically predisposed individuals [Figure 1].

Finally, the involvement of inflammation in autoimmunity was recently underlined by the positive effects of the administration of anakinra in autoimmune patients, leading to the amelioration of arthritis not only in rheumatoid arthritis, but also in SLE and in PM/DM patients. In addition, chloroquine, used widely in SLE, has been shown to decrease aberrant NLRP3 expression, a mechanism that might be related to its therapeutic effect.

CONCLUSIONS
From a pathogenic point of view, autoinflammatory and autoimmune diseases share the chronic activation of the immune system, which eventually leads to tissue inflammation. The specific effectors of damage are different in the two groups of diseases. Nevertheless, in the past decade we began to understand the involvement of AIF processes in AIM disease. However, the effects of IL-1β and IL-18 on lymphocyte programming and function have not been extensively studied and require further research to expand our knowledge in this field and to hypothesize new therapeutic approaches in autoimmune diseases.

Antifungal drug resistance evoked via RNAi-dependent epimutations

Microorganisms evolve via a range of mechanisms that may include or involve sexual/parasexual reproduction, mutators, aneuploidy, Hsp90 and even prions. Mechanisms that may seem detrimental can be repurposed to generate diversity. Calo et al. show that the human fungal pathogen *Mucor circinelloides* develops spontaneous resistance to the antifungal drug FK506 (tacrolimus) via two distinct mechanisms. One involves Mendelian mutations that confer stable drug resistance; the other occurs via an epigenetic RNA interference (RNAi)-mediated pathway resulting in unstable drug resistance. The peptidylprolyl isomerase FKBP12 interacts with FK506 forming a complex that inhibits the protein phosphatase calcineurin. Calcineurin inhibition by FK506 blocks *M. circinelloides* transition to hyphae and enforces yeast growth. Mutations in the fkbA gene encoding FKBP12 or the calcineurin cnbR or cnaA genes confer FK506 resistance and restore hyphal growth. In parallel, RNAi is spontaneously triggered to silence the fkbA gene, giving rise to drug-resistant epimutants. FK506-resistant epimutants readily reverted to the drug-sensitive wild-type phenotype when grown without exposure to the drug. The establishment of these epimutants is accompanied by generation of abundant fkbA small RNAs and requires the RNAi pathway as well as other factors that constrain or reverse the epimutant state. Silencing involves the generation of a double-stranded RNA trigger intermediate using the fkbA mature mRNA as a template to produce antisense fkbA RNA. This study uncovers a novel epigenetic RNAi-based epimutation mechanism controlling phenotypic plasticity, with possible implications for antimicrobial drug resistance and RNAi-regulatory mechanisms in fungi and other eukaryotes.

“Kindness is in our power, even when fondness is not”

Samuel Johnson (1709-1784), British poet, essayist, moralist, literary critic, biographer, editor and lexicographer
Enhanced Myelination in Autoimmunity and in Normal Development Induced by Glatiramer Acetate

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Multiple sclerosis (MS) is a complex multifaceted disease in which inflammatory autoimmune attack in the central nervous system (CNS) leads to the destruction of the myelin sheath and the formation of demyelinated lesions in the white matter [1]. Diffused molecular and cellular changes in normal-appearing white matter and cortical demyelination have also been recognized as a component of MS pathology. This widespread demyelination results in impaired nerve conductivity and neuroaxonal damage. Thus, the essential challenge for MS therapy is not only to ameliorate the inflammatory aspect of the disease, but to promote neuroprotective repair mechanisms beyond their limited spontaneous occurrence, in particular remyelination.

Glatiramer acetate (GA, Copaxone®, Israel) is an amino acid copolymer, an approved drug widely used as a first-line treatment for MS [2]. Cumulative evidences indicate that GA affects various levels of the innate and the adaptive immune response, inducing deviation from pro-inflammatory to anti-inflammatory pathways [3]. This includes competition for the binding of antigen-presenting cells, driving dendritic cells, monocytes, and B cells towards anti-inflammatory responses, induction of Th2/3 and T-regulatory cells, and down-regulation of both Th1 and Th17 cells. The immune cells induced by GA reach into the CNS and secrete in situ anti-inflammatory cytokines, thus alleviating the pathological inflammatory processes [4]. In addition to its immunomodulatory activity, GA treatment induces elevation in the CNS levels of several neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), suggesting neuroprotective repair consequences [5]. To examine whether GA can indeed affect the repair and remyelination we applied two systems: the animal model of MS, experimental autoimmune encephalomyelitis (EAE), and postnatal myelination in the developing CNS of newborn mice.

Remyelination in EAE

The effect of GA treatment on remyelination was investigated in relapsing-remitting EAE induced by myelin proteolipid protein (PLP) peptide in which widespread demyelination is the main pathological manifestation, and in chronic EAE induced by myelin oligodendrocyte glycoprotein (MOG) peptide in which degeneration processes prevail. We observed reduced myelin damage as detected by scanning electron microscopy (SEM) and immunohistochemistry in EAE-inflicted mice treated with a late therapeutic GA regimen (initiated when there was already extensive myelin damage), suggesting the induction of actual repair processes [6]. This was further confirmed by applying transmission electron microscopy (TEM), which facilitates the visualization of newly myelinated axons [7]. Indeed, quantitative spinal cord TEM analysis of remyelination compared to demyelination revealed a significant increase in the relative remyelination, by seven- and threefold over untreated EAE mice, when GA treatment was applied during the first or second disease exacerbation, respectively [Figure 1A].

This pattern was also evident on MRI using magnetization transfer ratio (MTR), which focuses on macromolecules, indicating myelin loss. Overall assessment of the whole brain by histogram analysis as well as detection of specific affected areas by voxel-based analysis revealed restoration of the MTR values to the normal level following GA treatment [8].

The effect of GA in this system is attributed to increased proliferation and survival of oligodendrocyte progenitor cells (OPCs) and their recruitment into injury sites [Figure 1B], thus enhancing myelin repair in situ. Furthermore, GA treatment induces a morphological transformation of OPCs from the earlier bipolar stage to the more mature multiprocessed form, suggesting an effect on the differentiation along the oligodendroglial maturation cascade towards myelin-producing cells [6].

Postnatal Myelination in the Developing CNS

It was subsequently questioned whether the remyelination demonstrated in EAE-induced mice is solely due to the anti-inflammatory activity of GA in the inflamed CNS. Addressing this issue we recently investigated whether GA can affect postnatal myelino genesis in the developing nervous system under
Figure 1. The effect of GA treatment on myelination in EAE [A,B] and on postnatal myelinogenesis [C,D]

[A] TEM micrograph of a remyelination zone in the spinal cord of a GA-treated EAE-induced mouse, depicting oligodendrocyte cell surrounded by newly remyelinated axons, and quantitative assessment of the remyelination to demyelination ratio of three mice per treatment group at each time point.

[B] Left panel: progenitor oligodendrocyte expressing NG2 (red), extending multiple processes, intertwines several transected nerve fibers (green)
Right panel: mature oligodendrocytes expressing O4 (red) accumulating in spinal cord lesions of GA-treated EAE-induced mice

[C] Representative spinal cord sections and quantitative assessment of myelinated axons compared to the total number of axons on postnatal day 14. More MBP-encircled axons are depicted in GA-injected compared to PBS-injected mice

[D] Left: representative TEM micrographs depicting larger axons with thicker myelin sheath in GA-injected mice
Right: linear regression of the interaction between the treatment and axonal diameter on myelin thickness, and the average g-ratio for six mice per treatment group
non-pathological conditions, when injected on postnatal days 7–21 [9]. Immunohistological and ultrastructural analyses revealed significant elevation in the number of myelinated axons as well as in the thickness of the myelin encircling them and the resulting g-ratios, in spinal cords of GA-injected mice compared to their phosphate-buffered saline (PBS)-injected littermates, on postnatal day 14 [Figure 1C & D]. GA induced also an increase in the axonal diameter, implying an effect on the overall development of the nervous system. It should be noted that when the myelination process was apparently completed (postnatal day 21), the extent of myelinated axons and their morphological appearance did not differ between the GA-injected and the PBS-injected mice. Thus, GA accelerates myelin development without inducing an excessive or aberrant myelination.

A prominent elevation in the amount of OPCs and their proliferation (detected by BrdU incorporation) as well as in mature oligodendrocytes indicated that similar to the findings in EAE, the effect of GA in postnatal myelination is linked to differentiation along the oligodendroglial maturation cascade. As previously found in EAE, GA-postnatal injection resulted in increased expression of BDNF and insulin-like growth factor (IGF-1), which are known to promote myelination, suggesting that the mode of action of GA can be linked to its neurotrophic effect. Notably, the GA-injected newborn mice exhibited better performance in a rotating rod test than their PBS-injected littermates, suggesting that the accelerated myelin development results in functional advantage in sensorimotor functions.

During remyelination, features of developmental myelination are recapitulated. For example, some exons of the MBP gene that are present during fetal development are expressed again in oligodendrocytes during demyelinating diseases [10]. The effect of GA on postnatal myelogenesis as well as on remyelination in EAE is therefore relevant to its therapeutic effect in MS, supporting the notion that the repair process in the CNS can be up-regulated by therapy, irrespective of the inflammatory process.

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References

Capsule

Chromatin state dynamics during blood formation

Chromatin modifications are crucial for development, yet little is known about their dynamics during differentiation. Hematopoiesis provides a well-defined model to study chromatin state dynamics; however, technical limitations impede profiling of homogeneous differentiation intermediates. Lara-Astiaso et al. developed a high sensitivity indexing-first chromatin immunoprecipitation approach to profile the dynamics of four chromatin modifications across 16 stages of hematopoietic differentiation. The authors identified 48,415 enhancer regions and characterized their dynamics. They found that lineage commitment involves de novo establishment of 17,035 lineage-specific enhancers. These enhancer repertoire expansions foreshadow transcriptional programs in differentiated cells. Combining our enhancer catalog with gene expression profiles, we elucidate the transcription factor network controlling chromatin dynamics and lineage specification in hematopoiesis. Together, these results provide a comprehensive model of chromatin dynamics during development.

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Eitan Israeli

“Others have seen what is and asked why. I have seen what could be and asked why not”

Pablo Picasso (1881-1973), Spanish painter, sculptor, printmaker, ceramicist, stage designer, poet and playwright. One of the greatest and most influential artists of the 20th century, he is known for co-founding the Cubist movement and collage, and for the wide variety of styles that he helped develop and explore.
Pregnancy in Antiphospholipid Syndrome: Can we Improve Patient Management?

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Antiphospholipid syndrome (APS) is characterized by thrombotic events and/or pregnancy morbidity in the presence of antiphospholipid antibodies (aPL). According to the 2006 classification criteria, the tests to detect aPL are: anti-beta2 glycoprotein I antibodies (aβ2GPI), anticardiolipin antibodies (aCL), and lupus anticoagulant (LA) [1]. Pregnancy morbidities included in the APS classification are defined as:

- At least one unexplained fetal death at ≥ 10 weeks of gestation with normal morphology on prenatal ultrasound examination or direct postnatal examination
- At least one preterm delivery of a morphologically normal infant before 34 weeks of gestation due to severe pre-eclampsia, eclampsia, or features consistent with placental insufficiency. Generally accepted features of placental insufficiency include: (i) abnormal or non-reassuring fetal surveillance test (e.g., non-reactive non-stress test), (ii) abnormal Doppler flow velocimetry waveform analysis (e.g., absent end-diastolic flow in the umbilical artery), (iii) oligohydramnios, or (iv) birth weight less than the 10th percentile for gestational age
- At least three unexplained consecutive spontaneous pregnancy losses < 10 weeks of gestation, after exclusion of maternal anatomic and hormonal abnormalities and paternal and maternal chromosomal abnormalities.

After more than 30 years of clinical experience, the treatment milestone of obstetric APS is still low dose aspirin (LDA) in association or not with low molecular weight heparin (LMWH). It is commonly recognized that despite treatment about 25% of APS women still suffer from recurrent pregnancy loss [2].

**STRATIFICATION OF RISK**

Many efforts have been made to identify patients at high risk of recurrence despite treatment. In terms of aPL profile, patients may have single, double or triple aPL positivity; single or multiple isotypes (IgG, IgM); and low vs. medium–high titers of antibodies. Which aPL profiles, if any, can predict adverse pregnancy outcome in women with APS is still debated; however, it is widely accepted that not all aPL profiles confer the same degree of obstetric risk. Among aPL tests, LA was found to have the highest predictive value according to a multicenter prospective study (PROMISSE: Predictors of PRegnancy Outcome: BioMarkers In Antiphospholipid Syndrome and Systemic Lupus Erythematosus). This was a multicenter observational study on pregnancy outcome of 144 patients with APS and/or systemic lupus erythematosus (SLE) who were aPL-positive. Poor pregnancy outcome was observed mainly in LA-positive women and in women with moderate to high titer IgG aCL; other aPL did not independently predict adverse pregnancy outcome. This study, however, showed some limitations. First, not all patients were tested for aβ2GPI. Second, the study design excluded patients whose pregnancies terminated before 12 weeks; therefore, it does not provide information about the aPL-mediated early miscarriages. Third, the therapeutic strategy was not homogenous among patients, casting some doubt on its possible effects on pregnancy outcome [3].

Another observational multicenter case-control study identified some independent risk factors for pregnancy morbidity. The researchers retrospectively considered 410 pregnancies and found three major independent risk factors for both pregnancy loss and pregnancy complications: (i) history of either thrombosis or previous pregnancy morbidity (without any relation to the type of previous obstetric morbidity), (ii) triple aPL positivity, and (iii) the presence of an underlying SLE or other autoimmune disease [4]. In the same report, patients with single aPL positivity without previous thrombosis seem to have lower risk of poor outcome if managed with conventional treatment [4].

**TREATMENT**

Identifying risk factors associated with pregnancy failure could be an important step in aiding clinicians to manage APS patients during pregnancy and avoid applying standard therapy to women with a high risk profile. Some patients may in fact require a personalized strategy, in addition to standard protocols, to improve their chances of a successful outcome [Table 1].
Proposed treatments for women with recurrent pregnancy loss have focused on thromboprophylaxis and immune modulation. The use of LDA and/or prophylactic dose of LMWH to treat women with APS has been guided by the original trials, which included mainly women with recurrent early miscarriage and totally excluded women with previous thrombosis, women with concomitant systemic autoimmune disease and LA-positive patients [5,6]. Therefore, we now understand that the studies conducted in the 1990s totally excluded patients with some of the known major risk factors for pregnancy morbidity in APS.

New pharmacological approaches subsequently explored tried to improve pregnancy outcome in patients refractory to standard therapy. As a first step, experts agreed to administer LMWH at a therapeutic dose, and the results were partially successful [7].

Corticosteroids are known to be associated with significant side effects, especially at a high dose; these include preterm delivery, preeclampsia, gestational diabetes, and hypertension. Despite this, it was demonstrated that women taking low doses of prednisone until 14 weeks of gestation plus standard therapy can experience favorable pregnancy outcome, with a higher live birth rate. [8]

Some controversial findings were seen following therapy with intravenous immunoglobulins G (IVIg). IVIg seem to be safe and effective, but since available data were from small series of patients only, few controlled trials support this approach and the results are therefore inconclusive [9]. With regard to plasma exchange in high risk pregnancies, only case reports have been published, and it is considered as a last-resort treatment.

Hydroxychloroquine (HCQ) should be considered for its antithrombotic and immunomodulatory properties. Its effectiveness was shown in experimental models [10]. A recently published multicenter European retrospective study assessed the effect of non-conventional treatment strategies on pregnancy outcomes in women with APS. A total of 196 pregnancies were evaluated and it was shown that the outcome of high risk pregnancies (previous thrombosis plus triple aPL positivity) significantly improved when additional treatments were applied. These treatments included IVIg and plasmapheresis, IVIg alone, plasmapheresis alone, IVIg and low dose corticosteroids, and IVIg and immunoadsorption. In particular, logistic regression analysis showed that additional treatments were the only independent factors associated with a favorable pregnancy outcome and a significant higher live birth rate.

A possible link between complement activation and pregnancy loss, growth restriction and preeclampsia, through a pro-inflammatory mechanism was recently defined in animal models. These complications could be prevented or reversed by complement inhibition. Those findings are interesting but the real effect of complement inhibition in human pregnancy is still unknown. Hence, this approach is not feasible.

In conclusion, as experts in the field have recently suggested, the use of low dose corticosteroids, LDA, LMWH, IVIg and plasma exchange, either alone or in combination, can improve pregnancy outcome in real life, even if only a few and relatively small randomized controlled trials, with doubtful results, have been published.

Table 1. Proposed management of APS patient with recurrent pregnancy loss despite standard treatment

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<th>Proposed treatments:</th>
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<td>LDA plus LMWH (prophylactic dose) or LDA plus LMWH (therapeutic dose) with or without low dose corticosteroids</td>
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Proposed management of APS patient with recurrent pregnancy loss despite standard treatment

- Identification of patients at high risk
- Proposed treatments:
  - LDA plus LMWH (prophylactic dose) or LDA plus LMWH (therapeutic dose) with or without low dose corticosteroids
  - with or without HCQ
  - with or without plasma exchange
  - with or without IVIg

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Anti-Saccharomyces cerevisiae Autoantibodies and Autoimmune Diseases: the Sweet and Sour of Baking Yeast

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Saccharomyces cerevisiae has long been used both to ferment the sugars of cereals to produce alcoholic drinks and in the baking industry to leaven dough until it became the gold-standard in brewing and baking. Thousands of years ago, humans were exposed to yeast, which accidentally “contaminated” flour or drinks. The flavorsome results have brought it into almost every meal nowadays depending on the culinary culture [1]. The probable physiopathologic mechanism of molecular mimicry of self-antigens behind the induction of anti-Saccharomyces cerevisiae autoantibodies (ASCAs) may indicate their pathogenic role in several associated autoimmune diseases. In recent decades, the pathogenesis of autoimmune diseases has increasingly come to be understood as a process involving several different factors. These factors constitute the interesting complexity of the mosaic of autoimmunity whose pieces include genetic, immunologic, hormonal and environmental factors, such as microbial agents among which yeasts are becoming apparent as part of the whole [2]. Evidence of the association between ASCAs and autoimmune disorders has increased over the past two decades.

EXPOSURE TO MICROBIAL AGENTS AND AUTOIMMUNITY

The immune system is exposed to different antigens owing to the burden of microorganisms. Therefore, the microbial pattern of each subject is unique. This physiologic process allows only mildly self-binding lymphocytes to survive according to positive selection, whereas those interacting too tightly with immunogenic molecular structures are negatively selected through a pathway leading to programmed cell death. It is noteworthy that microbial agents can induce autoimmunity through four main mechanisms:

• Molecular mimicry (cross-reactivity due to overlapping molecular sequences)
• Epitope spreading, which is the detection of new epitopes that differ from the original shared sequence after an antigen has been processed and presented on the cell surface by antigen-presenting cells (APCs)
• Bystander activation, which is based on the release of sequestered antigens as a consequence of tissue damage especially by microbial injury
• Persistent activation of the immune response, as may well occur during viral spread [1].

The gut-associated lymphoid tissue (GALT) is the main localization site of the recently defined CD4+ Th17 lymphocytes that release interleukin-17 (IL-17), involved in the response to extracellular bacteria and fungal infections. Saprophytic microbial agents can ordinarily preserve the dynamic Th17 T regulatory (Treg) balance in GALT. Nonetheless, dysregulated IL-17 secretion drives immune mediated pathology, notably inflammatory bowel diseases (IBD) in the gut [4]. Consequently, even the apparently non-pathogenic microbiota could trigger autoimmunity when the finely regulated balance is aberrantly fragile [5]. Critical data on the effect that dietary intake of “the brewer and baker’s yeast” Saccharomyces cerevisiae may induce on T helper (Th) 17 cells are still lacking.

IMMUNOLOGICAL ASPECTS AND CLINICAL-LABORATORY ANALYSIS

Yeasts are employed as efficient biological machinery that produces several antigenic components for vaccines needed to elicit protective immune responses. The phagocytosis of the yeast by dendritic cells is activated by the immunogenic cell wall molecules, such as β-1,3-D-glucan and mannan. These proteins can induce critical signals usually associated with microbial infection. In fact, this pair of events constitutes the first step and is followed by antigen degradation and its fragment presentation on the APC surface by major histocompatibility complex (MHC) I and MHC II molecules, interacting with the T cell receptor and prompting
co-stimulatory signals to lead the adaptive T cell (CD8+ or CD4+)-mediated response [1,8]. Subsequently, the development of the humoral immune response leads to the production of antibodies against the yeast by both B lymphocytes and plasma cells, thus it is not yet clear whether this outcome might be an epiphenomenon or could have a direct pathogenic role through a co-stimulatory CD80/86-CD28-mediated effect. By contrast, concerns have been raised regarding the current safety of vaccines due to the presence of adjuvants [3]. Shoenfeld and Agmon-Levin described the autoimmune/inflammatory syndrome induced by adjuvants (ASIA syndrome) in which adjuvants may trigger an autoimmune/inflammatory response in predisposed individuals. In this context, heat-killed Saccharomyces can act like common adjuvants, such as aluminium and silicone, when injected together with preventive vaccines [1,3,6]. ASCAs are directed against the cell wall mannan (phosphopeptidomannan) of S. cerevisiae. The assessment of ASCAs by enzyme-linked immunosorbent assay (ELISA) resulted in 50–79% sensitivity and 74–93% specificity in Australian patients with Crohn’s disease [7], depending on the commercial kits used such as those giving ASCA IgA/IgG-positive results at 10 U/ml with a detection threshold of 1 U/ml (ORG 545 ASCA IgG/IgA, ORGENTEC® Diagnostika GmbH, Germany).

**DATABANK SEARCH METHOD AND RESULTS**

The Protein Database of the National Center for Biotechnology Information (NCBI) was consulted, focusing on the most specific and significant results (highest identity/positivity). Table 1 summarizes the main findings from our group. We also evaluated the E value, which represents the number of different alignments with scores equivalent to or better than is expected for protein sequences. The percentage of sequence identities and/or positive substitutions expresses the extent to which the protein sequences are related.

**ASCA POSITIVITY IN AUTOIMMUNE DISEASES**

ASCA immunoglobulin A, G and M levels were measured by ELISA in 30 patients affected with RA and in 152 healthy adult controls. ASCA IgA prevalence was significantly higher in patients suffering from RA than in the healthy subjects (40% vs. 5.3%). In RA patients, ASCA IgA levels were also strongly correlated with C-reactive protein (CRP) (r = 0.695, P < 0.01) as well as erythrocyte sedimentation rate (ESR) (r = 0.708, P < 0.01) [1]. As shown in Table 1, significant similarities were observed between the sequence alignments of mannan and U2 snRNP B’ showing the best match. Considering several autoimmune disorders associated with ASCA positivity [1], we also discovered other autoantigens that might cross-react with antibodies against mannan of S. cerevisiae according to the percentages of sequence identities (ID) and/or positive substitutions (PS) such as GAD65 (ID 35%, PS 57%) and zinc-transporter 8 (ID 43%, PS 57%), α-enolase 1 in Behçet’s disease with ocular involvement (uveitis) (ID 63%, PS 88%), thyroid peroxidase (ID and PS 71%) for autoimmune thyroid disease, and calprotectin (ID 60%, PS 100%) for Crohn’s disease [1].

**ASCA POSITIVITY AND ATHEROSCLEROSIS**

Interestingly, several homologies have been detected by analyzing and matching the molecular sequence of S. cerevisiae phosphopeptidomannan with cardiac myosin (ID 63%, PS 88%). Complementary to this, elevated ASCA IgA and IgG levels were found in patients who had suffered an acute coronary syndrome (ACS) or acute myocardial infarction (AMI), suggesting that ASCA positivity in AMI could be identified as a useful marker for atherosclerotic plaque instability [10]. Therefore, since autoantibodies can be considered stable over time, they may be less dependent on the period between plaque rupture and AMI onset than other assessable inflammatory biomarkers [10]. Additionally, the assessment of these autoantibodies could be a valid addition for the careful screening of patients whose anti-cardiac troponin I (anti-cTnI) serum

<table>
<thead>
<tr>
<th>Autoimmune disease</th>
<th>ASCA</th>
<th>Antigen (Hom sapiens)</th>
<th>Comparison to mannan: # EDV13046,1</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>neg</td>
<td>gp130-RAPS</td>
<td>4/5, 80%</td>
</tr>
<tr>
<td>SLE</td>
<td>pos</td>
<td>U2snRNP B’</td>
<td>5/6, 83%</td>
</tr>
<tr>
<td>APS</td>
<td>pos</td>
<td>antiCL/β2GlI</td>
<td>7/11, 64%</td>
</tr>
<tr>
<td>ACS/AMI</td>
<td>pos</td>
<td>cardiac myosin</td>
<td>5/8, 63%</td>
</tr>
<tr>
<td>Behçet’s disease</td>
<td>pos</td>
<td>α-enolase 1</td>
<td>5/8, 63%</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>pos</td>
<td>Calprotectin</td>
<td>3/5, 60%</td>
</tr>
</tbody>
</table>

The table above shows the original selection of the most significant results from our exhaustive collection.

The percentage of sequence identities and/or positive substitutions expresses the extent to which the protein sequences are related to the yeast by both B lymphocytes and plasma cells, thus it is not yet clear whether this outcome might be an epiphenomenon or could have a direct pathogenic role through a co-stimulatory CD80/86-CD28-mediated effect. By contrast, concerns have been raised regarding the current safety of vaccines due to the presence of adjuvants [3]. Shoenfeld and Agmon-Levin described the autoimmune/inflammatory syndrome induced by adjuvants (ASIA syndrome) in which adjuvants may trigger an autoimmune/inflammatory response in predisposed individuals. In this context, heat-killed Saccharomyces can act like common adjuvants, such as aluminium and silicone, when injected together with preventive vaccines [1,3,6]. ASCAs are directed against the cell wall mannan (phosphopeptidomannan) of S. cerevisiae. The assessment of ASCAs by enzyme-linked immunosorbent assay (ELISA) resulted in 50–79% sensitivity and 74–93% specificity in Australian patients with Crohn’s disease [7], depending on the commercial kits used such as those giving ASCA IgA/IgG-positive results at 10 U/ml with a detection threshold of 1 U/ml (ORG 545 ASCA IgG/IgA, ORGENTEC® Diagnostika GmbH, Germany).
level may produce false negatives, bringing about a delay in non-ST segment elevation myocardial infarction (NSTEMI) diagnosis [10].

CONCLUSIONS
Our results strongly suggest that the role of ASCAs in clinical practice, as well as the dietary recommendations for specific groups of patients, should be further explored in order to evaluate their predictive or prognostic relevance. New manufacturing challenges may need to be considered for vaccines containing S. cerevisiae.

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Capsule

Parasites make it hard to fight viruses
Microbial co-infections challenge the immune system – different pathogens often require different flavors of immune responses for their elimination. Two teams studied what happens when parasitic worms and viruses infect mice at the same time. Reese et al. (Science 2014; 345: 73) found that mice already infected with parasitic worms were worse at fighting off viruses. In both cases, worms skewed the immune response so that the immune cells and the molecules they secreted created an environment favorable for the worm at the expense of antiviral immunity.

Eitan Israeli

Capsule

Angiotensin-neprilysin inhibition versus enalapril in heart failure
McMurray et al. compared the angiotensin receptor-neprilysin inhibitor LCZ696 with enalapril in patients who had heart failure with a reduced ejection fraction. In previous studies, enalapril improved survival in such patients. In this double-blind trial, the authors randomly assigned 8442 patients with class II, III, or IV heart failure and an ejection fraction of 40% or less to receive either LCZ696 (at a dose of 200 mg twice daily) or enalapril (at a dose of 10 mg twice daily) in addition to recommended therapy. The primary outcome was a composite of death from cardiovascular causes or hospitalization for heart failure, but the trial was designed to detect a difference in the rates of death from cardiovascular causes. The trial was stopped early, according to prespecified rules, after a median follow-up of 27 months, because the boundary for an overwhelming benefit with LCZ696 had been crossed. At the time of study closure, the primary outcome had occurred in 914 patients (21.8%) in the LCZ696 group and 1117 patients (26.5%) in the enalapril group [hazard ratio in the LCZ696 group 0.80, 95% confidence interval (CI) 0.73–0.87, P < 0.001]. A total of 711 patients (17.0%) receiving LCZ696 and 835 patients (19.8%) receiving enalapril died (hazard ratio for death from any cause 0.84, 95%CI 0.76–0.93, P < 0.001); of these patients, 558 (13.3%) and 693 (16.5%), respectively, died from cardiovascular causes (hazard ratio 0.80, 95%CI 0.71–0.89, P < 0.001). As compared with enalapril, LCZ696 also reduced the risk of hospitalization for heart failure by 21% (P < 0.001) and decreased the symptoms and physical limitations of heart failure (P = 0.001). The LCZ696 group had higher proportions of patients with hypotension and nonserious angioedema but lower proportions with renal impairment, hyperkalemia, and cough than the enalapril group.

Eitan Israeli
Obesity: an Additional Piece in the Mosaic of Autoimmunity

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The recent outbreak of autoimmune diseases in industrialized countries has brought into question the factors contributing to this increased prevalence. Given the constancy of genetics, growing attention has focused on environmental factors, especially the western lifestyle. Indeed, in the last few decades significant changes in western dietary habits have led to the parallel rise in obesity. White adipose tissue (WAT), previously believed to be an inert tissue, was recently found to secrete numerous soluble mediators called adipokines that are involved in many processes including immunity and inflammation. Thus, a link between obesity and autoimmune diseases was strongly suggested. In a systematic literature review we analyzed the relationship between obesity, adipokines and several immune mediated conditions.

FROM OBESITY TO AUTOIMMUNITY

Several mechanisms have been suggested to explain how obesity could promote autoimmune disease. One of them is the secretion by WAT of adipokines, which encompass both classical cytokines and specific molecules such as leptin, adiponectin, resistin and visfatin. In addition to their role in various physiologic processes, adipokines modulate the immune response and contribute to the “low grade inflammation state” in obese subjects. Leptin, resistin and visfatin exhibit pro-inflammatory activity and their levels are directly correlated with adipose mass. Conversely, adiponectin is an anti-inflammatory molecule and its secretion is reduced in obese persons. Other mechanisms may link obesity and autoimmunity, but these require further research. Thus, the frequently found vitamin D deficiency in obese subjects, alteration of gut microbiota due to a western diet, or obesity itself may be responsible for a dysregulation of T helper (Th)17/T-regulatory cell (Treg) balance, contributing to the development of autoimmunity. Some authors highlighted the role of the apoptosis inhibitory macrophage (AIM), a macrophage-derived blood protein whose circulating levels are increased in obesity. AIM has been found to promote autoantibody formation, M1-macrophage infiltration in WAT, as well as inflammasome activation, leading to both inflammation and autoimmune response.

OBESITY AND AUTOIMMUNE DISEASES

- Obesity and rheumatoid arthritis (RA)
  RA is an inflammatory autoimmune disease characterized by synovial inflammation and joint destruction. Most studies, including two recent large prospective studies, reported a higher risk of RA developing, especially anticitrullinated protein antibody (ACPA)-negative RA, among obese individuals with an odds ratio (OR) ranging from 1.2 to 3.4. Furthermore, obesity is associated with a more severe disease and a higher prevalence of comorbidities, as well as a worse therapeutic response, particularly for infliximab. This is consistent with pathophysiological data indicating that increased levels of pro-inflammatory adipokines, as found in obese subjects, correlate with severity parameters. Conversely, a paradoxical protective effect of obesity on radiographic joint damage was observed.

- Obesity and multiple sclerosis (MS)
  MS is the most common chronic inflammatory demyelinating disease of the central nervous system and it affects mainly young adults. A strong body of evidence supports obesity as a risk factor for developing MS. First, several large clinical studies found an overall twofold increased risk of MS associated with obesity during childhood or late adolescence – critical periods of susceptibility for MS. This risk seems to be more pronounced among women. Moreover, adipokine involvement in the pathogenesis of MS was strongly demonstrated, particularly for leptin. Thus this hormone, whose levels are elevated in obese subjects, is critical for induction and progression of murine models of MS, notably by promoting a Th1 pro-inflammatory profile and reducing Treg cells. Similarly, low adiponectin and high visfatin levels have been shown to promote the onset and severity of murine MS.

- Obesity, psoriasis and psoriatic arthritis (PsA)
  Psoriasis is a common chronic inflammatory skin disease that in a third of cases may be complicated by articular involvement, namely PsA. The association between obesity and both psoriasis and PsA is widely recognized. It appears to result
from bidirectional phenomena. First, psoriasis and PsA promote weight gain by changes in lifestyle such as depression, overeating and physical inactivity. Second, several large prospective studies have demonstrated that obesity is an independent risk factor for psoriasis and PsA (OR 1.48–6.46) [6]. In addition, obesity has been shown to worsen psoriasis and PsA evolution, increase cardiovascular comorbidities, and impair therapeutic response. This is likely partly due to the pro-inflammatory and pro-atherogenic effects of adipokines. Indeed, high rates of leptin and resistin were observed in the serum and skin of psoriatic patients and correlated positively with disease activity. In support of these data, weight loss has been shown to lessen disease severity and improve treatment efficacy.

- **Obesity and systemic lupus erythematosus (SLE)**
  SLE is a systemic autoimmune disorder. Data on the risk of occurrence of SLE under obese conditions are scarce and do not allow a definite conclusion. With regard to disease severity, although obesity has not been found to correlate with SLE activity scores, it appears to be associated more with lupus nephritis, cognitive impairment, cardiovascular morbidities, and an impaired quality of life [7]. The high levels of leptin and resistin and low levels of adiponectin observed in both SLE and obese subjects, by their pro-inflammatory and pro-atherogenic effects, are thought to contribute to this association.

- **Obesity and inflammatory bowel disease (IBD)**
  IBD is a group of inflammatory diseases affecting the gut whose main forms are Crohn’s disease (CD) and ulcerative colitis (UC). Despite some conflicting results, a body of clinical and experimental evidence points to obesity as a risk and aggravating factor of IBD. First, epidemiologic data highlight the simultaneous outbreak of both IBD [8] and obesity in western countries. In addition, several studies have correlated obesity with the risk of occurrence as well as a more severe course of IBD. Moreover, experimental data in mice models and patients support the involvement of adipokines, as found in obese individuals, in the pathogenesis of IBD. Indeed, high levels of leptin, resistin and visfatin in plasma, visceral adipose tissue or gut lumen were found to be associated with intestinal inflammation. Finally, obese IBD patients also exhibit a worse therapeutic response, especially to anti-tumor necrosis factor-α (TNFα) therapies.

- **Obesity and type 1 diabetes (T1D)**
  T1D is a juvenile-onset dysregulation of glucose metabolism resulting from autoimmune destruction of insulin-secreting β-cells. The impact of overweightness at different ages of life on the risk of subsequent T1D has been extensively investigated. Therefore, high birth weight, early weight gain in the first years of life, childhood obesity and adult obesity have been associated with a twofold increased risk of T1D on average [9]. However, it remains a matter of debate whether obesity acts as a real risk factor or just an accelerator shifting the onset of diabetes at an earlier age without changing the lifetime risk. Several mechanisms have been proposed. First, obesity-induced insulin resistance may lead to β-cell overload and apoptosis rendering them immunogenic. Moreover, adipokines have been found to promote an autoimmune response against β-cells as well as metabolic and vascular complications.

- **Obesity and Hashimoto thyroiditis (HT)**
  HT is a highly common autoimmune disease responsible for hypothyroidism and characterized by goiter with lymphocytic infiltration and thyroid autoantibodies. It is now recognized that obese subjects often exhibit high levels of thyroid-stimulating hormone (TSH). In most cases it is not related to hypothyroidism or to an autoimmune process, but more likely is an adaptive mechanism of the hypothalamic-pituitary axis partially regulated by leptin to increase energy expenditure. Nevertheless, several studies also demonstrated that obesity may promote the development of thyroid autoimmunity, especially HT-related autoantibodies, and this was correlated with leptin levels as well as Th17 cells, suggesting the involvement of leptin in the pathogenesis of HT [10].
Clearly, obesity has emerged as a new piece in the mosaic of risk of RA, MS, psoriasis and PsA, and is suggested to promote IBD, T1D and HT. Furthermore, obese patients exhibit a more severe course of RA, SLE, IBD, psoriasis and PsA and reduced therapeutic response in RA, IBD, psoriasis and PsA. Clearly, obesity has emerged as a new piece in the mosaic of autoimmune [Figure 1].

Conclusions
The recent increase in incidence of autoimmune diseases is gaining interest. A number of environmental factors have been shown to participate in this phenomenon. Following the discovery of the broad endocrine properties of WAT and particularly its immunomodulatory effects, numerous studies have highlighted the involvement of obesity and its adipokines in the pathogenesis of various autoimmune conditions. Thus, obesity is clearly associated with an increased risk of RA, MS, psoriasis and PsA, and is suggested to promote IBD, T1D and HT. Furthermore, obese patients exhibit a more severe course of RA, SLE, IBD, psoriasis and PsA and reduced therapeutic response in RA, IBD, psoriasis and PsA. Clearly, obesity has emerged as a new piece in the mosaic of autoimmune [Figure 1].

References

A neuropeptide kills patient’s motivation
Chronic pain is not only extremely disturbing and unpleasant, it can also make people depressed and demotivated. What causes these effects? Schwartz and co-researchers discovered that chronic pain causes changes in the way a neuropeptide called galanin affects certain neurons in a brain region called the nucleus accumbens. Galanin influences a variety of behaviors, including feeding and certain aspects of pain. In this case, it depresses synaptic transmission at specific excitatory synapses. It does so, in part, by changing the ratio of subunits of an important receptor protein. Science 2014; 345: 535

Combinations of antibiotics to fight bacteria
Is it possible to streamline the complex task of finding new drugs to fight resistant bacteria and other disease targets? Most biological processes are controlled by complicated regulatory networks, so combinations of two or more drugs are likely to be more effective than any single agent. Finding combinations that work means first screening enormous numbers of possibilities. Cheng et al. examined mixtures of genetic elements in millions of different combinations.

Those combinations with the desired effect in a biological test could be identified afterward by high-throughput sequencing capable of detecting associated DNA “barcode” identifier sequences. Results are promising and revealed combinations of transcription factors that enhanced lethal effects of an antibiotic by a millionfold. Proc Natl Acad Sci USA 2014;10.1073/pnas.140003911

“Being in the same room with people and creating something together is a good thing”
Robin Williams (1951-2014), American actor and comedian. His film career included acclaimed work such as Popeye (1980), The World According to Garp (1982), Good Morning, Vietnam (1987), Dead Poets Society (1989), Awakenings (1990), The Fisher King (1991), and Good Will Hunting (1997), as well as financial successes, Mrs. Doubtfire among others. Williams received numerous awards. He died of an apparent suicide at his home in California
Hereditary Angioedema and Autoimmunity

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KEY WORDS: autoantibodies, acquired angioedema (AAE), autoimmune disease, complement, hereditary angioedema (HAE)

Hereditary angioedema (HAE) is a rare autosomal dominant disorder that is defined by a deficiency of complement component 1 (C1) esterase inhibitor (C1-INH), a glycosylated serine protease inhibitor (serpin) that plays a regulatory role in the complement cascade, the contact system, and the intrinsic coagulation cascade [1]. Deficiency of this regulator causes chronic activation of the complement cascade with a resultant deficiency of C4 and C2 and the overproduction of bradykinin, leading to increased vascular permeability and severe edema [1].

HAE accounts for approximately 2% of clinical angioedema cases and affects approximately 1 in 50,000 people. HAE is due to mutations in the C1-INH gene (SERPING1) that result in deficiency or functional impairment of C1-INH. The most common is type I (85% of all HAE), which is related to a mutation of one copy of the SERPING1 gene and is characterized by low levels of both C1-INH antigen and functional activity. HAE type II (15%–20% of all HAE) is clinically indistinguishable from type I and is characterized by mutations of the SERPING1 gene, leading to the production of non-functional enzyme with normal or even high levels of low functional C1-INH antigen. HAE type III is a form of HAE not caused by C1-INH deficiency or dysfunction with normal C1-INH; it is found predominantly in women and may be due to known mutations in the factor XII gene or to unknown genetic mutations [1].

Data from the literature on HAE prevalence were recently confirmed in a report by the Italian Network for C1-INH-HAE (ITACA), established in 2012, in which our Division represents one of the 17 active centers. The ITACA database included 983 patients (53% female) with C1-INH-HAE from 376 unrelated families: the minimal prevalence of C1-INH-HAE in Italy in 2013 was 1.54:100,000 inhabitants, equivalent to a prevalence of 1:64,935 (median age of patients 45 years, with median age at diagnosis 26 years). The majority of patients (87%) had C1-INH-HAE type I; patients with type II comprised 13%. Clinical manifestations of C1-INH deficiency are a result of increased vascular permeability in subcutaneous and submucosal soft tissues and include recurrent episodes of non-pitting edema of the extremities, face, upper respiratory tract, gastrointestinal mucosa and genitals, with a variety of clinical complications and management issues [1]. The edema develops slowly over a period of up to 36 hours and resolves 1 to 3 days later. C1-INH deficiency may be also acquired. Angioedema due to acquired C1-INH deficiency, frequently referred to as acquired angioedema (AAE), has been reported rarely, usually in association with lymphoproliferative disorders or autoimmune, neoplastic, or infectious diseases [2]. The disease generally manifests in adulthood and is characterized by decreased activity of C1-INH, decreased but sometimes normal levels of C1-INH protein, decreased C4 and, frequently, decreased C1q.

Autoantibodies to anti-C1-INH seem to prevent the inhibitory activity of the C1-INH on target proteases and convert the inhibitor into a substrate that can be cleaved to an inactive form. Autoantibodies directed to C1-INH were also identified in patients without diseases, suggesting the existence of two forms of acquired C1-INH deficiency: type 1 associated with B cell disorders and type 2 with autoantibodies. This issue was later questioned and it is now clear that patients with C1-INH autoantibodies may have or may develop B cell malignancies. Monoclonal gammapathy of unknown significance (MGUS) is the most frequent condition associated with the acquired C1-INH deficiency [2]. In the context of the autoimmune mechanisms involving the complement components, autoantibodies directed to C1q, which recognize the collagen-like region (CLR) of C1q molecule, are frequently present in the serum of patients with autoimmune diseases, mainly systemic lupus erythematosus (SLE) and hypocomplementemic urticarial vasculitis syndrome, contributing to clinical manifestations in those conditions [3]. Several investigators have demonstrated the presence of anti-C1q autoantibodies in sera of patients with rheumatoid arthritis, in patients with renal diseases as well as in healthy individuals [3].

Advances in our understanding of immunologic pathways in the complement system provide new insights on the immunoregulatory disorders as well as the autoimmune manifestations in HAE patients [4]. Occasional reports have linked HAE with autoimmune diseases and only a few studies have been conducted on large patient populations, with controversial results. In 1986, Brickman et al. [5] systematically evaluated 157 patients with HAE-C1-INH for manifestations of autoim-
munity and reported an increased frequency of autoimmune disease. In 1987, Muhlemann et al. [6] screened 91 patients with HAE-C1-INH for thyroid antibodies and found that the prevalence of thyroglobulin antibodies and thyroid peroxidase in the group of young female patients with HAE-C1-INH was higher than expected. A low frequency of autoimmune disease was found by Agostoni and Cicardi [7], who identified only one case of rheumatoid arthritis and a single case of Sjögren’s syndrome (SjS) among 226 HAE-C1-INH patients. In 1996, Nielsen et al. [8] revealed no statistically significant differences between HAE-C1-INH patients and their healthy relatives concerning the prevalence of autoantibodies, although rheumatic complaints were reported by 53% of HAE-C1-INH patients and 12% of their unaffected relatives [8]. Farkas et al. analyzed clinical data and immunoserologic parameters of 130 HAE-C1-INH and 174 non-C1-INH-deficient patients with angioedema, but did not find a higher prevalence of immunoregulatory disorders among HAE-C1-INH patients [9]. Interestingly, immunoserology screening revealed a greater prevalence of anticardiolipin IgM among HAE-C1-INH patients than in those with non-C1-INH-deficient angioedema [9].

To advance our understanding of immunoregulatory disorders in HAE patients, we recently performed a large population study in HAE patients in collaboration with the Allergy and Clinical Immunology Units at medical centers in Israel [10]. We characterized the profile of autoantibodies in a group of HAE patients in terms of rheumatoid factor, antinuclear autoantibodies, anticardiolipin, anti-tissue transglutaminase, anti-endomyosial, anti-Saccharomyces cerevisiae, anti-thyroid and anti-neutrophil cytoplasmic antibodies. We also analyzed the memory B cell phenotype, the Toll-like receptor (TLR)-9 expression and total phosphorysine in B cells isolated from HAE patients. We demonstrated that HAE patients have enhanced production of autoantibodies due most probably to the increased activation of B cells, which was found to be associated with a high expression of TLR-9.

According to our database, which collects medical histories and laboratory findings of HAE patients referred to our Division, we systematically reviewed the medical records of 143 patients with HAE for manifestations of autoimmunity. Characteristics of those 143 patients are as follows: 72 women [65/72 (90.3%) type I HAE; 7/72 (9.7%) type II HAE; mean age at diagnosis 28.13 ± 18.7 years] and 71 men [61/71 (86%) type I HAE; 10/71 (14%) type II HAE; mean age at diagnosis 27 ± 17.7 years]. Among these patients, we diagnosed autoimmune diseases in 6 (4.2%): antiphospholipid syndrome in 2 patients with type I HAE, systemic sclerosis in a man with type II HAE, psoriatic arthritis in a man with type I HAE, SLE in a woman with type I HAE, and mixed connective tissue disease in a man with type I HAE. It should be noted that most of the patients with autoimmune conditions were affected by type I HAE and that for all these patients a familial history for HAE was regis-

| Table 1. Characteristics of patients with angioedema and autoimmune diseases referred to our Division |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Patient | Type of AE | Gender | Age at AE diagnosis (yr) | Autoimmune disease | AE disease duration* (yr) |
| 1 | Type I HAE | M | 29 | APS | 9 |
| 2 | Type I HAE | F | 29 | APS | 9 |
| 3 | Type II HAE | M | 20 | SSc | 22 |
| 4 | Type I HAE | M | 51 | PsA | 10 |
| 5 | Type I HAE | F | 6 | SLE | 29 |
| 6 | Type I HAE | M | 27 | MCTD | 30 |
| 7 | AAE | F | 45 | SjS | 1 |

*AE disease duration at the time of the autoimmune disease diagnosis
HAE = hereditary angioedema, AE = angioedema, AAE = acquired angioedema, APS = antiphospholipid syndrome, SSc = systemic sclerosis, PsA = psoriatic arthritis, SLE = systemic lupus erythematosus, MCTD = mixed connective tissue disease, SjS = Sjögren syndrome

References
Comprehensive molecular characterization of gastric adenocarcinoma

Gastric cancer is a leading cause of cancer deaths, but analysis of its molecular and clinical characteristics has been complicated by histological and etiological heterogeneity. The Cancer Genome Atlas Research Network describe a comprehensive molecular evaluation of 295 primary gastric adenocarcinomas as part of The Cancer Genome Atlas (TCGA) project. The authors propose a molecular classification dividing gastric cancer into four subtypes: tumors positive for Epstein-Barr virus, which display recurrent PIK3CA mutations, extreme DNA hypermethylation, and amplification of JAK2, CD274 (also known as PD-L1) and PDCD1LG2 (also known as PD-L2); microsatellite unstable tumors, which show elevated mutation rates, including mutations of genes encoding targetable oncogenic signaling proteins; genomically stable tumors, which are enriched for the diffuse histological variant and mutations of RHOA or fusions involving RHO-family GTPase-activating proteins; and tumors with chromosomal instability, which show marked aneuploidy and focal amplification of receptor tyrosine kinases. Identification of these subtypes provides a roadmap for patient stratification and trials of targeted therapies. Nature 2014; 513: 202

Eitan Israeli

AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer

The androgen-receptor isoform encoded by splice variant 7 lacks the ligand-binding domain, which is the target of enzalutamide and abiraterone, but remains constitutively active as a transcription factor. We hypothesized that detection of androgen-receptor splice variant 7 messenger RNA (AR-V7) in circulating tumor cells from men with advanced prostate cancer would be associated with resistance to enzalutamide and abiraterone. Antonarakis et al. used a quantitative reverse-transcriptase-polymerase chain reaction assay to evaluate AR-V7 in circulating tumor cells from prospectively enrolled patients with metastatic castration-resistant prostate cancer who were initiating treatment with either enzalutamide or abiraterone. We examined associations between AR-V7 status (positive vs. negative) and prostate-specific antigen (PSA) response rates (the primary end-point), freedom from PSA progression (PSA progression-free survival), clinical or radiographic progression-free survival, and overall survival. A total of 31 enzalutamide-treated patients and 31 abiraterone-treated patients were enrolled, of whom 39% and 19%, respectively, had detectable AR-V7 in circulating tumor cells. Among men receiving enzalutamide, AR-V7-positive patients had lower PSA response rates than AR-V7-negative patients (0% vs. 53%, P = 0.004) and shorter PSA progression-free survival (median 1.4 months vs. 6.0 months, P = 0.001), clinical or radiographic progression-free survival (median 2.1 vs. 6.1 months, P < 0.001), and overall survival (median 5.5 months vs. not reached, P = 0.002). Similarly, among men receiving abiraterone, AR-V7-positive patients had lower PSA response rates than AR-V7-negative patients (0% vs. 68%, P = 0.004) and shorter PSA progression-free survival (median 1.3 months vs. not reached, P < 0.001), clinical or radiographic progression-free survival (median 2.3 months vs. not reached, P < 0.001), and overall survival (median 10.6 months vs. not reached, P = 0.006). The association between AR-V7 detection and therapeutic resistance was maintained after adjustment for expression of full-length androgen receptor messenger RNA.


Eitan Israeli

“Keep your eyes on the stars, and your feet on the ground”

Theodore Roosevelt (1858-1919), American politician, author, naturalist, explorer, and historian who served as the 26th President of the United States

2013; 87: 323-32.
Old Obstacles but New Hopes: Trying to Understand the Fibromyalgia Construct

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KEY WORDS: fibromyalgia, compliance, drug adherence, diagnosis, pain

Fibromyalgia is a chronic debilitating disorder characterized by widespread pain, allodynia and hyperalgesia on one hand, with fatigue, unrefreshing sleep accompanied by mood and cognitive disturbances on the other. It affects 5% of the population worldwide with a clear female preponderance [1]. More than two decades ago fibromyalgia was acknowledged and defined by classification criteria that underlined the somatic aspects of the disorder. This set of criteria was adopted by the American College of Rheumatology (ACR) [2]. Within the last 4 years the diagnosis of fibromyalgia has progressed, emphasizing the importance of the symptoms beyond pain. The new suggested criteria take into consideration additional symptoms mentioned above [3].

Despite significant achievements in the field of fibromyalgia research linking it to various neurophysiological mechanisms, many physicians still regard fibromyalgia as a controversial entity since there is no objective test to confirm the diagnosis [4]. The diagnosis is further complicated by the stigmatization of this disorder among treatment providers, the health insurance industry, and the general population. The immense financial and emotional burden of this syndrome reflects the complexity of the disease, its comorbidities, and the difficulties in its diagnosis [1].

The financial burden of fibromyalgia is substantial, with reported health care costs (for 12 months) ranging from $2274 to $9573 in the United States and up to $2298 in Canada, not including indirect costs such as disability claims and loss of work days [5]. The health care system is utilized at significantly higher rates by fibromyalgia patients due to more frequent visits to the physician, laboratory and imaging tests and visits to the emergency department. In addition, these patients are more likely, as mentioned, to suffer from comorbidities and are more prone to receive pain-related medications [1,3,6,7].

TREATMENT

Treatment of fibromyalgia is a complex issue, encompassing a wide diversity of therapies – both pharmacological and non-pharmacological [6]. The most substantiated pharmacological treatments, with an A1 level of evidence, are norepinephrine serotonin reuptake inhibitors (milnacipran, duloxetine), gabapentinoids (pregabalin, gabapentin), tricyclic antidepressants (amitryptiline), and Υ-amino butyrate. To date, no specific medication has been proven significantly more efficient than another, but most medications show an amelioration of 30–50% in pain in up to half the patients [1,6]. Many non-pharmacological therapies have been studied such as exercise, education, and cognitive behavioral therapy, the latter being the most investigated and the most substantiated [1,6]. In addition, many alternative and complementary therapies are offered, although there is a paucity of good evidence due to different problems in study design. Most guidelines emphasize the importance of education on the nature of the disease. Empowerment of an active patient stance towards the disease and its implication is seminal in order to achieve therapeutic success; such an intervention should include physical activity and cognitive behavioral therapy as crucial adjuncts to pharmacological therapies [1,6,8].

ADHERENCE TO TREATMENT

There is little research on adherence in fibromyalgia. In general it has been shown that adherence is higher in acute pain conditions compared to chronic conditions, and that improving adherence leads to reduced health care costs and improved patient quality of life [9,10]. Various barriers to adherence have been studied, including cognitive barriers such as fears regarding analgesic use (fear of addiction, etc.); concern of appearing weak to family, physicians and others; and a belief that pain is an inevitable part of the disease [10]. Other obstacles to improved compliance are psychological factors, patient-
physician discordance, not being under a rheumatologist’s care, comorbidities, and others. There is sparse literature on adherence specifically in fibromyalgia, and even less on adherence to specific medications. Of the little that is known, only 33% of patients prescribed duloxetine were considered highly adherent, with a higher adherence to lower dosages. When compared to pregabalin, duloxetine had better adherence rates with less titration of dosage during the first year.

It is of utmost importance to investigate adherence in fibromyalgia, addressing the many issues that affect it in order to better our practice. Addressing compliance and adherence, particularly in these patients, might lead to reduced health care costs and improved quality of care. More data on this issue are therefore warranted.

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References

Evolution of Ebola virus over time
The high rate of mortality in the current Ebola epidemic has made it difficult for researchers to collect samples of the virus and study its evolution. Gire et al. describe Ebola epidemiology on the basis of 99 whole-genome sequences, including samples from 78 affected individuals. The authors analyzed changes in the viral sequence and conclude that the current outbreak probably resulted from the spread of the virus from central Africa in the past decade. The outbreak started from a single transmission event from an unknown animal reservoir into the human population. Two viral lineages from Guinea then spread from person to person into Sierra Leone.

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Eitan Israeli

Loss of oncogenic Notch1 with resistance to a PI3K inhibitor in T cell leukemia
Mutations that deregulate Notch1 and Ras/phosphoinositide 3 kinase (PI3K)/Akt signaling are prevalent in T cell acute lymphoblastic leukemia (T-ALL), and often coexist. Dail and colleagues show that the PI3K inhibitor GDC-0941 is active against primary T-ALLs from wild-type and KrasG12D mice, and addition of the MEK inhibitor PD0325901 increases its efficacy. Mice invariably relapsed after treatment with drug-resistant clones, most of which unexpectedly had reduced levels of activated Notch1 protein, down-regulated many Notch1 target genes, and exhibited cross-resistance to γ-secretase inhibitors. Multiple resistant primary T-ALLs that emerged in vivo did not contain somatic Notch1 mutations present in the parental leukemia. Importantly, resistant clones up-regulated PI3K signaling. Consistent with these data, inhibiting Notch1 activated the PI3K pathway, providing a likely mechanism for selection against oncogenic Notch1 signaling. These studies validate PI3K as a therapeutic target in T-ALL and raise the unexpected possibility that dual inhibition of PI3K and Notch1 signaling could promote drug resistance in T-ALL.

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Eitan Israeli

“Quality is never an accident. It is always the result of intelligent effort”
John Ruskin (1819-1900), leading English art critic of the Victorian era, also an art patron, draughtsman, watercolorist, a prominent social thinker and philanthropist. Today, his ideas and concerns are widely recognized as having anticipated interest in environmentalism and sustainability
Treating Inflammatory Bowel Disease: from Helminths to Ova

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KEY WORDS: helminth, inflammatory bowel disease (IBD), colitis, hygiene hypothesis

During the last century, the western lifestyle had led to a decrease in the infectious burden. On the other hand, there is a high rate of autoinflammatory disorders expressed by a higher prevalence of autoimmune and autoinflammatory diseases and allergies. Strachan, who first proposed “The Hygiene Theory” while following more than 17,000 British children born in 1958, noticed an inverse correlation between hay fever and the number of older siblings. This hypothesis states that limited exposure to microorganisms such as helminths and microbes in childhood will eventually lead to an off-balanced immune system [1]. The eradication of helminths was shown to increase atopic skin sensitization in Venezuela, Gabon, and Vietnam. Moreover, the disappearance of malaria due to mosquito-eradication programs was linked to the increase of multiple sclerosis in Sardinia with respect to the high genetic susceptibility of human leukocyte antigen (HLA) DR3 on the island.

Helminths aim to survive in the host and therefore try to induce a tolerance scenario. Yet, it is important to keep in mind that immunomodulation is affected by several key elements such as the helminth’s species and the host’s immune system response. In most cases helminths will induce tolerance, but in some scenarios they may cause inflammatory responses. Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the digestive tract and manifests primarily as Crohn’s disease (CD) and ulcerative colitis (UC). CD may involve inflammation in any part of the gastrointestinal tract (from mouth to anus), while UC is confined to the large intestine (the colon and rectum) [2]. In the late 1990s, Weinstock [3] raised the “inflammatory bowel disease hygiene hypothesis,” based on the increasing prevalence of IBDs which was in reverse correlation to the prevalence of helminths in the United States. He proposed that exposure to helminths might protect against IBDs [3]. Further proof was given by a study carried out in sub-Saharan Africa where helminth intestinal infestation is frequent. There was a low incidence and prevalence of IBD that could not be explained by genetic factors, since the incidence of IBDs in the black populations of the USA and Britain was approaching that of the Caucasian population [3].

Helminths, their ova and their antigens have been used in many studies that have attempted to treat IBDs in murine models as well as in humans [3-8] [Table 1]. One model of experimental colitis is interleukin-10 (IL-10) knockout mice. IL-10 is an important immunoregulatory cytokine that down-modulates macrophage activation, thus IL-10⁻/⁻ deficient mice develop spontaneous chronic colitis. Colonization of Heligmosomoides polygyrus in IL-10 knockout mice with piroxicam-induced colitis was shown to suppress established inflammation and to decrease lymphocytic infiltration. Moreover, in vitro analysis of lamina propria mononuclear cells (LMP) showed that the cells from mice bearing H. polygyrus did not release interferon-gamma (IFNy) or IL-12p40, unlike control mice LMP [6]. Rag-deficient mice (mice born without mature T and B cells) injected with IL-10⁻/⁻ T cells fed with ovalbumin displayed intense inflammation of the small bowel and colon.

Table 1. Treating inflammatory bowel disease with helminths and their derivatives

<table>
<thead>
<tr>
<th>Helminth</th>
<th>Model</th>
<th>Treatment type</th>
<th>Proposed mechanism</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heligmosomoides polgyrus</td>
<td>Piroxicam-induced colitis in IL-10 knockout mice</td>
<td>Colonization</td>
<td>Reduces IL-17 production and IFNy, IL-12 release</td>
<td>[6]</td>
</tr>
<tr>
<td></td>
<td>Rag-deficient mice injected with IL-10⁻/⁻ T cells fed with ovalbumin</td>
<td>Colonization</td>
<td>Suppression of IL-17, IFNy and induction of IL-10 modulates intestinal dendritic cell function to act as regulatory agents</td>
<td>[5,6]</td>
</tr>
<tr>
<td>Schistosoma mansoni</td>
<td>TNBS-induced colitis in mice</td>
<td>Ova</td>
<td>Reduced IFNy and IL-4 levels</td>
<td>[8]</td>
</tr>
<tr>
<td></td>
<td>TNBS-induced colitis in mice</td>
<td>S. mansoni-homogenized proteins</td>
<td>Decreased inflammation and MPO activity</td>
<td>[8]</td>
</tr>
<tr>
<td>Trichinella spiralis</td>
<td>DNBS-induced colitis in mice</td>
<td>T. spiralis antigens from frozen skeletal muscle larvae</td>
<td>Down-regulation of MPO, IL-1β and iNOS, Up-regulation of IL-13 and TGFβ</td>
<td>[7]</td>
</tr>
<tr>
<td>Trichuris suis</td>
<td>IBD patients</td>
<td>Ova</td>
<td>Not tested</td>
<td>[3]</td>
</tr>
</tbody>
</table>

TNBS = 2,4,6-trinitrobenzene sulfoxide acid, DNBS = dinitrobenzenesulfonic acid, IBD = inflammatory bowel disease, IL = interleukin, IFNy = interferon-gamma, TGFβ = transforming growth factor-beta, MPO = myeloperoxidase
When infected with *H. polygyrus*, gut inflammation was abrogated, with suppression of IL-17 and IFNγ and induction of IL-10 [6]. It was demonstrated that direct interaction with innate immune system cells by *H. polygyrus* can inhibit colitis, without direct interactions with T or B cells [5]. Moreover, *H. polygyrus* induced tolerogenic dendritic cells in the intestinal of infected Rag-deficient mice [5].

Another model of experimental colitis is trinitrobenzene sulfonic acid (TNBS)-induced colitis, characterized by ulcer formation, infiltration of the lamina propria with chronic inflammatory cells, and transmural lymphocytic inflammation [8]. *Schistosoma mansoni* ova exposure attenuated TNBS-induced colitis and protected BALB/c mice from lethal inflammation. IFNγ levels were reduced while IL-4 and IL-10 mRNA levels were enhanced due to production by αCD3-stimulated spleen and mesenteric lymph node cells [8]. Furthermore, treatment with *S. mansoni*-derived proteins during TNBS-induced colitis in mice showed a significant decrease in macroscopic inflammation score as well as a decrease in colonic inflammation and myeloperoxidase (MPO) activity. The effect resulted in reduction of gastrointestinal motility [8].

Treatment with *Trichinella spiralis* frozen skeletal muscle larvae prior to dinitrobenzene sulphonlic acid (DNBS)-induced colitis in C57BL/6 mice significantly reduced the severity of colitis. MPO activity was down-regulated, as well as IL-1β production and iNOS expression. IL-13 and tumor growth factor-beta (TGFβ) production in colon was up-regulated [7].

Moreover, Pineda et al. [9] found a compound, ES-62, secreted from the rodent nematode *Acanthocheilonema vitae* which has immunoregulatory capabilities. ES-62 is surrounded by a moiety of phosphorylcholine (PC) attached to the core by glycans. The immunomodulatory effect of ES-62 was attributed to PC [9]. Recently we successfully employed helminth PC-based conjugates to treat colitis in a mice model [10].

Furthermore, the use of toll-like receptor (TLR)-signaling antagonists and TLR-negative regulator agonists from helminths or helminth products should be considered as treatment for IBD. TLR signaling may contribute to destructive host responses and chronic inflammation, while helminths may play an important role in down-regulation of gene activation to control overwhelming inflammation and pro-inflammatory cytokine production [9].

Murine studies have led to human therapy trials with helminth ova. A preliminary study conducted in the early 2000s indicated that *Trichuris suis* (pig whipworm) seemed to be safe and possibly effective in the treatment of inflammatory bowel disease. *T. suis* met the safety requirements. It can colonize humans but only for a short time. A single dose of *Trichuris suis* ova (containing up to 7500 ova) was well tolerated and did not result in short- or long-term treatment-related side effects [3].

Summers and co-authors [3] studied seven IBD patients. In an initial treatment and observation period, a single dose of 2500 live TSO was given orally and the patients were followed for 12 weeks. Six of them achieved remission. The benefit was temporary in some patients with a single dose, but it could be prolonged with maintenance therapy every 3 weeks. In a later TSO study involving 29 patients with active CD who ingested 2500 live TSO every 3 weeks for 24 weeks, disease activity was monitored. The results were impressive: at week 24, 23 patients responded and 21/29 remitted. Furthermore, in a randomized, double-blind placebo-controlled trial, 54 patients with active UC received 2500 TSO or placebo orally at 2 week intervals for 12 weeks. The results demonstrated improvement according to the intent-to-treat principle in 13 of 30 patients with ova treatment as compared to 4 of 24 patients given placebo. Improvement was also found to be significant by week 6 [3].
New Potential Biomarkers for Disease Activity and Fibrosis in Systemic Sclerosis

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The Inflammatory Response in SSc

One of the major pathogenetic mechanisms of SSc is the inflammatory process. It is composed mainly of activated T cells, which release pro-inflammatory and pro-fibrogenic cytokines. Defective T-regulatory (Treg) cells have also been described in these patients, thus the immunoregulation in this disease is flawed. It is believed that transforming growth factor-beta (TGFβ), platelet-derived growth factor (PDGF), and several other interleukins are released from early inflammatory infiltrates and are crucial for the activation of fibroblasts and the transition of fibroblasts to myofibroblasts [2], thus contributing to the process of fibrosis. Semaphorin 3A (sema3A), a secreted member of the semaphorin family, is now recognized as a potent immunoregulator during all immune response stages – from early initiation to the late phase of inflammatory processes [3].

Sema3A expression on Treg cells has been recognized as a suppressive marker, contributing to the regulatory properties of these cells [4]. In 2010 Catalano [5] was the first to report on the defective expression of sema3A in CD4+ T cells derived from patients with rheumatoid arthritis (RA). The altered expression on T cells was shown to correlate with the progression of RA [5]. Recently we reported the presence of low serum semaphorin3A levels in systemic lupus erythematosus (SLE) patients, in correlation with SLEDAI score and reflecting disease activity [6]. As a front player in the regulation of immune responses and the maintenance of self-tolerance, sema3A should be expected to be involved in the pathogenesis of many autoimmune diseases. Thus we designed a study in which we evaluated the level of sema3A in the serum and its expression on Treg cells in SSc patients, healthy controls and SLE patients as the disease control, and correlated the results with clinical and serological parameters. Serum levels of sema3A were lower in SSc patients compared to healthy controls (14.38 ± 5.7 vs. 27.14 ± 8.4 ng/ml, P < 0.0001) and similar to SLE patients (15.7 ± 4.3 ng/ml). The expression of sema3A on Treg cells was also lower in SSc patients compared to healthy controls (61.7 ± 15.7% vs. 88.7 ± 3.7%, P < 0.0001). Semaphorin 3A serum level correlated inversely with the duration of disease (r = -0.4, P = 0.036) and with low C4 level (r = 0.66, P = 0.026). SCL-70 antibody positivity was associated with a lower sema3A level in serum (difference in mean of 3.44, P = 0.06). Sema3A expression was found in this study to be lower in SSc serum and also on Treg cells, in inverse correlation with disease duration. The finding of reduced expression of sema3A on Treg cells in SSc is in line with former studies that suggested either increased or decreased numbers of these cells but denoted inefficient T regulatory activity in autoimmune diseases such as SSc [7-9]. Thus, the finding of reduced sema3A expression on Treg cells in SSc patients may reflect their impaired regulatory function, thereby contributing to our understanding of the immune pathogenesis of the disease and this sema3A level may serve as a future target for follow-up and treatment.

Development of Fibrosis in SSc

Fibrosis is the main complication of SSc and its pathophysiology is complex. Despite our growing understanding of this process and the many available targets, our therapeutic success in ameliorating fibrosis in SSc is minimal [10]. Moreover, even today, assessment of skin fibrosis is usually determined by the modified Rodnan skin score (mRSS), a score based on clinical inspection of 17 parts of the body, which has significant interobserver variability and is rather subjective, hence the need for other objective and specific markers for assessing fibrosis. Collagen I is the most abundant structural protein of connective tissues such as the skin. The formation of collagen is an active

Key Words: systemic sclerosis (SSc), fibrosis, inflammation, semaphorin 3A (sema3A), lysyl-oxidase (LOX)

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process that reflects a balance between degradation and synthesis. Under physiological conditions, the chemical cross-linking of collagen molecules incorporated in collagen fibrils is critical for the mechanical stability of these fibrils. Moreover, the presence of chemical cross-links makes fibril-incorporated collagen molecules more resistant to proteolysis. Formation of cross-links is an enzymatic process catalyzed by lysyl-oxidase (LOX).

LOX is a copper-dependent amine oxidase that initiates the covalent cross-linking of collagen and elastin by catalyzing oxidative deamination of lysine and hydroxylysine residues to aminoadipic semi-aldehydes. These highly reactive semi-aldehydes can spontaneously condense to assure extracellular matrix (ECM) stability. LOX activity is essential to maintain the tensile and elastic features of connective tissues of skeletal, pulmonary, and cardiovascular systems, among others. LOX is synthesized as a pre-pro-LOX and is secreted into the extracellular environment where it is proteolytically processed to release the mature and active 32 kDa form and its pro-peptide.

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LOX has been evaluated in patients with diffuse cutaneous systemic sclerosis by immune staining of the skin and was found to be increased in interstitial fibroblastic cells compared with normal skin, but was not increased in SSc patients with skin atrophy [14]. LOX has been evaluated in other states of fibrosis, including primary myelofibrosis (PMF), hepatic and myocardial fibrosis [10], and was found to be overexpressed in the relevant tissues. We therefore conducted a study to evaluate LOX serum levels in SSc patients compared to normal controls and patients with PMF, as a disease control, and correlated these levels with clinical parameters. The study population comprised 26 SSc patients who were compared with healthy and primary myelofibrosis patients as the disease control. Ten SSc patients had diffuse disease with lung fibrosis and 16 had limited cutaneous disease. LOX serum concentration in SSc patients was higher than in healthy controls and similar to the disease control (58.4 ± 4.8 vs. 28.4 ± 2.5 ng/ml vs. 44.6 ± 9.4 ng/ml respectively, P < 0.001). LOX serum level was significantly higher in patients with diffuse vs. limited disease (73 ± 6.6 vs. 49.3 ± 5.5 ng/ml, P < 0.01). LOX serum concentration correlated with mRSS (P < 0.01) and with severity score (P < 0.001) in patients with SSc. This was the first study to demonstrate high serum levels of LOX in SSc patients, which specifically correlate with skin fibrosis and disease severity.

These correlations suggest that LOX levels can serve as a novel biomarker for fibrosis and severity in SSc. Future studies are warranted to determine LOX as a potential therapy target in SSc.

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**Capsule**

**Statin treatment rescues FGFR3 skeletal dysplasia phenotypes**

Gain-of-function mutations in the fibroblast growth factor receptor 3 gene (FGFR3) result in skeletal dysplasias, such as thanatophoric dysplasia and achondroplasia (ACH). The lack of disease models using human cells has hampered the identification of a clinically effective treatment for these diseases. Yamashita et al. show that statin treatment can rescue patient-specific induced pluripotent stem cell (iPSC) models and a mouse model of FGFR3 skeletal dysplasia. The authors converted fibroblasts from thanatophoric dysplasia type I (TD1) and ACH patients into iPSCs. The chondrogenic differentiation of TD1 iPSCs and ACH iPSCs resulted in the formation of degraded cartilage. They found that statins could correct the degraded cartilage in both chondrogenically differentiated TD1 and ACH iPSCs. Treatment of ACH model mice with statin led to a significant recovery of bone growth. These results suggest that statins could represent a medical treatment for infants with children and TD1 and ACH.

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Eliran Israeli
The Many Faces of B Regulatory Cells

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KEY WORDS: B regulatory cells (Bregs), self-tolerance, autoimmunity, interleukin-10 (IL-10), transforming growth factor-beta (TGFβ)

Autoimmune diseases develop when high titers of auto-antibodies against self-antigens are continuously produced. More than 5% of the entire world population suffers from at least one of numerous autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), among others. The role of B cells in the development of autoimmune diseases has been growing persistently. Apart from being the source of autoantibodies, they are efficient antigen-presenting cells and producers of pro-inflammatory cytokines. Many studies focused on the complexity of B cell overactivity, namely, the overproduction of B cell-activating factor (BAFF), the escape of autoreactive B cells from apoptosis, and the unbalanced production of various inflammatory and protective cytokines. On the other hand, B cells are a source of inhibitory cytokines such as interleukin-10 (IL-10) and transforming growth factor-beta (TGFβ). Depending on the signals that B cells receive, pro- or anti-inflammatory cytokines are produced, and the shift towards an inflammatory or a protective/suppressive response is induced.

B regulatory cells (Bregs) were first reported in a murine model of experimental autoimmune encephalomyelitis (EAE) showing that B cells are not required for the induction of EAE but they may contribute to immune regulation, resulting in complete recovery from acute EAE. In a later study, the stimulation of arthritogenic B cells with an agonistic anti-CD40 and collagen generated a subset of IL-10-producing B cells. The transfer of these B cells to syngeneic immunized mice prevented the induction of arthritis and ameliorated established disease by down-regulating Th1 cytokines [1]. During the last 5 years Bregs were intensively investigated in healthy humans and in many autoimmune diseases. Different phenotypic Bregs, inducing their regulatory functions via many different pathways and thereby influencing the course of viral infections, malignancies and autoimmune diseases, were identified.

HUMAN B REGULATORY CELLS

The question how to identify Bregs with membrane markers or transcription factors is yet unresolved. Also unresolved is how to better stimulate them in order to improve their regulatory properties. CD19+CD25+ B cells were the first subset of human B cells previously suggested to have a regulatory function. They were characterized as expressing high levels of immunoglobulins compared with CD19+CD25- B cells, but they lacked the ability to secrete them. They were defined as memory B cells (being CD27+) that secrete high levels of the inhibitory cytokine IL-10 compared with CD25- B cells [2]. Later, CD19+CD25high B cells were reported to be significantly higher in patients with anti-neutrophil cytoplasmic antibodies (ANCA)-related vasculitis when in remission, but much lower when disease was active. In an elegant study by Mauri et al [3], the presence of human Bregs, namely CD19+CD24highCD38high B cells, were identified as being able to suppress the differentiation of naïve T cells into T helper-1 (Th1) and Th17 cells. They also converted CD4+CD25- T cells into regulatory T cells (Tregs) in a CD40-dependent way and through the production of IL-10 but not TGFβ. In healthy individuals, CD19+CD24highCD38high B cells suppressed CD4+CD25- T cell proliferation as well as the release of interferon-gamma (IFNγ) and tumor necrosis factor-alpha (TNFα) [Figure 1A]. This suppressive capacity was blocked by the addition of CD80 and CD86 monoclonal antibodies. When analyzed in SLE patients, these cells were refractory to further CD40 stimulation, produced less IL-10,
and were incapable of suppressing Th1 proliferation compared to their ability in healthy individuals [3]. Later we characterized Breg cells as CD25highCD1dhighIL-10highTGF-βhigh. These were able to down-regulate CD4+ T cell proliferation when a co-culture, in a cell-to-cell-dependent way, suggesting that this regulatory function is CD86-dependent. Our other finding in this study was that Bregs were efficient in up-regulating autologous Treg cell properties, namely, enhancing FoxP3 and CTLA-4 expression in these Treg cells following Breg/Treg cell-to-cell co-culture [Figure 1B] [4].

Aiming to improve the characteristics of Bregs, we found that other regulatory markers were highly expressed on these cells. We showed that molecules such as semaphorin3A (a regulatory protein) and C72 (a regulatory B cell co-receptor) were mainly expressed on CD19CD25highIL-10high, suggesting CD19+CD25highCD72+sema3Ahigh cells to be a subset of Breg cells in humans; this needs further attention [5]. Trying to identify other markers for Bregs, IL-10 and TGFβ-producing B cells were found to highly express Foxp3 and CD5, suggesting CD19CD5highFoxP3highIL-10high to be a different subset of Breg cells. Looking into their possible involvement in inflammatory diseases, they were found to have a regulatory role in non-immunoglobulin E (IgE)-mediated food allergy and in atopic dermatitis [6]. In line with this, CD19+CD25+Foxp3+B cells were noticed to play a role in multiple sclerosis (MS). They were significantly higher in relapsing-remitting MS during relapse symptoms when compared to non-clinically active MS patients [Figure 1C]. Further evidence is required to establish the true presence of Foxp3+B cells and to prove that these are indeed Bregs.

**“KILLER” B REGULATORY CELLS**

The FasLigand/Fas receptor axis has been studied extensively as a mechanism of killing CD4+ T cells and other immune cells, thereby preventing autoimmunity and cancer. Evidence has emerged that in addition to activated cytotoxic T cells (CTL) and natural killer (NK) cells, B lymphocytes were also found to express FasL, thus mediating cell death of many overactivated immune cells. Among B cell subsets, the expression of both FasL and IL-10 was highest on CD5+ B cells, suggesting this subset to be a unique one in the field of human Bregs. This subset of cells was found to be higher in aggressive forms of B cell lymphoma and during persistence of viral infections such as Epstein-Barr virus, suggesting their expansion to be one of the mechanisms by which tumor and infected cells may escape efficient immune responses. The above explains why this subset of Breg cells is defined as “killer”/regulatory cells, which serve to protect against the development of autoimmunity [Figure 1D] [7].

Granzym B (GzmB) represents a major component of the granules of NK cells and CTL. Classically, GzmB has been linked primarily to the induction of apoptosis in target cells after attack by CTL. Various autoimmune diseases have been linked to elevated levels of IL-21 and GzmB, which were shown to play an immunosuppressive and thereby protective role in the early phase of SLE. With this in mind, CD5high B cells were demonstrated to be IL-10 and GzmB producers playing an additional role in suppressing autoimmune responses. Aiming to assess the relationship between CD5+ B cells, IL-21 and GzmB in SLE patients, both IL-21 and GzmB serum levels were evaluated. Here, in vitro experiments showed that IL-21 directly induces GzmB expression and secretion by CD5+ B cells, suggesting again that CD5+GzmB+ are important disease-modifying players in the early phase of SLE [8].

**IL-35 AND IL-21 AND B REGULATORY CELLS**

In addition to their ability to produce IL-10 and TGFβ, Bregs were reported to produce IL-35, which is essential for their suppressive/regulatory function. In one study, IL-35 increased the ability of Bregs to suppress experimental autoimmune uveitis. Moreover, recombinant IL-35 inhibited lipopolysaccharide-driven B cell activation while inducing IL-10 production, thus suggesting IL-10high B cells to be IL-35high [9]. To identify signals that regulate IL-10 producing B cells in vivo, purified B cells were cultured with cytokines known to influence B cell function. Stimulation with IL-21, but not IL-4, IL-6, IL-12 and IL-23 induced 4.4 to 5.3-fold more IL-10 secretion at 48 and 72 hours respectively. IL-21 also induced a threefold increase in IL-10+ B cells within the spleen CD1dhighCD5high B cell subset, but did not induce IL-10+ B cells among the CD5 subset. To verify that T cell-derived IL-21 and CD40 signals drive B10 cell expansion and IL-10 production, B cells were cultured with anti-CD40 antibodies and B lymphocyte stimulator (BLyS) in the presence of IL-4. B cells were then cultured with exogenous IL-21 for 5 days, which was essential to optimally expand IL-10+ B cells and induce IL-10 production. The transfer of CD5+ B cells markedly reduced EAE disease severity in wild-type mice, explaining in part why EAE is exacerbated in the absence of IL-21 [10].

**CONCLUSIONS**

A better characterization of Bregs – their membrane markers, cytokine profile and contribution to self-tolerance – is needed. How to achieve their maximal regulatory effect should also be the subject of future studies.

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Capsule

Refilling drug delivery devices

Drugs delivered throughout the body often cause collateral damage to healthy tissues. When disease or injury is localized, patients can avoid this problem by using a drug delivery device implanted in the target tissue. However, such devices eventually run out of drugs and must be removed surgically and refilled. Brudno et al. designed a drug-delivery device that can be refilled non-invasively and tested it in a mouse tumor model. They made the device from a gel tethered to short DNA sequences. To refill it, they coupled gel strands to drugs and tethered them to complementary DNA sequences, then injected the strands intravenously into the mice. Because of the complementary DNA sequences, the strands homed directly to the device.

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Eitan Israeli

Capsule

Flu survivors are an inflammatory club

Kill it: That is the immune system's response to most viral infections, including influenza. Eliminating infected cells rids the body of the infection. Heaton and group report that a special type of epithelial cell in the lungs of mice – called club cells – survive influenza infection. How do they do it? Gene expression analysis suggests that club cells express high amounts of antiviral genes in response to infection. Although this process probably helps the animal contain the virus during early infection, club cells also produced pro-inflammatory molecules that cause lung pathology. Whether club cells play a role in inflammation-induced mortality, as seen in the H5N1 and H1N1 influenza pandemics, remains to be seen.


Eitan Israeli

Capsule

An acetate switch regulates stress erythropoiesis

The hormone erythropoietin (EPO), which is synthesized in the kidney or liver of adult mammals, controls erythocyte production and is regulated by the stress-responsive transcription factor hypoxia-inducible factor-2 (HIF-2). Xu and co-authors previously reported that the lysine acetyltransferase CREB-binding protein (CBP) is required for HIF-2α acetylation and efficient HIF-2-dependent EPO induction during hypoxia. Now the authors (show that these processes require acetate-dependent acetyl CoA synthetase 2 (ACSS2). In human Hep3B hepatoma cells and in EPO-generating organs of hypoxic or acutely anemic mice, acetate levels rise and ACSS2 is required for HIF-2α acetylation, CBP-HIF-2α complex formation, CBP-HIF-2α recruitment to the EPO enhancer and efficient induction of EPO gene expression. In acutely anemic mice, acetate supplementation augments stress erythropoiesis in an ACSS2-dependent manner. Moreover, in acquired and inherited chronic anemia mouse models, acetate supplementation increases EPO expression and the resting hematocrit. Thus, a mammalian stress-responsive acetate switch controls HIF-2 signaling and EPO induction during pathological states marked by tissue hypoxia.

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Eitan Israeli
Mortality Due to sepsis In Patients with Systemic Lupus Erythematosus and Rheumatoid Arthritis

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KEY WORDS: systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), infection, sepsis

SYSTEMIC LUPUS ERYTHEMATOSUS AND INFECTION

The survival rate of patients with SLE has improved significantly over the last five decades, from less than 50% at 5 years in 1955 to 85% at 10 years in recent studies [1]. This improvement in SLE survival rates is the result of a continuously increasing survival in the general population, advances in therapeutic modalities, more judicious use of existing therapies (particularly steroids and cytotoxic agents), and the change in prognostic factors. Despite this encouraging progress, patients with SLE followed at various centers in North America have a 2.4 to threefold increased risk of death compared with the general population. This increased mortality is the result of infections, cardiovascular disease, and irreversible damage to target organs [1].

Infections contribute significantly to the morbidity and mortality of patients with SLE. It is estimated that at least 50% of SLE patients will suffer a severe infectious episode during the course of their disease [2]. Up to 30% of deaths in SLE are due to infections, although a recent mortality study from Hong Kong showed infection to be the main cause of death in 60% of the cases [3]. Similar to infections in the general population, major infections in SLE include common infectious diseases, such as pneumonia, urinary tract infection, cellulites and septicemia. In addition, patients with SLE are susceptible to infections associated with immune suppression, including opportunistic infections, tuberculosis, herpes zoster, as well as disseminated infections. In a study from Sao Paulo, the observed number of deaths due to tuberculosis, septicemia and pneumonia was significantly higher among SLE patients as compared to age- and gender-matched controls [1].

Identifying predictor variables for major infection in SLE is crucial for developing management plans to further improve the survival of SLE patients. A wide range of demographic, clinical and laboratory variables have been associated with increased risk of infections in SLE. These include low socioeconomic status, race, nephritis, antiphospholipid syndrome, high disease activity, damage measures and many others. The degree of immunosuppression and SLE disease are among the most important of those variables [4].

The clinical features of very active SLE may mimic those of infection and occasionally it is difficult to distinguish between SLE infection and SLE flare. Early diagnosis and treatment of a suspected infectious process is highly important since a delay in diagnosis may result in a rapid and fatal course.

RHEUMATOID ARTHRITIS AND INFECTION

RA is a chronic systemic inflammatory disease characterized by proliferative synovitis of diarthrodial joints, serositis, lymphocytic infiltration in various tissues, vasculitis of small vessels, and production of autoantibodies. The management of RA includes the use of non-steroidal anti-inflammatory drugs, steroids, disease-modifying anti-rheumatic drugs, and a variety of biologic therapies. Those therapies are used in combination and this is associated with significant immunosuppression. Indeed, infectious diseases were one of the three leading causes of premature death in several RA cohorts.

Similar to patients with SLE, RA patients are at increased risk for serious infectious disease, including pneumonia, urinary tract infections, septicemia and septic arthritis [5]. Therapy with tumor necrosis factor inhibitors (TNFi) is associated with an increased risk of active tuberculosis (TB). In one study, the gender- and age-adjusted incidence rate of active TB among TNFi-treated patients was 117 per 100,000 patient-years and the standardized incidence ratio (SIR) was 12.2. Tuberculosis developed within a few months of beginning TNFi therapy as a result of reactivation of latent TB infection or due to recent
primary infection following exposure to patients with active TB [6].

Variables associated with significantly increased risk for infection among patients with RA include older age, smoking, and presence of other comorbid diseases such as chronic lung and kidney diseases and diabetic mellitus, as well as the use of immunosuppressive medications [5,7].

SEPSIS

Systemic inflammatory response syndrome (SIRS) is an inflammatory state in which various mechanisms of the immune system are activated. This response is frequently associated with infection. Sepsis refers to the existence of these processes in the presence of infection [8,9]. Sepsis is the second leading cause of mortality among patients admitted to the ICU [9]. During the last decade, there was a steady increase in the incidence rate of sepsis in the general population, and the mortality rate as a result of sepsis is still around 70% [9,10]. Case-fatality rates of patients with sepsis is 1.5 to 2.5-fold more common than those of patients admitted to the ICU due to other causes [9,10]. In 2000, 28 day mortality rates were 7% for SIRS, 16–25% for sepsis and 20–54% for severe sepsis and septic shock. Mortality rates from sepsis correlate with the number of organ systems involved. The mortality rate is 15% risk for death when there is no sign of organ dysfunction or tissue hypoperfusion, and 70% in patients presenting a dysfunction in three or more organ systems [8-10]. Despite the vast number of papers studying infections in SLE and RA, a literature review did not reveal large controlled studies of sepsis in SLE and RA.

Clalit Health Services is the largest health fund in Israel and seven major hospitals are affiliated to this organization. SEPSIS-ISR is an ongoing prospective study that collects data on all patients admitted with the diagnosis of sepsis to the ICUs of all seven Clalit hospitals during the period 2002–2012. From the database we identified all patients with SLE and RA aged 18 years or more who were diagnosed with sepsis and admitted to the general ICU [10].

This ongoing study aims to assess whether SLE and/or RA are independent risk factors for short- and long-term mortality in patients admitted to the ICU with sepsis. Age- and gender-matched subjects with a diagnosis of sepsis but without SLE or RA were selected for each patient with SLE/RA at a 1:3 and 1:2 ratio respectively. The diagnosis of sepsis was based on well-defined criteria.

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References
Narcolepsy – Genes, Infections and Vaccines: the Clues for a New Autoimmune Disease

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KEY WORDS: narcolepsy, autoimmune disease, environmental factors, autoantibodies

Narcolepsy is a sleep disorder resulting from the lack of orexin, an essential neurotransmitter involved in the equilibrium between sleep and wakefulness. Postmortem analyses of narcoleptic patients demonstrate the loss of orexin-producing neurons in the hypothalamus. As a consequence of this disruption, narcoleptic patients suffer from uncontrollable sleep attacks characterized by a rapid eye movement (REM) sleep pattern which is not preceded by the non-REM stage. In some patients the sleep disturbance is accompanied by other symptoms, such as cataplexy (loss of muscle tone), sleep paralysis and hallucinations [1].

Currently, narcolepsy is considered a rare disease with a world prevalence ranging from 25 to 50 per 100,000 people [2]. Its prevalence varies from one region to another, suggesting the importance of genetic and environmental factors [1]. In recent years scientists and clinicians have suspected that narcolepsy has an autoimmune nature, mostly due to its strong association with specific polymorphisms in immune related genes. Namely, the allele frequency of human leukocyte antigen (HLA) DQB1*06:02 among narcoleptic patients is 82–99% [1], while only 12–38% of healthy individuals are carriers. Later reports found additional associations between narcolepsy and other gene polymorphisms (e.g., TCRα) [3]. However, the fact that the rate of disease concordance among monozygotic twins is between 20% and 35% suggests that, as in other autoimmune diseases, environmental factors play a key role [1].

ENVIRONMENTAL ROLE: AH1N1 VACCINE, INFLUENZA AND STREPTOCOCCAL INFECTIONS

The relation between infections and autoimmunity is well documented. The best example is streptococcal group A (SGA) bacterial infection, which is able to generate super-antigens that may stimulate autoreactive B and T cells leading to the production of autoantibodies (e.g., rheumatic fever). Moreover, SGA infections have also been related to other autoimmune neurological conditions, as well as to the presence of autoantibodies against neuronal proteins. Data suggest an association between infections, mainly Streptococcus sp. or influenza virus, and the onset of narcolepsy [4]. Childhood streptococcal throat infections were shown to be a risk factor for narcolepsy, and elevated anti-streptococcal antibodies were demonstrated in the sera of newly diagnosed narcoleptic patients [1,5].

Most striking was the discovery of a temporal association between narcolepsy and sleep-related disturbances, and the AH1N1 infection and its vaccination. In the 1918 H1N1 influenza pandemic, an increase in sleep disturbances as well as extreme sleepiness were noted in flu patients [1]. Ninety-one years later, a seasonal pattern of H1N1 infection in the 2009 Chinese pandemic was followed by a parallel increment in the incidence of narcolepsy, which returned to the usual incidence later [1,5]. However, the strongest evidence of an environmental association with narcolepsy was observed after immunization with the AS03-adjuvanted AH1N1 vaccine after the AH1N1 2009 pandemic [5]. This vaccine was designed based on the A/California/7/2009 (H1N1) v-like strain, and it was the one most used in Europe and the only one that contained the adjuvant AS03 [6]. Initially, an increment in narcolepsy cases related to the H1N1 vaccine was documented in Finland in 2010. As a result, a retrospective epidemiological analysis of historical reports and medical records demonstrated that the AS03 H1N1 vaccine was a risk factor for narcolepsy in Finland, Denmark, Sweden, France and England [6]. This association seemed to be stronger in genetically susceptible individuals, as HLA evaluation of new cases of narcolepsy/cataplexy post-vaccination showed they were mainly carriers of DQB1*06:02 [1,5]. Thus, the mechanisms by which the vaccine may be involved were explored. A recent study suggests that α-tocopherol (a component of the adjuvant AS03) may influence neuronal mouse cells, inducing over-production of orexin peptides as well as increased activity of the proteoso-
nal system. Masoudi et al. [7] suggest that this up-regulation might lead to the presentation of orexin peptides by the HLA, inducing an autoreactive response.

**IMMUNE SYSTEM INVOLVEMENT IN NARCOLEPSY**

Despite the recent reports of higher levels of inflammatory cytokines (i.e., granulocyte colony-stimulating factor and interleukin-8) in the plasma of narcolepsy patients [8], there is no evidence of an inflammatory process, including lymphocytic infiltration in the hypothalamus. A major obstacle is the inability to analyze brain specimens of patients at early stages of the disease [1,5]. The genetic polymorphisms associated with the disease are related to the immune system, and two of them are related to antigen presentation: the HLA DQB1*06:02 and the TCRα polymorphism. This may indicate the importance of the T cell response in the pathogenesis of narcolepsy, as it interacts directly with the HLA. It is possible that in predisposed individuals pathogenic T cells escape from the central tolerance process in the thymus. As a result, these cells may be able to be stimulated by external factors such as H1N1 vaccine or infections and finally evolve into an autoimmune response against orexin neurons. In fact, functional analyses of CD4+ lymphocytes from narcoleptic patients, but not from controls, showed that these cells were able to recognize orexin peptides when they were presented by dendritic cells (homozygous for DQA1*01:02/DQB1*06:02 haplotype); however, the authors failed to repeat these results [9]. Nevertheless, these controversial results do not clarify the mechanisms by which orexin peptides may be presented by CD4+ cells since it is currently unknown whether or not lymphocytes infiltrate the brain in narcolepsy patients. Furthermore, the role of CD8+ should also be further clarified, as should the mechanism whereby these cells pass the blood-brain barrier (BBB) [1,5].

The importance of B cell-mediated response has also been evaluated in narcolepsy. So far, three independent studies have shown that passive immunization with antibodies from narcoleptic patients can induce narcoleptic behavior and activity in mice [1,10], but none of them evaluated the sleep pattern of the immunized animals. Our group passively transferred total immunoglobulin G (IgG) from narcoleptic patients into mice [1,10], but none of them evaluated the sleep pattern of the immunized animals. A major obstacle is the inability to analyze brain specimens of patients at early stages of the disease [1,5]. The genetic polymorphisms associated with the disease are related to the immune system, and two of them are related to antigen presentation: the HLA DQB1*06:02 and the TCRα polymorphism. This may indicate the importance of the T cell response in the pathogenesis of narcolepsy, as it interacts directly with the HLA. It is possible that in predisposed individuals pathogenic T cells escape from the central tolerance process in the thymus. As a result, these cells may be able to be stimulated by external factors such as H1N1 vaccine or infections and finally evolve into an autoimmune response against orexin neurons. In fact, functional analyses of CD4+ lymphocytes from narcoleptic patients, but not from controls, showed that these cells were able to recognize orexin peptides when they were presented by dendritic cells (homozygous for DQA1*01:02/DQB1*06:02 haplotype); however, the authors failed to repeat these results [9]. Nevertheless, these controversial results do not clarify the mechanisms by which orexin peptides may be presented by CD4+ cells since it is currently unknown whether or not lymphocytes infiltrate the brain in narcolepsy patients. Furthermore, the role of CD8+ should also be further clarified, as should the mechanism whereby these cells pass the blood-brain barrier (BBB) [1,5].

The importance of B cell-mediated response has also been evaluated in narcolepsy. So far, three independent studies have shown that passive immunization with antibodies from narcoleptic patients can induce narcoleptic behavior and activity in mice [1,10], but none of them evaluated the sleep pattern of the immunized animals. Our group passively transferred total immunoglobulin G (IgG) from narcoleptic patients into mice brain, by intracerebroventricular injection. This procedure induced narcoleptic-like attacks and other behavioral changes in the mice and demonstrated the loss of orexin neurons in their hypothalamus [10]. Nonetheless, similar to the involvement of T lymphocytes, the mechanism by which antibodies can induce narcolepsy is not known; neither is the identity of the specific autoantigen involved [5].

**CONCLUSIONS**

It is well known that the development of an autoimmune disease in genetically susceptible individuals can be triggered by contact with specific environmental factors, especially infections. Autoimmune diseases develop through four stages: inherited factors, interaction with environmental factors, breakdown of autoimmune tolerance defined by the appearance of autoreactive B or T cells, and the clinical stage when the disease is active. For narcolepsy, heritable factors as well as the relationship with environmental factors are well documented. However, the two final stages are not understood. Further research in this field is essential to understand the mechanisms by which the orexin neurons are lost in the brain of narcolepsy patients.

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Arresting Beta-Cell Destruction in Type 1 Diabetes Mellitus: Mobilizing Homuncular Autoimmunity to Treat Autoimmune Disease

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KEY WORDS: autoimmunity, Homunculus, autoantibodies, T cells

The immunological Homunculus – or Immunculus – designates particular sets of self-antigens to which healthy individuals manifest autoantibodies and autoreactive T cells [1-3]. We recently demonstrated in a phase 3 clinical trial that the subcutaneous administration of 1 mg of a self-peptide three times a year to patients with new-onset type 1 diabetes mellitus (T1D) could significantly arrest the autoimmune destruction of their residual beta cells and enhance metabolic control of their diabetes – all with apparently no undesirable side effects [4]. The effective peptide p277 (in DiaPep277) is derived from the human heat shock protein-60 (HSP60) molecule; how can so small a dose of a self-peptide antigen have such a marked effect on an advanced autoimmune process? Here, I will briefly summarize the fact that the p277 peptide of human HSP60 is a homuncular self-antigen. The p277 peptide treatment of T1D evolved over 20 years of basic and clinical research [5], but the take-home lesson is clear: Homuncular autoimmunity can be mobilized to safely and effectively treat a serious autoimmune disease; in place of global suppression of the immune system with its attendant toxicity, we can now reason with the system, as it were, using its own molecular language.

THE AUTOANTIBODY HOMUNCULUS

A global view of natural autoantibodies capable of binding to self-antigens was first obtained using gel-separated extracts of healthy tissues arrayed on gels [2]. This “Panama Blot” technology paved the way for the development of microarray devices that could test microliter volumes of fluid for the binding of antibodies and autoantibodies to hundreds of known proteins, peptides, lipids, carbohydrates, and nucleic acids arrayed on glass slides [6]. Examination of sera from healthy persons, including young mothers and their newborn babies (cord blood), helped characterize the basic autoantibody Homunculus. Human babies obtain from their mothers immunoglobulin (Ig) G autoantibodies to many self-antigens; however, newborn humans during healthy development in utero actively produce IgM and IgA autoantibodies to common sets of self-antigens – a congenital Homunculus [6]. The point here is that HSP60 and its p277 peptide are prevalent homuncular self-antigens. Indigenous homuncular autoimmunity can be beneficial; the presence in unimmunized NOD male mice of antibodies to HSP60 peptide p277 was correlated with natural resistance to the development of T1D [7].

THE AUTOACTIVE T CELL HOMUNCULUS

It is only recently that advances in sequencing and informatics analysis have made it possible to characterize T cell repertoires. My laboratory has collaborated with Nir Friedman and his group to study the CDR3 segments of the T cell receptor (TCR) beta chain repertoire in 28 healthy C57BL/6 mice. It turns out that several hundreds of public CDR3 segments are shared by all, or almost all mice [8]. We found that the TCR CDR3 sequence (C9) of NOD mice that responds to HSP60 peptide p277 [9] was also a public TCR segment in our database of healthy C57BL/6 mouse T cell repertoires [8]. The C9 sequence manifests a large clone size and a high degree of convergent recombination. Because of the degeneracy of the nucleic acid codons for each expressed amino acid, the C9 sequence is encoded by an average of over 50 different nucleic acid sequences; private CDR3 segments are encoded by one nucleic acid recombination, on average [8]. Thus, HSP60 and its p277 peptide are members of a highly shared public TCR repertoire prevalent in variously different mouse strains. It appears that major histocompatibility complex differences are reflected more in V-gene usage than in CDR3 segments [8].

A SHIFT IN PARADIGM

Initially, self-reactive lymphocytes were thought to be forbidden [10]; we now know that self-recognizing T cells and B cells, components of the Homunculus, are present from birth in all individuals. Indeed, there seems to be strong positive selection...
for public autoreactive repertoires [8]. The recent observation that one such public homuncular peptide epitope, p277, can signal the immune system to desist from an autoimmune attack in T1D [4] suggests that some homuncular self-antigens may function as immune modulators. The immune system responds to these self-molecules as biomarkers for regulating inflammation; indeed, the immune system seems to be poised to recognize HSP60 and peptide p277 using many different receptors, innate TLR as well as somatically generated antigen receptors. Whether homuncular self-molecules can be used to treat other autoimmune diseases is an open question; we are now developing treatments based on HSP70 and HSP90 homuncular molecules.

References

Capsule

**Practice makes perfect — or does it?**

How do we learn from past errors? Herzfeld et al. found that when we practice a movement, the human brain has a memory for errors that is then used to learn faster in new conditions. This memory for error exists in parallel with motor memory’s two traditional forms: memory of actions and memory of external perturbations.

They also proposed a mathematical model for learning from errors. This model explained previous experimental results and predicted other major findings that they later verified experimentally. *Science* 2014; 345: 1349

Eitan Israeli

Capsule

**The alarmin IL-33 promotes regulatory T cell function in the intestine**

FOX3+ regulatory T cells (Treg cells) are abundant in the intestine, where they prevent dysregulated inflammatory responses to self and environmental stimuli. It is now appreciated that Treg cells acquire tissue-specific adaptations that facilitate their survival and function; however, key host factors controlling the Treg response in the intestine are poorly understood. The interleukin (IL)-1 family member IL-33 is constitutively expressed in epithelial cells at barrier sites, where it functions as an endogenous danger signal, or alarmin, in response to tissue damage. Recent studies in humans have described high levels of IL-33 in inflamed lesions of inflammatory bowel disease patients, suggesting a role for this cytokine in disease pathogenesis. In the intestine, both protective and pathological roles for IL-33 have been described in murine models of acute colitis, but its contribution to chronic inflammation remains ill defined. Schiering and team show in mice that the IL-33 receptor ST2 is preferentially expressed on colonic Treg cells, where it promotes Treg function and adaptation to the inflammatory environment. IL-33 signaling in T cells stimulates Treg responses in several ways. First, it enhances transforming growth factor (TGF)-β1-mediated differentiation of Treg cells and, second, it provides a necessary signal for Treg cell accumulation and maintenance in inflamed tissues. Strikingly, IL-23, a key pro-inflammatory cytokine in the pathogenesis of inflammatory bowel disease, restrained Treg responses through inhibition of IL-33 responsiveness. These results demonstrate a hitherto unrecognized link between an endogenous mediator of tissue damage and a major anti-inflammatory pathway, and suggest that the balance between IL-33 and IL-23 may be a key controller of intestinal immune responses. *Nature* 2014; 513: 564

Eitan Israeli
Harmonization of Autoimmune Diagnostics with Antinuclear Antibody Testing Algorithm: Approach of Appropriateness and Clinical Relevance

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KEY WORDS: diagnostic algorithm, autoimmune rheumatic disease, antinuclear antibodies (ANA), anti-extractable nuclear antigens (ENA), anti-double stranded DNA (dsDNA)

Limiting inappropriate test requests and identifying a balance between available economic resources and growing health needs is crucial for health care services today. In this context, harmonizing testing algorithms is a goal. As such, the clinical laboratory plays a critical role to help interpret clinical presentations that are often misleading in these pathologies. Recent advances in diagnostic technologies for autoimmune diseases have had a vital impact on the diagnostic approach to these pathologies.

The presence of antinuclear antibodies (ANA) in the serum, anti-extractable nuclear antigens (ENA) and anti-double stranded DNA (dsDNA) is one of the diagnostic criteria for autoimmune rheumatic disease. The test request for these assays has grown exponentially, due to increasing knowledge of the pathogenetic and diagnostic value of autoantibodies in autoimmune diseases and the inappropriate use of laboratory diagnostics. The latter problem is not precisely defined as yet, and is mainly a consequence of several factors, including inadequate collaboration or audit between physicians and laboratory personnel, availability of different techniques and methodologies in laboratory practice for assessing the same marker, and lack of a uniform terminology and diagnostic algorithms when performing autoantibody testing. Furthermore, a reduction in the number of clinically inappropriate requests and the establishment of a reasonable balance between available economic resources and increasing needs is a principal target of health care services worldwide.

To improve the appropriateness of the test requests in autoantibody testing, reliable and universally accepted diagnostic algorithms need to be defined and implemented; these algorithms should have been developed using the available guidelines found in the current scientific literature and should be shared by all physicians working in clinical immunology. The most appropriate strategy for requesting autoimmune rheumatic disease laboratory testing should encompass selective criteria; it should begin from a clinical suspicion, followed by a logical succession of analyses performed with sensitive tests at an early stage and specific tests for confirmation.

In view of its solid diagnostic performance, the ANA test has been proposed by several authors as a first-level test in the laboratory diagnosis of autoimmune rheumatic diseases, whereas tests for antibodies to specific nuclear antigens can only be detected when ANA screening is positive or if the patient has clear signs and symptoms suggesting a systemic rheumatic disease (second-level tests).

METHODS

In the light of this background, we conducted a multicenter study in the northwestern region of Emilia Romagna, supported by a regional grant for innovative research projects during the years 2008–2010. This observational research aimed at comparing the number of ANA, anti-dsDNA and anti-ENA testings as well as the percentage of positive test results before and after implementation of the diagnostic algorithm in hospitalized patients, performing ANA at the first level. A multidisciplinary team consisting of clinical immunology and laboratory scientists was established to collect and analyze diagnostic criteria, clinical needs, laboratory report formats, analytical procedures, as well as the number of tests performed. The laboratory results and the clinical protocol were both validated by clinical data emerging from the clinical follow-up studies. With regard to cost/management efficiency in terms of the number of tests performed, a significant reduction in the number of anti-dsDNA (26%) and anti-ENA (15%) tests was observed when comparing the production statistics of the first term of 2008 with those of 2009, whereas the ANA determination increased by 10%, following the trend of increasing requests recorded for autoimmunity tests.
Regarding the diagnostic specificity of the algorithm, the percentages of positivity observed for the second-level tests increased after application of the diagnostic algorithm, particularly for the ENA test (13% vs. 17%). This is attributed to the fact that this test can be more easily standardized in the various centers as compared with the anti-dsDNA tests (9% vs. 11%). As expected, the percentages of positivity did not significantly change for the ANA test since it was used as the "baseline" test.

RESULTS
Following this multicenter study, on 1 March 2013 the Emilia-Romagna region accepted and adopted though legislation the diagnostic ANA algorithm (creating a new test called ANA Reflex) with the aim of promoting the appropriate use of laboratory investigations. This will serve as a common guide for autoantibody testing, placing the ANA test at the first level with subsequent steps to be carried out directly by the laboratory. This algorithm has since been implemented in every autoimmune laboratory in the region, for both hospitalized patients and outpatients. Figure 1 depicts the diagnostic protocol.

THE PROTOCOL SPECIFICALLY IMPLIES THAT:
- The ANA test is the first-level test performed by an indirect immunofluorescence assay on HEp-2 cells. The screening dilution used is 1:80. In our clinical protocol we also suggest that the ANA test be performed only when there is a consistent clinical suspicion of autoimmune rheumatic disease
- When ANA testing is negative or positive to dilution < 1:160, no additional tests for antibodies to specific nuclear antigens (anti-dsDNA and anti-ENA) should be performed. These tests, even when not requested, are performed when ANA is positive for dilution ≥ 1:160 with any fluorescence pattern. These tests are performed even if the ANA test is negative, when a specific clinical request has been sent to the laboratory (request form or computerized note on request file).

During the first year of ANA Reflex use we observed that the requests for ANA Reflex represented a significant percentage of total ANA requests in both Parma and Modena (46% and 49% respectively). We also found that ENA requests during this period showed a reduction of 22% in Parma and 21% in Modena, accompanied by a substantial increase in positivity. DNA showed a reduction of 14% in Parma and 26% in Modena before and after implementation of ANA Reflex, with a substantial improvement in selected positive cases.

The definition and application of a diagnostic algorithm may help in test requesting and interpretation of laboratory findings throughout the challenging patient management.

In our experience, a diagnostic protocol including both screening and confirmation tests should allow a cascade of tests in the diagnosis of autoimmune rheumatic disease, thereby improving the appropriate use of tests for specific autoantibodies. Moreover, the inappropriate use of immunological tests can lead to misdiagnosis, inappropriate therapy, and waste of health care resources.

CONCLUSIONS
Close collaboration and audit between clinicians and laboratory personnel will enable standardization and widespread implementation of diagnostic algorithms for a more efficient use of immunological tests in the diagnostic evaluation, prognostic assessment, and monitoring of patients with systemic rheumatic diseases.

The percentage of second-level test positivity increased for both ENA and dsDNA after implementation of the diagnostic protocol. Furthermore, the introduction of this diagnostic algorithm led to a significant decrease in the number of second-level tests.

Efficiency and efficacy are strongly linked. We expect that the clinical protocol for autoantibody testing as presented in this article will be effective in improving both patient outcome (i.e., efficacy) and efficiency over a broad geographic area.

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References


**Capsule**

**Moral homeostasis in real life vs. the lab**

Individuals who witnessed a moral deed are more likely than non-witnesses to perform a moral deed themselves and are also more likely to allow themselves to act immorally. Hofmann et al. asked smartphone users to report their encounters with morality. Most moral judgment experiments are lab-based and don't allow for conclusions based on what people experience in their daily lives. This field experiment revealed that people experience moral events frequently in daily life. A respondent’s ideology influenced the kind of event reported and the frequency, which is consistent with moral foundations theory. *Science* 2014; 345: 1340

Eitan Israeli

**Capsule**

**Origin of the spine lies in a worm**

The notochord, the developmental backbone precursor, defines chordates – the group of animals to which humans belong. The origin of the notochord remains mysterious. Lauri and co-workers report the identification of a longitudinal muscle in an annelid worm that displays striking similarities to the notochord regarding position, developmental origin, and expression profile. Similar muscles, termed axochords, are found in various invertebrate phyla. These data suggest that the last common ancestor of bilaterians already possessed contractile midline tissue that, via stiffening, developed into a cartilaginous rod in the chordate line. *Science* 2014; 345: 1385

Eitan Israeli

**Capsule**

**Rationale for co-targeting IGF-1R and ALK in ALK fusion-positive lung cancer**

Crizotinib, a selective tyrosine kinase inhibitor (TKI), shows marked activity in patients whose lung cancers harbor fusions in the gene encoding anaplastic lymphoma receptor tyrosine kinase (ALK), but its efficacy is limited by variable primary responses and acquired resistance. In work arising from the clinical observation of a patient with ALK fusion-positive lung cancer who had an exceptional response to an insulin-like growth factor 1 receptor (IGF-1R)-specific antibody, Lovly and fellow researchers define a therapeutic synergism between ALK and IGF-1R inhibitors. Similar to IGF-1R, ALK fusion proteins bind to the adaptor insulin receptor substrate 1 (IRS-1), and IRS-1 knockdown enhances the antitumor effects of ALK inhibitors. In models of ALK TKI resistance, the IGF-1R pathway is activated, and combined ALK and IGF-1R inhibition improves therapeutic efficacy. Consistent with this finding, the levels of IGF-1R and IRS-1 are increased in biopsy samples from patients progressing on crizotinib monotherapy. Collectively these data support a role for the IGF-1R-IRS-1 pathway in both ALK TKI-sensitive and ALK TKI-resistant states and provide a biological rationale for further clinical development of dual ALK and IGF-1R inhibitors.

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Eitan Israeli

“Choose your corner, pick away at it carefully, intensely and to the best of your ability and that way you might change the world”

Charles Eames (1907-1978), American designer who (with his wife Bernice Alexandra “Ray”) made major contributions to modern architecture and furniture. They also worked in the fields of industrial and graphic design, fine art and film. The “Eames chair” has become iconic with Modern style design; an example is in the permanent collection of New York’s Museum of Modern Art.
Psoriatic arthritis (PsA) is a chronic inflammatory arthropathy of unknown etiology [1] that affects as many as one-third of patients with psoriasis. Together with enteropathic arthritis (EA), reactive arthritis (ReA), ankylosing spondylitis (AS) and undifferentiated spondyloarthropathy (uSpA), it is a member of the spondyloarthritis (SpA) family of rheumatic diseases [2] whose overlapping features include arthritis of the axial skeleton, inflammatory back pain, uveitis, dermatological and gastroenterological involvement, and a genetic association with human leukocyte antigen (HLA)-B27. It affects men and women equally (although the presence of axial disease is three times more frequent in men), and appears mainly between the ages of 30 and 50 years. Its various manifestations include mono-oligoarthritis, an erosive and destructive polyarthritis that cannot be distinguished from rheumatoid arthritis, and spondyloarthropathy with axial involvement or enthesitis, but it is also complicated by comorbidities such as cardiovascular and metabolic diseases.

ETIOPATHOGENESIS

The etiopathogenesis of PsA is still unclear but involves both genetic and environmental factors. Pro-inflammatory cytokines are major mediators of systemic and local inflammation, and high interleukin 1 (IL-1), IL-6 and tumor necrosis factor (TNF) levels have been observed in psoriatic skin lesions and the synovial tissue of patients with rheumatoid arthritis (RA) or PsA [3]. The synovial infiltrate associated with both diseases has a similar number of fibroblast-like synoviocytes, but the synovium of PsA patients is characterized by a less hyperplastic lining and fewer monocytes/macrophages [3].

Although there are also considerably fewer T cells, T cells are probably involved in the pathogenesis of both psoriasis and PsA because a subset of specific T cells may be sufficient to promote inflammation and regulatory T cells may have anti-inflammatory effects. A recent study of abatacept (a selective inhibitor of T cell activation as a result of its competitive binding to CD80 or CD86) has shown that it is efficacious for joints but has less effect on skin lesions [6]. It is also known that T-helper (Th) cells producing IL-17 (Th17 cells, which also produce TNF, IL-21 and IL-22) play a role in chronic inflammatory conditions and are stimulated by IL-23, which is highly expressed in psoriatic plaques [3]. The role of IL-23 in PsA is not clear, but the Th17-related cytokines IL-17 and IL-23 are expressed in the joints of PsA and RA patients, and ongoing clinical studies of the Th17 axis are investigating whether it can be used in the treatment of PsA.

The expression of TNFα, IL-1β, IL-6 and IL-18 is equally high in patients with PsA and in those with psoriasis, and published data support the view that blocking TNFα together with IL-1β, IL-6, IL-18 and IL-23 may be effective in PsA.

TREATMENT

Due to the clinical heterogeneity of PsA, selecting the most appropriate treatment is a challenge. Some countries have published treatment recommendations, as have international groups such as the Group for Research and Assessment of Psoriasis and Psoriatic Arthritis (GRAPPA) and the European League Against Rheumatism (EULAR). The GRAPPA recommendations consider five domains (peripheral arthritis, skin and nail involvement, enthesitis, dactylitis, axial arthritis) and use a grid approach to account for various levels of disease activity and severity [4]. The EULAR recommendations use an algorithmic approach that mainly considers peripheral arthritis, with dactylitis, enthesitis, dactylitis and skin and nail involvement being considered separately [5].

The aims when initiating PsA treatment are to alleviate signs and symptoms, inhibit structural damage, and maximize the patient’s quality of life. Mild PsA is often successfully treated with non-steroidal anti-inflammatory drugs (NSAIDs), although local intra-articular injections of corticosteroids may be used if only a few joints are involved. Neither, however, inhibit the development of structural joint damage.

PsA treatments have often been borrowed from RA, but there is a lack of randomized and controlled trials (RCTs) evaluating the impact of disease-modifying anti-rheumatic drugs (DMARDs) on PsA [6], although the findings of observational studies indicate that traditional DMARD therapy has little control over structural damage.
• **DMARDs**
  One observational cohort study of 23 patients treated with methotrexate (MTX) for 2 years found no reduction in radiological progression in comparison with matched controls [7], although other authors have found that the early administration of high dose MTX significantly decreases actively inflamed joint counts and psoriasis and somewhat reduces radiological progression. An open-label study comparing MTX with MTX + infliximab in patients with early disease observed not only very good joint and skin responses in the patients receiving the combination, but also substantial improvements in those receiving MTX alone, which is in line with everyday clinical experience. Leflunomide is effective, and was formally approved for the treatment of PsA in Europe. Cyclosporin can rapidly ameliorate psoriatic skin lesions, but there is little evidence that it is effective in musculoskeletal disease, and its use is limited by the adverse effects of hypertension and renal insufficiency. However, it has been used in combination with anti-TNF agents such as etanercept [8].

• **Anti-TNF Drugs**
  A number of studies have shown that PsA patients have high TNF levels in synovial fluid and the synovium, and it has been demonstrated that all anti-TNF agents slow radiographic progression and improve the patient’s quality of life [8].
  
  TNF inhibitors are effective in joints, skin, enthesitis and dactylitis in PsA patients, inhibit structural damage, and significantly improve function and quality of life [8], and it is presumed that they are as effective in the spine as they are in patients with AS. Furthermore, RCTs involving PsA patients have shown that they are also effective in reducing active joint inflammation and radiographic damage [8]. However, some patients with severe PsA are resistant to anti-TNF agents or develop adverse events and require alternative treatment.

• **Rituximab**
  One recent study has shown the presence of B cell lymphoid aggregates in PsA synovial tissue, and the partial remission of psoriasis has been reported in patients receiving rituximab for non-Hodgkin lymphoma [6,9]. Furthermore, Cohen has described a case in which rituximab led to a dramatic clinical improvement and possible structural effect in a patient with severe PsA [6].
  
  A number of small open-label cohorts of PsA patients have received rituximab administered with the same regimen as that used in RA (two intravenous injections of 1000 mg separated by an interval of 2 weeks), some of whom showed a slight improvement in joint counts although there was little impact on skin lesions. One logical off-label application of rituximab would be to treat a patient with PsA and current or recent lymphoma in whom other agents are contraindicated. However, the possible use of rituximab to treat PsA and psoriasis still needs to be confirmed by clinical trials.

• **Tocilizumab**
  Tocilizumab is a recombinant humanized monoclonal antibody (mAb) that inhibits the signal transduction of IL-6 by preventing it from interacting with both the membrane-expressed receptor and its soluble counterpart. It has been approved for the treatment of moderate and severe RA in adults who have inadequately responded to or been intolerant of previous DMARD or anti-TNF therapy (in Europe in January 2009 and the United States in 2010). Treatment with tocilizumab alone or in combination with MTX for 24 weeks is superior to MTX alone in reducing disease activity in RA patients [6,9,10], and has also been reported to be efficient in treating Castleman’s disease, adult-onset Still’s disease, Crohn’s disease and juvenile inflammatory arthritis.
  
  However, a pilot study (RCT) of the effects of IL-6 inhibitors on PsA did not lead to good results, and a recent case study of two patients treated with tocilizumab for 6 months did not ameliorate arthritis or the skin lesions, although it did lead to a decrease in serum C-reactive protein (CRP) in both patients [10]. The tolerability of tocilizumab seems to be acceptable.

• **Abatacept**
  A phase II placebo-controlled study of the effect of various intravenous doses of abatacept on PsA found that 6 months treatment with the RA-labelled dose of 10 mg/kg led to ACR 20 responses in 48% of the patients. The clinical response data were corroborated by a significant improvement in magnetic resonance imaging (MRI) scores, although the improvement in the skin lesions was less marked. Nevertheless, the ACR and skin responses were maintained in the abatacept-treated group after 12 months, and the patients originally receiving placebo showed similar responses [6].

• **Ustekinumab**
  Ustekinumab is a fully human mAb that has been approved for the treatment of psoriasis and PsA. It blocks the activity of p40, a protein subunit shared by IL-12 and IL-23, thus neutralizing their biological activity. It has been shown that it decreases the mRNA expression of IL-12p40, IL-23p19 and interferon-gamma (IFNγ) in the skin, inhibits IL-12 and IL-23-induced IFNγ, IL-17A, TNFα, IL-2 and IL-10 secretion, and is generally safe and well tolerated.

• **IL-17 inhibitors**
  IL-17, an inflammatory cytokine secreted by Th17 T cells and other cells, has been identified in psoriatic plaques and inflamed entheses [6]. Three IL-17 inhibitors are currently being tested in advanced-phase clinical trials: secukinumab and ixekizumab are mAbs against IL-17A, and brodalumab
is a mAb against IL-17 receptor A (IL-17RA). They have all been shown to improve skin psoriasis: a phase Ib RCT of secukinumab found a psoriasis area severity index (PASI) of 75 and PASI improvements in respectively 81% and 57% of the patients after 12 weeks, compared with 9% for placebo; and a randomized dose-finding study of ixekizumab showed significant PASI improvements in > 77% of patients compared with 8% for placebo [6]. Brodalumab has also been studied in PsA: subcutaneous doses of 140 mg and 280 mg respectively led to 12 week ACR 20 responses (36.8% and 39.3% of the patients, compared with 18.2% for placebo). However, further longer-term studies are necessary to define the effects of IL-17 inhibitors on the various manifestations of PsA [6].

**APREMILAST**

Apremilast is an oral phosphodiesterase-4 inhibitor that regulates inflammatory mediators. Phosphodiesterase-4, the dominant phosphodiesterase expressed in immune cells, degrades cyclic AMP (cAMP) into AMP [6]. Thus, its inhibition increases intracellular cAMP levels, which can down-regulate the inflammatory cytokines IL-12, IL-23, TNFα and IFNγ, increasing the expression of the anti-inflammatory mediator IL-10. Apremilast has been used in several studies of PsA patients and was shown to be efficacious and well tolerated, with minimal effects on any laboratory parameters.

**CONCLUSIONS**

Although pharmacological treatment often begins with MTX, anti-TNF therapies remain the gold standard. However, since many patients become refractory to current treatment options or develop side effects, it is important to find new and effective drugs for the treatment of PsA.

**References**

Subcutaneous Immunoglobulin G in Idiopathic Inflammatory Myopathies: Therapeutic Implications

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POLymyositis (PM) and dermatomyositis (DM) are systemic autoimmune diseases of unknown etiology that primarily affect the skeletal muscle [1]. Despite the improvement achieved in recent years with new therapeutic options, the prognosis remains poor, with high rates of morbidity and mortality. Conventional first-line treatment is based on glucocorticoids. However, their use in many patients requires long-term administration, which increases the probability of side effects developing. Thus, there is often the need to add immunosuppressive or immunomodulatory agents to improve the disease’s response to treatment and to reduce the long-term complications linked to glucocorticoids [2]. Among the treatment options, the use of immunoglobulin, by both the intravenous (IVIg) and the subcutaneous (SCIg) route, is still under debate [3].

IVIg as replacement therapy was first introduced in the middle of the 20th century for the treatment of immunodeficiencies, for which it is the treatment of choice [4]. Several mechanisms have been proposed to explain how IVIg acts by interaction with the humoral and cellular components of the immune system. However, its exact mechanism of action is not thoroughly understood [5,6]. In immune mediated disorders, IVIg is a therapeutic option considered as a steroid-sparing agent and for reducing side effects related to the use of immunosuppressants. In inflammatory myopathies (IIM), only IVIg has demonstrated statistically significant improvement in scores of muscle strength compared to placebo in high quality randomized controlled trials [2,7]. However, the use of IVIg is associated with several issues, namely, the need for an intravenous route of administration necessitates hospitalization, which leads to high costs and potentially severe systemic side effects (volume overload, anaphylactic reactions) [4].

SCIg is a blood product containing immunoglobulin G from healthy subjects, initially used in primary immuno-deficiency diseases and more recently in immune mediated disorders or neurological conditions. In primary immuno-deficiency SCIg has been demonstrated to reduce adverse reactions with reliable efficacy and improved quality of life [8]. We describe here our experience with the use of SCIg in patients with PM and DM.

METHODS

In our clinic, for each patient with a suspected IIM the biopsy-proven diagnosis was based on Bohan and Peter’s criteria. Each patient was then followed with clinical evaluation (MRC muscle strength score and Rankin modified score) and laboratory and instrumental monitoring (creatine kinase serum levels and electromyography-electroneurography).

We administered SCIg according to two different treatment schedules:

- the “sequential IVIg-SCIg protocol” given to patients with severe disease (dysphagia, head drop, high levels of CK, severe weakness). It is based on a 6 month period of IVIg (1 g/kg/month given on 2 consecutive days each month) followed by maintenance treatment with SCIg (0.2–0.8 g/kg/month).
- the “direct SCIg protocol” for patients with moderately active PM/DM having either a new onset or recently relapsed disease, as add-on treatment to glucocorticoids [10].

In the last few years we used 20% SCIg (Hizentra®, CSL Behring GmbH, Marburg) infusions. The 20% SCIg has high purity (> 98% IgG), higher IgG content (20%) and lower viscosity. This permits low infusion volumes and high infusion rates, shorter length of infusion, and convenient product conservation compared to 16% SCIg.

At our Center of Clinical Medicine we observed the efficacy of the sequential IVIg-SCIg treatment in six patients (all females, mean age 48 years, three PM and three DM) who had clinical and laboratory remission after 12 months of follow-up. With sequential administration of IVIg, the maximum response to therapy in a short time was achieved, followed by a maintenance period with SCIg (0.2–0.8 g/kg/month). The first SCIg dose was administered 2 weeks after the last IVIg infusion [9,10].

Regarding the direct protocol, we documented its efficacy and safety using SCIg as add-on treatment to glucocorticoids in seven
patients (five females and two males, mean age 55 years) with new-onset or recently relapsed PM/DM. SCIg infusions were given as previously described. In all patients we documented improvement in the MRC score and Rankin modified score, associated with a reduction in serum creatine kinase levels.

In both trials most of the patients were able to significantly reduce the dose of prednisone and, if applicable, of immunosuppressants as well.

**CONCLUSIONS**

Based on our experience, the use of SCIg may be a valid option alternative to IVIg in patients with refractory PM/DM or as first-line treatment when the use of glucocorticoids or immunosuppressants is contraindicated or if the patient is poorly compliant with these drugs and IVIg therapy [Table 1].

The sequential protocol with IVIg-SCIg in patients with new-onset myositis allows complete clinical and functional recovery with a long-term stable remission. The direct protocol should be considered in moderately active inflammatory myopathies as add-on treatment to glucocorticoids in patients with either new-onset or recently relapsed PM/DM or refractory disease.

### Table 1. Recommendations for SCIg use as first-line treatment in idiopathic inflammatory myositis

<table>
<thead>
<tr>
<th>Possible indications for SCIg as first-line therapy in patients with PM/DM</th>
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<tbody>
<tr>
<td>• Cancer-associated myositis, with recent cancer or with pre-neoplastic disease</td>
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<tr>
<td>• Contraindications to the use of immunosuppressants (hepatitis C virus, hepatitis B virus, human immunodeficiency virus, common variable immunodeficiency and/or other situation of immunodepression)</td>
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<tr>
<td>• Young women who wish to fall pregnant</td>
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<tr>
<td>• Contraindications to continued glucocorticoid treatment (i.e., poorly controlled diabetes, arterial hypertension, osteoporosis)</td>
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<tr>
<th>Possible indications for SCIg as preferred to IVIg in subjects with</th>
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<tr>
<td>• Difficulty of venous access</td>
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<tr>
<td>• Past thromboembolic events</td>
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<td>• Selective IgA deficit</td>
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<tr>
<td>• Impaired renal function</td>
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<td>• Heart involvement due to myositis or other causes</td>
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**Capsule**

**Endothelial cell FAK targeting sensitizes tumors to DNA-damaging therapy**

Chemoresistance is a serious limitation of cancer treatment. Until recently, almost all the work done to study this limitation has been restricted to tumor cells. Tavora et al. have identified a novel molecular mechanism by which endothelial cells regulate chemosensitivity. The authors established that specific targeting of focal adhesion kinase (FAK, also known as PTK2) in endothelial cells is sufficient to induce tumor cell sensitization to DNA-damaging therapies and thus inhibit tumor growth in mice. The clinical relevance of this work is supported by our observations that low blood vessel FAK expression is associated with complete remission in human lymphoma. This study shows that deletion of FAK in endothelial cells has no apparent effect on blood vessel function per se, but induces increased apoptosis and decreased proliferation within perivascular tumor cell compartments of mice treated with doxorubicin and radiotherapy. Mechanistically, we demonstrate that endothelial cell FAK is required for DNA damage-induced NF-κB activation in vivo and in vitro, and the production of cytokines from endothelial cells. Moreover, loss of endothelial cell FAK reduces DNA damage-induced cytokine production, thus enhancing chemosensitization of tumor cells to DNA-damaging therapies in vitro and in vivo. Overall, their data identified endothelial cell FAK as a regulator of tumor chemosensitivity. Furthermore, the authors anticipate that this proof-of-principle data will be a starting point for the development of new possible strategies to regulate chemosensitization by targeting endothelial cell FAK specifically.

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Eitan Israeli
Modulation of Adaptive Immune Response following Intravenous Immunoglobulin Therapy in Common Variable Immunodeficiency

Marzia Dolcino PhD, Antonio Puccetti MD PhD, Andrea Ottria MD, Alessandro Barbieri PhD, Giuseppe Patuzzo MD PhD and Claudio Lunardi MD

I. Introduction

Common variable immune deficiency (CVID) is the most frequent symptomatic primary immune deficiency in adults with a prevalence of approximately 1 in 25,000 in the general population [1]. Indeed, patients frequently become symptomatic later in life; however, their clinical history may reveal features of the disease dating back to early childhood. The disorder is characterized by recurrent and/or severe infections and may be associated with autoimmunity and increased risk of lymphoid malignancies. CVID probably represents a heterogeneous group of disorders culminating in late-onset antibody failure. The genetic basis of CVID has been identified in only a minority of patients; it is likely that in the majority of patients the disease may have a polygenic origin [2]. Since the cause of CVID is unknown and the clinical features variable, there is no universally accepted definition of the disorder and several diagnostic criteria have been proposed [3]. The diagnostic criteria of the ESID (European Society of Immunodeficiencies)/PAGID (Pan American Group for Immune Deficiency) comprise three aspects: (i) hypogammaglobulinemia with immunoglobulin G (IgG) levels 2 standard deviations below the mean value, (ii) impaired vaccine responses or absent isohemagglutinins, and (iii) exclusion of other causes of hypogammaglobulinemia.

The typical defect in CVID is the failure of B lymphocytes to differentiate into switched memory B cells and plasma cells [4]. Several abnormalities of T cells have also been described in CVID, including oligoclonal expansion of CD8+T cells and decreased numbers of CD4+T cells [5]. Moreover, T lymphocytes show an impaired secretion of several soluble mediators. The current standard of care for patients with CVID is lifelong replacement with intravenous immunoglobulin preparations (IVIg) that reduce the frequency of infections and the progression of complications, including suppurative lung disease. IVIg use is increasing rapidly, given its efficacy also in patients with autoimmune and inflammatory disorders. IVIg therapeutic effect seems to be related not only to antibody replacement but also to active modulation of immune responses [6]. Such immunomodulatory effects have been hypothesized but never proven. The aim of our study was to address this issue by using a gene expression approach.

II. Methods

We analyzed the effect of IVIg treatment in 10 patients with CVID by evaluating the gene-expression profiles from Affimetrix HG-U133A [7]. The gene array results were validated by real-time polymerase chain reaction (RT-PCR) and by the detection of soluble mediators by enzyme-linked immunosorbent assay. Moreover, we performed fluorescence-activated cell sorting (FACS) analysis of blood mononuclear cells from the patients enrolled in the study.

III. Results

Seventy-seven genes were differentially expressed in CVID patients before IVIg therapy, including the CD14 molecule (CD14, FC 2), leptin receptor (LEPR, FC 22.5), CD38 molecule (CD38, FC 22.4), RGS1 (FC 11.3), TNFRSF25 (FC 2.2), interleukin-4 (IL-4, FC2.9), CXCR4 (FC 3.1), CCR3, (FC 7.4), and IL-8 (FC 26.9) [Figure 1A]. The vast majority (26/77) of these modulated transcripts belong to the innate and acquired immune response gene categories [Figure 1B]. We then studied the effect of IVIg infusion on the immune response in CVID patients by analyzing the gene expression profiles obtained from the same CVID patients 3 days after IVIg therapy. Interestingly, 23 of 77 genes returned to an expression level similar to that of normal controls [Figure 1A & B].

RT-PCR of selected genes and serum levels of soluble mediators (IL-4, IL-8 and CXCR4) before and after therapy changed...
Figure 1. Gene expression profiles of modulated genes and flow cytometric analysis of T and B cell populations in CVID patients before and after IVIG treatment
[A] Global representation of genes related to innate and adaptive immune responses
[B] Representation of genes involved in T and B cell immune response.

Figure shows FC in gene expression in peripheral blood mononuclear cells (PBMC) isolated from 10 patients with CVID before (orange bars) and after (blue bars) IVIG infusion. Y axis represents the fold change level of gene modulation. Asterisks indicate genes returned to the level of healthy controls after IVIG infusion.

[C,D,E] The histograms show the percentages of CD4+, CD8+ T and CD19+CD5-CD23-CD27-IgM-IgD- B (centrocytes) cell populations before (blue bars) and after (red bars) IVIG infusion, respectively.

Data are representative of the mean values obtained for all the 30 subjects studied.
according to gene array results. Moreover, FACS analysis demonstrated a marked decrease of CD8+ T cells and an increase of CD4+ T cells following treatment [Figure 1 C & D]. Interestingly, we also observed an increase of centrocytes (CD23+ CD27-IgM– IgD– B cells) [Figure 1 E]. The phenotypic modifications observed with the FACS analysis are in agreement with the gene expression data. Indeed, the expression of the TNFRSF25 gene, able to promote CD8+ T cell survival, was up-regulated before therapy and returned to baseline levels after IVIg infusion, in accordance with the reduction of CD8+ T cells. The LEPR gene, which has a crucial role in CD4+ T cell proliferation, was down-regulated before infusion returned to baseline levels after the treatment. Therefore, the modulation of TNFRSF25 and of LEPR genes may be related to the decrease of CD8+ cells and to the increase of CD4+ T cells after IVIg. Similarly, modulation of genes involved in B cell development such as RGS1 may result in modification of the B cell phenotype induced by IVIg infusion, i.e., increased number of switched memory B cells.

CONCLUSIONS

Our results provide further support to the hypothesis that the benefits of IVIg therapy [8-10] are not only related to antibody replacement but also to its ability to modulate the immune response in common variable immunodeficiency.

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References

Capsule
A long non-coding RNA protects the heart from pathological hypertrophy

The role of long non-coding RNA (lncRNA) in adult hearts is unknown; also unclear is how lncRNA modulates nucleosome remodeling. An estimated 70% of mouse genes undergo antisense transcription, including myosin heavy chain 7 (Myh7), which encodes molecular motor proteins for heart contraction. Han and group identified a cluster of lncRNA transcripts from Myh7 loci and demonstrated a new lncRNA-chromatin mechanism for heart failure. In mice, these transcripts, which they named myosin heavy chain-associated RNA transcripts (Myheart, or Mhrt), are cardiac-specific and abundant in adult hearts. Pathological stress activates the Brg1-Hdac-Parp chromatin repressor complex to inhibit Mhrt transcription in the heart. Such stress-induced Mhrt repression is essential for cardiomyopathy to develop: restoring Mhrt to the pre-stress level protects the heart from hypertrophy and failure. Mhrt antagonizes the function of Brg1, a chromatin-remodeling factor that is activated by stress to trigger aberrant gene expression and cardiac myopathy. Mhrt prevents Brg1 from recognizing its genomic DNA targets, thus inhibiting chromatin targeting and gene regulation by Brg1. It does so by binding to the helicase domain of Brg1, a domain that is crucial for tethering Brg1 to chromatinized DNA targets. Brg1 helicase has dual nucleic-acid-binding specificities: it is capable of binding lncRNA (Mhrt) and chromatinized – but not naked – DNA. This dual-binding feature of helicase enables a competitive inhibition mechanism by which Mhrt sequesters Brg1 from its genomic DNA targets to prevent chromatin remodeling. A Mhrt-Brg1 feedback circuit is thus crucial for heart function. Human MHRT also originates from MYH7 loci and is repressed in various types of myopathic hearts, suggesting a conserved lncRNA mechanism in human cardiomyopathy. These studies identified a cardioprotective IncRNA, defined a new targeting mechanism for ATP-dependent chromatin-remodeling factors, and established a new paradigm for lncRNA-chromatin interaction.

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Eitan Israeli
Belimumab for the Treatment of Refractory Systemic Lupus Erythematosus: Real-Life Experience in the First Year of Use in 18 Italian Patients

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To date, it has been estimated that more than 50% of patients affected by systemic lupus erythematosus (SLE) have suboptimal disease control: while 40% of them have chronic active disease (CAD), the remaining 10% suffer from relapsing-remitting disease (RRD) with frequent exacerbations [1,2]. This situation requires frequent changes of therapy and, in particular, increased steroid dosage, along with the obvious risk of one or more of the well-known related side effects [3,4]. In this scenario the need for new treatment options is even more evident than in other rheumatic diseases.

After 50 years with no new drug licensed for SLE, belimumab was recently approved for the treatment of active and refractory SLE. Belimumab is a human immunoglobulin G (IgG)1λ monoclonal antibody specific for soluble human B lymphocyte stimulator protein (BLyS) able to inhibit the survival of B cells, including autoreactive B cells, and it reduces the differentiation of B cells into Ig-producing plasma cells. It is indicated in active SLE in the presence of hypocomplementemia and anti-ds-DNA antibody positivity, in addition to the standard treatment regimen. The recommended dose is 10 mg/kg belimumab in intravenous administration on day 0, 14 and 28, and once a month thereafter.

The development of belimumab represents the largest trial ever conducted in SLE patients, with 2200 patients enrolled in clinical studies with long-term treatment, in some cases for more than 7 years [5]. The aim of the study was to evaluate the efficacy and safety of belimumab in the real-life experience of a single tertiary referral center of rheumatology after the first year of licensed use in Italy.

METHODS
The study group comprised 18 SLE patients with active disease despite standard therapy. They met the American College of Rheumatology (ACR) classification criteria for SLE. The patients received belimumab (10 mg/kg) in addition to their current treatment. Hypocomplementemia was considered if serum levels of C3 and/or C4 were low. Anti-dsDNA antibodies were tested by Farr assay and classified as "high titer" if exceeding three times the normal values. CAD was defined as the presence of a SLEDAI-2K ≥ 2 (excluding isolated serology) in at least two of three evaluations performed in a single year, and RRD as a SLEDAI-2K ≥ 2 in at least one of three [2]. A disease flare was defined as measurable increases in disease activity in one or more organs and systems with the onset or a worsening of signs and symptoms and/or laboratory parameters [6]. SLE clinical and serological manifestations and mean steroid dosage were compared between baseline and different time points using the Mann-Whitney test for unpaired data.

RESULTS
All the patients were female, and their mean age at first drug administration was 39.6 years (range 25–55) and mean disease duration 12.3 years (range 1–26). At baseline, 15 patients (83.3%) presented hypocomplementemia and 15 (83.3%) had positive anti-dsDNA antibodies (7 at low titer, 8 at high titer). Fourteen patients (77.8%) had positive antiphospholipid antibodies (in 9 cases a single test was positive, in 3 two tests, and in 2 patients all three tests were positive) but only 2 of the patients had a history of associated manifestations (deep vein thrombosis in one and HELLP syndrome in the other). At baseline, 17 patients (94.4%) were treated with one or more disease-modifying anti-rheumatic drugs (DMARDs) according to standard of care (12 patients were taking hydroxychloroquine, 7 mycophenolate mofetil, 4 methotrexate and 6 azathioprine), while 1 patient was treated solely with prednisone because she was intolerant or not responsive to immunosuppressive drugs.
Seven patients (38.8%) presented CAD while 11 (61.2%) showed RRD and experienced a disease flare in the year prior to belimumab administration (articular in 5 cases, cutaneous in 3, renal in 2 and constitutional – fever, malaise, weight loss – in 1 case). During the first 6 months of therapy we recorded five disease flares: one cutaneous, three articular and one cardiovascular (pericarditis). We also observed infectious adverse events in seven cases (four infections of the upper respiratory tract, in one case recurrent; two gastroenteric infections, and one urinary tract infection). A lower limb deep vein thrombosis (DVT) was diagnosed in a patient with high positive antiphospholipid antibodies (aPL) soon after the first infusion and warfarin was initiated. No significant differences were observed between anti ds-DNA, C3 and C4 values at baseline and after 3 (t3), 6 (t6), and 9 (t9) months from the start of therapy. SLEDAI-2K showed a significant decrease from t0 to t3 ($P = 0.002$), maintained also at t6 and t9 evaluation ($P = 0.012$). The mean dose of prednisone administered required 9 months of therapy to show a significant reduction ($P = 0.045$), even though it showed a trend towards decrease also at t3 (77.2 vs. 80 mg/week) and t6 (65 vs. 80 mg/week). The detailed data are shown in Table 1.

The administration of the drug was discontinued in 3 patients (16.7%), in 2 cases because of inefficacy and in 1 case due to recurrent infections of the upper respiratory tract (after 7 infusions). The two patients stopped belimumab after 6 months (seven infusions) because of the persistence of thrombocytopenia and hand vasculitis, and after 5 months (six infusions) because of persistent arthritis, respectively. One patient was lost to follow-up because she moved to another town.

**CONCLUSIONS**

Although SLE is a multifaceted disease, some disease patterns can be identified depending on the clinical and serological activity. CAD defines a patient whose activity persists for at least one year, while RRD, with alternating phases of activity and inactivity and quiescence, is identified when the disease remains inactive for at least one year [2]. A recent evaluation demonstrated that fewer than 50% of SLE patients reached and maintained a stable remission [1,2]. Despite the huge advances in diagnosis and therapy, an increase in mortality remains part of the natural history of SLE, with affected patients still having a three times higher risk of death than the general population [7]. Moreover, both disease activity and side effects of the drugs used to control SLE significantly influence the quality of life [8].

The recent introduction of belimumab has benefited patients who, despite the proper use of standard therapy, continue to present uncontrolled disease and are therefore exposed to the possible adverse events of the drugs taken as well as to the risk of developing chronic and irreversible damage of one or more organs or systems. Data on the use of the drug in real life are still limited, but our preliminary experience seems to confirm good tolerability and safety as shown in clinical trials.

Regarding effectiveness, based on our experience of the first few months of treatment we offer some relevant preliminary observations. We noted a significant reduction in the SLEDAI-2K score after 3 months of treatment followed by a significant decrease in steroids intake after 9 months of treatment, suggesting that the effects of belimumab can be seen over time for disease activity control and steroid-sparing at different time points. Changes in serology (reduction of anti-dsDNA and increase in complement levels) were not found to be significant as pooled data. However, this lack of significance can be due to the fact that nearly half the patients had low titers of anti-dsDNA and/or slightly reduced complement. In patients with an active serology these parameters improved markedly after a few months of treatment. The long-term follow-up will likely demonstrate the true effects of the drug in terms of clinical stabilization, improvement of laboratory parameters, lack of chronic damage accrual, and stable reduction of steroid intake.

Treatment dropouts occurred in patients whose clinical history had always been characterized by closely repeated relapses. High disease activity prompted the patients and physicians to discontinue the treatment, but we should question whether a 6 month period is sufficient to assess whether belimumab is working or not. It is also important to underline that a patient who discontinued the drug for ineffectiveness after 6 months was not taking any standard therapy except for steroids due to intolerance to multiple drugs. Belimumab was stopped as a cautionary measure in a patient with recurrent infection of the respiratory tract. However, no definite attribution to the drug could be made. In fact, the patient was concomitantly taking multiple immunosuppressive drugs and a generous steroid dosage, often spontaneously increased by the patient herself. Regarding the patient who developed a DVT soon after the

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**Table 1. Quarterly data on disease activity, serology and steroids intake from baseline to 9 months of treatment**

<table>
<thead>
<tr>
<th></th>
<th>t0</th>
<th>t3</th>
<th>t6</th>
<th>t9</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of treated patients</strong></td>
<td>18</td>
<td>17</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Prednisone (mg/week)</td>
<td>66.3 (58–100)</td>
<td>62.5 (45–105)</td>
<td>62.5 (43.1–96.9)</td>
<td>46.9* (30.3–64.1)</td>
</tr>
<tr>
<td>Anti-dsDNA Abs (normal &gt; 7 UI/ml)</td>
<td>25.7 (13.7–76.5)</td>
<td>18.1 (8.5–78.1)</td>
<td>27.0 (18.1–55.3)</td>
<td>24.6 (12.4–34.5)</td>
</tr>
<tr>
<td>C3 (normal 80–160 mg/dl)</td>
<td>69.3 (55.3–83)</td>
<td>73.5 (62.4–91)</td>
<td>69.5 (59.3–89.2)</td>
<td>79 (65.5–86.3)</td>
</tr>
<tr>
<td>C4 (normal 10–40 mg/dl)</td>
<td>12.1 (8.3–15.4)</td>
<td>11.6 (9.6–16.6)</td>
<td>11 (7.6–15.8)</td>
<td></td>
</tr>
<tr>
<td>SLEDAI 2K</td>
<td>9 (8–10.8)</td>
<td>6* (5–8)</td>
<td>6* (5.3–7.8)</td>
<td>6* (4.3–6)</td>
</tr>
</tbody>
</table>

Values are expressed as median and interquartile range

*Significant reduction as compared to baseline ($P < 0.05$, Mann-Whitney test).
Animal behavior follows dopamine rewards

In auditory fear conditioning, mice learn to associate auditory cues, such as a tone, with mild footshocks. Forming associations like this, then remembering them long-term (called fear memory consolidation), is an important strategy for navigating one’s environment. To understand the molecular basis of fear memory consolidation, Dias et al. investigated the contribution of micro-RNAs, small bits of RNA that modulate gene expression. They discovered an important role for the microRNA-34a, which targeted components of a particular signaling pathway (the so-called Notch pathway) that is normally involved in development. MicroRNA-34a caused a transient decrease in Notch-dependent signaling in the amygdala, which was important for fear memory consolidation.

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Eitan Israeli

The G protein α subunit Gas is a tumor suppressor in Sonic hedgehog-driven medulloblastoma

Medulloblastoma, the most common malignant childhood brain tumor, exhibits distinct molecular subtypes and cellular origins. Genetic alterations driving medulloblastoma initiation and progression remain poorly understood. He et al. identified GNAS, encoding the G protein Gas, as a potent tumor suppressor gene that, when expressed at low levels, defines a subset of aggressive Sonic hedgehog (SHH)-driven human medulloblastomas. Ablation of the single Gnas gene in anatomically distinct progenitors in mice is sufficient to induce Shh-associated medulloblastomas, which recapitulate their human counterparts. Gas is highly enriched at the primary cilium of granule neuron precursors and suppresses Shh signaling by regulating both the cAMP-dependent pathway and ciliary trafficking of Hedgehog pathway components. Elevation in levels of a Gas effector, cAMP, effectively inhibits tumor cell proliferation and progression in Gnas-ablated mice. Thus, these gain- and loss-of-function studies identify a previously unrecognized tumor suppressor function for Gas that can be found consistently across Shh-group medulloblastomas of disparate cellular and anatomical origins, highlighting G protein modulation as a potential therapeutic avenue.

Nature Med 2014; 20: 1035
Eitan Israeli

“No one means all he says, and yet very few say all they mean, for words are slippery and thought is viscous”

Henry Brooks Adams (1838-1918), American historian and member of the Adams political family, being descended from two U.S. Presidents
The Autoimmune Side of Rheumatic Fever

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KEY WORDS: rheumatic fever, rheumatic heart disease (RHD), autoantibodies, antiphospholipid syndrome, anti-endothelial cell antibodies

Acute rheumatic fever (ARF) is a multisystemic disease caused by an abnormal immunological response after group A Streptococcus (GAS) pharyngitis in predisposed people. Among the major criteria for diagnosis of ARF (Jones criteria) are carditis, polyarthritis, Sydenham chorea, erythema marginatum and subcutaneous nodules [1]. The disease usually presents with one or more acute episodes, while in 30–50% of all recurrent cases ARF may lead to chronic rheumatic heart disease (RHD) with progressive and permanent damage of the cardiac valves. Despite a notable reduction in the disease prevalence in industrialized countries, RHD remains one of the major causes of morbidity and mortality in developing countries. Over 15 million cases of RHD are estimated worldwide, with 282,000 new cases and 233,000 deaths annually.

The pathogenesis is not yet completely clarified; however, RHD may represent the most convincing model of an autoimmune disease triggered by infections, since the infectious agent is proven [Table 1]. The endocardial valve tissue is the main localization of cardiac damage. Peripheral T lymphocytes, interacting with adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1), infiltrate a non-vascularized tissue. Roberts et al. [2] demonstrated that VCAM-1 was expressed on the valvular endothelium in rheumatic valves, and that at the

Table 1. Summary of pathophysiological mechanisms

- Exposure to Streptococcus pyogenes (group A Streptococcus by the Lancefield system), usually after a throat infection
- HLA class II molecules which participate in antigen presentation to T cell receptors
- Antigenic mimicry (M, T, and R surface proteins associated with bacterial adherence to throat epithelial cells) and between streptococcal M protein and several cardiac proteins (cardiac myosin, tropomyosin, keratin, laminin, vimentin)
- Antibodies against group A Streptococcus
- Other antibodies: anti-endothelial cells/antiphospholipid antibodies
- Complement activation (via lectin pathway)
- Aberrant T cell activation

Valve surface CD4+ and CD8+ T lymphocytes were adherent to valve endothelium and penetrated through the subendocardial layer. Thus, T cell extravasation into the valve through the surface valvar endothelium appeared to be an important event in the development of RHD [2]. Furthermore, the evidence that the disease has an autoimmune background derives from the presence of anti-GAS antibodies as one of the major features in patients with rheumatic valvar disease. Antibodies and complement deposits have been detected in the heart of RHD patients, suggesting an autoimmune process [3]. The pathogenesis of RHD results from an immune response consisting of humoral and cellular components after exposure to Streptococcus pyogenes. The classic triad of the disease comprises the presence of the rheumatogenic group A streptococcal strain, a genetically susceptible host, and aberrant host immune response. There is a closer association of human leukocyte antigen (HLA) class II molecules (which participate in antigen presentation to T cell receptors) with an increased risk of acute rheumatic fever or rheumatic heart disease than class I molecules.

The autoimmune hypothesis was suggested in a 2001 study in which Lewis rats immunized with recombinant type 6 streptococcal M protein (rM6) developed valvulitis as well as focal lesions of myocarditis. Indeed, streptococcal M protein is structurally and immunologically similar to cardiac myosin working as antigenic mimicry. Interestingly, the valvular lesions initiated at the main surface endothelium spread into the valve, and Anitschkow cells and verruca-like lesions were present. T lymphocytes from rM6-immunized rats proliferated in the presence of purified cardiac myosin, but not skeletal myosin, while a T cell line produced from rM6-treated rats proliferated in the presence of cardiac myosin and rM6 protein. It was suggested that streptococcal M protein can induce an autoimmune cell-mediated immune attack on the heart valve in such an animal model [4].

Furthermore, antibodies against group A Streptococcus were demonstrated to react with human heart preparations. It is suspected that after binding to the antigenic peptide, the specific HLA complexes can initiate aberrant T cell activation. Molecular mimicry is another key step since it takes place between streptococcal M protein and several self cardiac proteins (tropomyosin, keratin, cardiac myosin, laminin, vimentin). The complement cascade seems to be activated through the lectin pathway. Indeed, mannose-binding lectin (MBL), by acting as a soluble
pathogen recognition receptor and binding to the carbohydrates on the surface of the pathogen, allows its opsonization, enhancing phagocytosis and activating the complement cascade. In RHD, MBL binds to N-acetylglucosamine, a molecule present on the Streptococcus cell wall and on human heart valves. It was observed that high levels of MBL- and MBL2-associated genotypes were associated with RHD, and in another study MBL2 genotypes associated with the high production of MBL seemed to be involved in the pathogenesis of rheumatic carditis and its progression to RHD [5].

One interesting observation is the linkage between antiphospholipid syndrome (APS) and ARF/RHD. Blank et al. [6] considered several common characteristics linking these conditions. Both diseases have central nervous system (CNS) and heart involvement; there is a molecular mimicry between the pathogen and the origin of the disease. Cross-reacting antibodies were found between the pathogen and self molecules, and endothelial cell activation was found to occur at the ‘scene of the crime’ – the valves. Finally, some patients with RHD have circulating antiphospholipid antibodies (aPL), while APS may be associated with streptococcal infection. It was recently demonstrated that cross-reactivity occurs between antibodies directed to the streptococcal M-protein and its synthetic derivative in rheumatic fever and antibodies derived from APS patients targeting the beta-2-glycoprotein-1 (β2GPI) and a β2GPI-related synthetic peptide [6]. Antibodies to β2GPI were found in 24.4% of ARF patients. Antibodies against various β2GPI-related peptides were found in 1.1–36.7% of the patients. The immunoglobulin (Ig) G sera from ARF patients possessed significant anti-β2GPI activity, while sera from APS patients contained considerable anti-streptococcal M protein activity. Affinity-purified anti-β2GPI and anti-β2GPI-related peptide antibodies from ARF patients cross-reacted with streptococcal M protein and M5 peptide, while β2GPI and β2GPI-related peptides from RF patients inhibited anti-streptococcal M protein activity. These data support the hypothesis that common pathogenic mechanisms underlie the development of cardiac valve lesions and CNS abnormalities in APS and ARF [7]. Figueroa et al. [8] observed a significant association between IgM antarcardiolipin antibodies (aCL) and carditis: all patients with valvulitis had IgM aCL (100%) vs. 37% of patients without valvular involvement (P = 0.02). This evidence suggested that antiphospholipid antibodies (aPL) may play a role in the pathogenesis of some clinical manifestations of acute rheumatic fever [8].

It was also demonstrated clinically that chorea in APS patients was associated with rheumatic fever and thrombocytopenia [9]. Indeed, the association between RF and APS, although quite rare, is of great clinical importance and APS should be included in the differential diagnosis of RF, especially when these patients suffer from stroke, or when echocardiogram does not show intracavitary thrombi.

Finally, potentially pathogenic anti-endothelial cell antibodies (AECA) were demonstrated in 40% of RHD patients [10]. As rheumatic valve damage may begin on the surface of valvular endothelium, AECA, possibly using a mechanism of molecular mimicry, could contribute to this damage by promoting endothelial stress and exposure of the underlying basement membrane/extracellular matrix antigens. Indeed, immunoproteomic analysis was able to characterize the autoantibodies directed against endothelial antigens in RHD patients. Interestingly, vimentin was identified as an endothelial autoantigen in RHD and revealed to be cross-reactive to streptococcal antigens. These antibodies also had functional effects on human coronary microvascular endothelial cells (HMVEC-C) [3]. In a preliminary study, we induced experimental myocarditis in Lewis rats by passive transfer of synthesized cross-reactive anti-vimentin antibodies. Cross-reactive anti-streptococcal/vimentin antibodies may represent a possible pathogenic actor in RHD, suggesting that molecular mimicry is fundamental in the development of the disease.

CONCLUSIONS
Taken together, all these data strengthen the idea that ARF/RHD is an autoimmune disease, and unveiling all the mechanisms leading to the disease onset may clarify the link between infections and autoimmunity. Nonetheless, in autoimmunity, everything is infectious until proven otherwise.

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References
Serpins (serine protease inhibitors) are a superfamiliy of evolutionary old, structurally conserved molecules that are widespread among all branches of life. They might have derived from a common ancestor, were lost throughout evolution in simpler organisms but gained important features among metazoans [1,2]. Beyond inhibition of serine proteases, serpins have several functions in homeostasis of living organisms [1], particularly regarding regulation of apoptosis and cellular survival [3].

All serpins share a common tertiary structure that is cored around the reactive center loop, driving serpin specificity and function [1,2]. To date, 37 serpins have been characterized in humans, mainly acting at the extracellular level and belonging to separate clades (termed A-I) according to their phylogenetic relationship. Serpins belonging to clade A (alpha-1 antitrypsin-like) and B (ovalbumin-serpins) represent the largest groups [2]. Unlike the majority of serpins, those belonging to clade B are localized intracellularly and their major task appears to be cellular protection against cytotoxic molecules which can leak into the cytoplasm [4], thereby exerting a direct cytoprotective effect. Moreover, clade B serpins and particularly SERPINB3 are endowed with an anti-apoptotic capability, further contributing to cellular survival [1,3].

The prosurvival role of SERPINB3 had previously emerged due to detection of high levels of SERPINB3 in the presence of squamous cell carcinomas [5] and more recently in liver carcinoma [6], though the mechanisms exploited in apoptosis modulation have not been fully unraveled. SERPINB3 was initially suggested to interfere either with mitochondrial release of cytochrome C or with caspase 3 activation or upstream proteins, due to decrease in caspase 3 activity following cellular transduction with SERPINB3 cDNA [7]. Interestingly, SERPINB3 may exert a double-faced effect regarding modulation of cell death as it was proven to promote endoplasmic reticulum stress and eventually apoptosis [1]. Whether a cell is driven toward uncontrolled survival or stress-induced death may depend on persistence of stressing stimuli. Hence, SERPINB3 is likely to modulate apoptosis following environment influxes as well.

SERPINB3 expression is displayed by several kinds of cells, including mononuclear cells and particularly B lymphocytes [1]. Recently, expression of SERPINB3 on peripheral blood mononuclear cells (PBMC) was analyzed in patients affected either with hepatitis C virus (HCV)-chronic liver disease or systemic lupus erythematosus (SLE) [8]. Interestingly, surface levels of SERPINB3 on PBMC were significantly lower in HCV-carriers than in healthy controls and the lowest levels were found on B lymphocytes of patients with SLE [8]. These findings led the authors [8] to propose a unifying role for interferon type I (IFN-I) and IFN-1-induced genes in dampening SERPINB3 expression on mononuclear cells both in HCV hepatitis and lupus.

In fact, reduced SERPINB3 expression in SLE and possibly in other systemic autoimmune disorders may account for alterations in apoptosis, which may intervene in the exposition of autoantigens in the extracellular space, thereby evoking an aberrant autoimmune response. Early data collected by our group on SERPINB3 administration in murine models of lupus suggested that lupus-like glomerulonephritis could be hindered by increased SERPINB3 levels [9]; however, exogenous administration of SERPINB3 may diverge from its intracellular function. Hence, more experiments investigating the potential of SERPINB3 restoration in lupus models either as a preventive or therapeutic strategy are being carried out.

References
Different studies have proved the concept of autoimmune/inflammatory syndrome induced by adjuvants (ASIA) in animal models [1]. Hence, vaccination remains a concern especially in genetically susceptible patients or those with immune mediated diseases such as systemic lupus erythematosus (SLE), as autoimmune diseases can be triggered by environmental factors. Therefore, it is important to determine if the use of vaccines or adjuvants in susceptible populations can initiate or aggravate the course of these diseases. For this reason, analyzing environmental effects in animal models is a good approach to identify risk in this particular population.

Hepatitis B and Human-Papilloma Virus Vaccines

Previously, both hepatitis B virus vaccine (HBVv) and human-papilloma virus vaccine (HPVv) were associated with SLE diagnosis or exacerbation in a minority of patients [2-4]. In the case of HBVv, we described 10 cases of SLE where the mean latency period from first vaccination to onset of autoimmune symptoms was 56.3 days [2]. In this case series, two patients had a personal history of autoimmunity and two a familial history of autoimmunity. Neurological symptoms were the most common manifestation in this group, present in 8 of 10 patients. Interestingly, 70% of patients continued with vaccination although adverse events were documented [2]. The aggravation of post-vaccine adverse phenomena in subjects who previously reacted adversely to vaccine administration has been documented previously by our group and others [3-5]. For instance, a case series of six patients with SLE post-HPVv was recorded, where several common features were observed among the patients, namely, personal or familial susceptibility to autoimmunity and an adverse response to a prior dose of the vaccine, both of which were associated with a higher risk of post-vaccination full-blown autoimmunity [4].

These observations from case series prompted further research in animal models to determine whether there might indeed be more solid proof for a causal association between vaccination and subsequent development of autoimmunity. Initially we evaluated the effects of immunization with HBVv and its adjuvant, aluminum, on NZBWF1 mice which are genetically prone to develop SLE-like disease. We chose this approach since it appears that in many cases there seems to be a genetic predisposition towards development of autoimmunity, which explains the relative rarity of such adverse phenomena when compared to the number of individuals receiving routine vaccinations. Our study demonstrated acceleration of the disease following immunization with the HBVv. Three groups of animals were immunized with HBVv, aluminum hydroxide or phosphate-buffered saline. The results demonstrated a differential effect of immunization. The group immunized with the whole vaccine, which contains HBV surface antigen, showed advanced kidney damage with severe inflammation, the presence of crescents and higher deposition of immunoglobulin, as well as higher urine protein levels and anti-ds-DNA antibodies. In contrast, immunization with the adjuvant alone was not associated with kidney aggravation. Nonetheless, there were notable hematological and neurobehavioral manifestations in the group of mice that received aluminum. In particular, neurocognitive deficits and brain inflammation were observed following both HBVv and aluminum injections, suggesting that these effects may have been induced by the aluminum adjuvant component of the HBVv [6]. Notably, similar neurocognitive effects were observed among C57BL/6 naïve mice 3 months following immunization with HPVv or aluminum (unpublished data).

Adjuvant Relevance

Recent experiments in animal models have revealed that injected nano-aluminum adjuvant particles have a unique capacity to travel to distant organs including the spleen and brain [7], inciting deleterious immuno-inflammatory responses in neural tissues [8,9]. In particular, following injection, antigen-presenting cells (APCs) avidly take up aluminum particles, and in so doing, become long-lived cells, impeding aluminum solubilization in the interstitial fluid. Thus, a pro-
portion of aluminum nanoparticles escape the injected muscle, mainly within immune cells, travel to regional draining lymph nodes, then exit the lymphatic system to reach the bloodstream, eventually gaining access to distant organs, including the spleen and the brain where aluminum deposits were still detected one year after injection [7]. Moreover, the "Trojan horse" mechanism by which aluminum loaded in macrophages enters the brain results in its slow accumulation due to lack of recirculation and is likely responsible for neurocognitive adverse manifestations previously associated with administration of aluminum-containing vaccines [8,9].

The bioaccumulation of aluminum in the brain appears to occur at a very low rate in normal conditions, potentially explaining the presumably good overall tolerance of this adjuvant despite its strong neurotoxic potential. Nonetheless, according to Khan et al. [7], continuously increasing doses of the poorly biodegradable adjuvant may become insidiously unsafe, especially in cases of repetitive, closely spaced vaccinations and an immature/altered blood-brain barrier. In this context, the latest research by Lujan et al. [8] who described a severe neurodegenerative syndrome in commercial sheep, linked to the repetitive inoculation of aluminum-containing vaccines, is noteworthy. In particular, the "sheep ASIA syndrome" mimics in many aspects human neurological diseases linked to aluminum adjuvants. Notably, the adverse chronic phase of this syndrome affects 50–70% of flocks and up to 100% of animals within a flock. It is characterized by severe neurobehavioral outcomes (restlessness, compulsive wool biting, generalized weakness, muscle tremors, loss of response to stimuli, ataxia, tetraplegia, stupor, coma and death), inflammatory lesions in the brain, and the presence of aluminum in central nervous system tissues [8]. In summary, the ability of aluminum adjuvants to penetrate the blood-brain barrier, its subsequent retention in the brain where the adjuvant has the capacity to trigger severe neurological damage, may in part explain why the vast majority of reported adverse reactions following vaccinations are neurological and neuropyschiatric [3]. These observations are further consistent with the results obtained from our NZBWF1 mice model [6].

CONCLUSIONS
With respect to autoimmune manifestations, the importance of genetic background in autoimmune diseases is well documented. For instance, many human leukocyte antigen polymorphisms have been associated with different autoimmune diseases as well as many other polymorphisms in genes related to immune processes (innate immunity, T and B cell function and differentiation). In addition, ancestry plays a major role in the etiology of autoimmune diseases. For example, Amerindian ancestry has a higher risk for SLE. Moreover, autoimmune diseases are usually aggregated among closer relatives. And finally, the presence of one autoimmune disease in an individual implies a greater risk of developing another [10]. Therefore, susceptible individuals may be at higher risk for developing post-vaccine autoimmunity compared to individuals with no predisposition. Thus, in prophylactic approaches such as vaccinations, potential risks must be carefully considered and evaluated, especially in individuals who may be inherently more prone towards developing autoimmune diseases either because of their genetic background or prior history of adverse reactions to vaccinations.

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“Nature is often hidden, sometimes overcome, seldom extinguished”
Francis Bacon (1561-1626), English philosopher, statesman, scientist, jurist, orator, essayist, and author. Named the “father of empiricism,” he established inductive methodologies for scientific inquiry (known as the Baconian or scientific method). His demand for a planned procedure of investigating all things natural marked a new turn in the rhetorical and theoretical framework for science, much of which still surrounds conceptions of proper methodology today.
Cryopyrin-Associated Periodic Syndrome
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Cryopyrin-associated periodic syndrome (CAPS) includes three overlapping disorders: familial cold autoinflammatory syndrome (FCAS, also known as familial cold urticaria [FCU]), Muckle-Wells syndrome (MWS), and neonatal onset multisystem inflammatory disorder (NOMID, also known as chronic infantile neurological cutaneous and articular [CINCA] syndrome). Once considered separate entities, these hereditary autoinflammatory disorders have been found to share a common genetic basis, pathogenesis and treatment and are therefore now considered a continuous clinical spectrum of a single entity [1].

Prevalence
No formal studies have been conducted to assess the prevalence of CAPS, but it is estimated to be 1–10 cases per million in different countries, with the clinical severity reported to vary greatly. Caucasians seem to be affected more than other races, and there is no male/female preponderance. In Israel there are approximately 30 known CAPS patients, with no particular ethnic predilection. However, since the separate clinical features are common (urticaria, arthralgia) and most physicians are unaware of the syndrome, misdiagnoses and delays in diagnosis occur frequently in CAPS. The assumed prevalence is therefore far greater.

Genetics and Pathogenesis
All cryopyrinopathies are caused by dominantly inherited or de novo gain-of-function mutations in the NLRP3 (also known as CIAS1) gene, located on chromosome 1q44, with a variable penetrance [2]. The NLRP3 gene encodes the NALP3 protein (also known as cryopyrin), which is a family member of the intracellular nucleotide-binding oligomerization domain (NOD)-like receptors (NLRS). NLRS are pattern recognition receptors (PRRs) that can recognize different danger-associated molecular pattern molecules (DAMPs) and pathogen-associated molecular pattern molecules (PAMPs). All NLRS contain a NACHT domain that enables them to aggregate and to oligomerize. Upon activation, NALP3 oligomerizes and recruits other proteins such as ASC and caspase-1, creating a multi-protein assembly called the inflammasome. The inflammasome induces inflammation through the activation and secretion of interleukin (IL)-18 and IL-1β. IL-1β is a potent inflammatory cytokine that causes fever, vasodilation and systemic inflammation through other inflammatory cytokines. Mutations in the NACHT domain of the NLRP3 lead to increased activation of the inflammasome and to increased inflammation [3]. Overall, the different mutations display a strong genotype-phenotype correlation, although a specific mutation may be associated with different phenotypes of varying severity. In a small subset of patients no mutation is found, despite a definitive clinical picture of CAPS. In some of these cases further tests have revealed somatic mosaicism, suggesting a role for somatic mutations.

Clinical Manifestations
CAPS is characterized by chronic or recurrent systemic inflammation, involving the skin, muscles, skeleton, joints, eyes and central nervous system (CNS), as well as by progressive hearing loss. Historically, three different syndromes were separately described: FCAS [4], MWS [5] and NOMID [6]. FCAS is the mildest CAPS disorder, in which exposure to cold results in a systemic inflammatory response including fever, an urticarial rash, conjunctival infection and arthralgia. Urticaria followed by fever and leukocytosis can appear from 30 minutes [4] up to 48 hours [7] following cold exposure. Symptoms develop in the first year of life, specifically in the newborn period in over 90% of cases. The severity and length of attacks vary widely depending on the duration of cold exposure, and tend to relent after a few hours, usually resolving within 24 hours. Affected individuals may experience daily rashes, fatigue, headache, myalgias and conjunctivitis even in the absence of any clear challenge. In contrast to other CAPS phenotypes, secondary amyloidosis is uncommon in FCAS.
MWS is an intermediate phenotype characterized by chronic or intermittent episodes of fever, headache, urticarial rash, arthralgias or arthritis in the absence of a specific trigger. The febrile attacks commence in early childhood and patients also develop progressive sensorineural hearing loss as well as secondary amyloidosis leading to proteinuria and renal failure.

NOMID, the most severe phenotype of CAPS, is characterized by chronic inflammation with numerous flare-ups involving the skin, joints and the CNS. Mortality is high, sometimes before adulthood. Chronic urticaria-like rash is the presenting symptom, appearing shortly after birth. Musculoskeletal involvement includes cartilaginous proliferation at growth plates and epiphyses and arthritis of knees, ankles and feet, elbows, wrists and hands. CNS symptoms include chronic meningitis, headaches, seizures, spasticity of legs, as well as cognitive and mental deficits. Progressive sensorineural deafness develops earlier than in MWS. Ocular involvement includes uveitis, papillary involvement, conjunctivitis and optic neuritis. Typical morphological features of NOMID neonates are short stature, head enlargement, saddle-back nose and short and thick extremities with clubbing of fingers [8].

Due to the considerable overlap, CAPS is considered today as a single clinical entity. Few studies have been conducted on all CAPS patients. A recent survey of a large European registry [9] confirmed earlier reports of CAPS symptoms and provides new data on European CAPS patients. In this cohort, median age at onset was 0.8 years (interquartile range 0.1–5), while age at diagnosis was 15 years (IQR 5–36), reflecting a considerable diagnosis delay. These results confirm earlier studies in 30 FCAS patients from the United States, where 44% were reported to have another diagnosis prior to the identification of CAPS. Forty percent of patients experience a recurrent course, 40% a chronic course, and 20% a chronic course with exacerbations. Most attacks resolve within 24 hours (48%), but a substantial portion (36%) last more than 3 days. Similarly, half the patients suffer fewer than 12 attacks a year, but in 40% of the patients the frequency is more than 24 attacks/year. The trigger is usually cold (85%), but some report other triggers such as infection, trauma, food and fatigue.

The skin is the most commonly affected organ in CAPS (97%), but only 90% have the classic urticarial rash. Fever is frequent (84%) as well as other constitutional symptoms (fatigue, malaise, mood disorders, failure to thrive). Musculoskeletal complaints (arthralgia and myalgia) are common (86%), but only 36% suffer from arthritis. Forty percent of CAPS patients suffer from neurological symptoms, such as morning headaches (29%), meningitis (26%) and papilledema (27%). Severe neurological involvement, such as seizure, hydrocephalus or mental retardation is reported in 12% of patients. Ocular involvement is common (70%), mostly in the form of conjunctivitis (66%) and far less commonly uveitis (7%), optic nerve atrophy, cataract, glaucoma and impaired vision. Hearing loss is reported by more than 40% of patients. Amyloidosis, a severe late complication, develops in 4%.

Laboratory results in CAPS are not specific and reflect systemic inflammation. Erythrocyte sedimentation rate and C-reactive protein are elevated, as are levels of serum amyloid A. Acute attacks are characterized by a marked leukocytosis, and meningitis is associated with increased intracranial pressure and cerebrospinal fluid pleocytosis. Anemia and thrombocytosis are common. X-rays may reveal patellar hypertrophy/overgrowth, epiphyses overgrowth, and complications of arthritis.

Diagnosis is determined by genetic testing for NRLP3 mutations. Some mutations correlate with phenotypes and can predict outcome [Table 1]. Diagnosis should be suspected in any patient with recurrent episodes of fever, urticaria, unexplained systemic inflammation, a positive family history, and early onset of symptoms. Differential diagnosis includes other periodic inflammatory disorders, such as tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS), hyper-immunoglobulinemia D with periodic fever syndrome (HIDS), juvenile systemic granulomatosis, and other common rheumatological disorders such as juvenile idiopathic arthritis. In Israel, familial Mediterranean fever (FMF) is the most common periodic fever syndrome, especially among certain ethnic groups. Behçet’s disease, another autoinflammatory disorder common in Mediterranean populations, can also mimic CAPS.

### Table 1. NRLP3 mutations and phenotype correlation

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Prevalence</th>
<th>Phenotype</th>
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| R260W   | 25%        | • Late age of onset (median > 2 yr)  
• Positive family history  
• Cold-triggered attacks  
• 40% undergo a chronic course |
| T348M   | 15%        | • Early age at onset (median < 2 mo)  
• 85% undergo a chronic course  
• Hearing loss (70%) |
| V198M   | 10%        | • Low penetrance  
• Median age of onset ~ 1.5 yr  
• Neurological involvement uncommon |
| A439 V  | 10%        | • Median age of onset ~ 4 yr  
• Neurological involvement uncommon  
• Positive family history |
| E311 K  | 7%         | • Median age of onset ~ 2 yr  
• High rate of hearing loss  
• Neurological involvement uncommon |
| Q703 K  | 7%         | • Considered a polymorphism (5% of healthy Caucasians)  
• Median age of onset ~ 6 yr (rarely before 12 mo)  
• Very mild disease  
• No arthritis or neurological involvement (with the exception of morning headaches)  
• No hearing loss |
| Rarer mutations or no mutation | 25% | • Severe disease  
• Early age of onset  
• Severe neurological manifestations  
• Severe musculoskeletal involvement  
• Hearing loss |

Based on European registry data, Levy et al. [8]
Shinar et al. [10] report the diagnosis of CAPS in a three-generation Jewish Turkish family, previously diagnosed with Behçet’s disease due to mucosal ulcers and human leukocyte antigen-B51 carriage. This case emphasizes the importance of physician awareness of the possible diagnosis of CAPS in all periodic inflammatory disorders.

TREATMENT

In the past, different anti-inflammatory drugs were used in CAPS with limited success. With the introduction of anti-IL-1 agents, therapy has become more effective with a marked improvement in patients’ quality of life. The three commercially available anti-IL-1 drugs today are anakinra, rilonacept, and canakinumab.

Anakinra (Kinnere®, SOBI, Sweden), an IL-1 receptor antagonist, is given subcutaneously on a daily basis. Anakinra prevents cold-induced attacks, markedly reduces daily symptoms, and ameliorates proteinuria caused by renal amyloidosis. Some have also reported a partial recovery in hearing loss. Unfortunately, some severe cases of NOMID do not always respond to anakinra. Similarly, bone and joint abnormalities do not adequately respond to anakinra.

Rilonacept (Acralyl®, Regeneron, USA) is an IL-1 trap given by weekly subcutaneous injections. This drug was studied in FCAS and MWS, showing a significant reduction in symptom scores compared with placebo. The drug is well tolerated, and the most common adverse effects were injection site reactions.

Canakinumab (Ilaris®, Novartis, Switzerland) is a human anti-IL-1 beta monoclonal antibody given by subcutaneous injections every 8 weeks. Canakinumab has been shown to be a potent therapeutic agent in CAPS, inducing complete remission in 97% of patients, though severe cases may require a dose escalation. Its main side effect was a substantial incidence of infections [1].

Another drug studied in CAPS is a caspase1 inhibitor (VX-765, Vertex Pharmaceuticals, USA) [1], but despite initial success in a small open-label trial no further studies have been conducted. Other drugs such as thalidomide and an anti-IL-6 receptor antibody were found to be beneficial in CAPS but have never been tested in larger studies.

SUMMARY

CAPS is a rare autoinflammatory disease associated with mutations in the NLRP3 gene that result in over-activation of the inflammasome, increased secretion of IL-1β and IL-18, and systemic inflammation. Genetic testing has allowed for grouping of the three, previously distinct clinical syndromes of FCAS, MWS and NOMID, into a single syndrome termed CAPS. The clinical features include urticarial rash and fever, CNS and musculoskeletal involvement, ocular disorders and progressive deafness. Onset, severity and complications (mainly retarda
tion, seizures, destructive arthropathy and amyloidosis) depend on the specific mutation. Diagnosis is determined by genetic tests but is often delayed due to lack of awareness. In Israel, the relative abundance of other autoinflammatory disorders (FMF, Behçet’s disease) may result in misdiagnosis. Treatment is based on IL-1 antagonism, which usually results in prompt clinical response and may prevent amyloidosis.

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References

“Don’t judge each day by the harvest you reap but by the seeds that you plant”
Robert Louis Stevenson (1850-1894), Scottish novelist, poet, essayist, and travel writer. His most famous works are Treasure Island, Kidnapped, and The Strange Case of Dr Jekyll and Mr Hyde

“Please all, and you will please none”
Aesop (620–564 BCE), ancient Greek fabulist or storyteller credited with a number of fables now collectively known as Aesop’s Fables. Although his existence remains uncertain and no writings by him survive, numerous tales credited to him were gathered across the centuries and in many languages in a storytelling tradition that continues to this day. Many of the tales are characterized by animals and inanimate objects that speak, solve problems, and generally have human characteristics
The Hyperferritinemic Syndromes and CD163: a Marker of Macrophage Activation

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**KEY WORDS:** hyperferritinemic syndromes, ferritin, CD163, macrophage activation syndrome (MAS), catastrophic antiphospholipid syndrome (CAPS), adult-onset Still’s disease (AOSD)

Ferritin is a key protein in iron metabolism. Its involvement in iron detoxification and iron storage is known, but recently, a new role in the pathogenesis of different autoimmune and autoinflammatory syndromes was hypothesized [1]. Four immune mediated conditions sharing the feature of marked hyperferritinemia have been gathered under the single term “Hyperferritinemic Syndromes” [1]. Such syndromes include macrophage activation syndrome (MAS), catastrophic antiphospholipid syndrome (CAPS), septic shock, and adult-onset Still’s disease (AOSD). These conditions constitute the scenario of the so-called cytokine storm and share a marked hyperferritinemia as well as clinical and laboratory features.

As far as we know, with regard to its structure, ferritin is composed of two different subunits, H and L, whose ratio is not fixed as it varies among several inflammatory and infectious conditions. Ferritin in spleen and liver, as well as in serum, is largely composed of L-subunits (involved in iron storage), while in heart and kidney the H-subunits (involved in iron detoxification) are predominant. Recently, an increase in the H-subunit expression, driven by different inflammatory stimuli, was demonstrated [2]. Furthermore, a possible role for ferritin in the regulation of immune response was suggested by Recalcati et al. in 2008 [3]. Indeed, the H-ferritin subunit can inhibit lymphoid and myeloid cell proliferation; and a specific ferritin receptor named TIM-2, present on several immune effector cells in murine models, has been identified [4]. Ruddell et al. reported in 2009 [5] that ferritin may behave similarly to pro-inflammatory cytokines, binding to the TIM-2 receptor in hepatic cell media. In doing so it may activate the hepatic cells, inducing enhanced production of several cytokines such as interleukin (IL)-1β, IL-18, tumor necrosis factor-alpha (TNFα), interferon-gamma (IFNγ), macrophage-colony stimulating factor (M-CSF) and IL-6. It was initially believed that the main passive source of ferritin was its leakage from damaged cells during inflammatory conditions. Ghosh et al. [6] later described an active production of ferritin L-subunit through a classical secretory pathway. More recently, Cohen et al. [7] reported the significant contribution of macrophages in ferritin production owing to the proven ability of these cells to actively secrete this protein through a non-classical secretory pathway. Such findings support the idea of active production of ferritin in the course of specific autoinflammatory conditions and, thus, a possible role other than second acute inflammatory reactant.

**ADULT-ONSET STILL’S DISEASE**

Adult-onset Still’s disease (AOSD) is a rare systemic inflammatory syndrome characterized by a typical triad of symptoms comprising a spiking fever, maculopapular rash and arthritis. Apart from laboratory features, marked neutrophilic leukocytosis and increased cytokine production (such as IL-18, one of the main cytokines driving the inflammatory response) [8], hyperferritinemia is one of the main findings. Indeed, over the course of AOSD, ferritin serum values are more than five times above the upper limit of normal, reaching extremely high levels in some cases (> 50,000 µg/L). For this reason, a fivefold increase in ferritin serum levels was noted to have a specificity and sensitivity for AOSD diagnosis of 41% and 80% respectively. Mehta et al. [9] speculated on the possible pathogenic function of ferritin in AOSD, suggesting the existence of a mutated form with defective iron release. A possible role has been proposed for the histiocyte-macrophagic system and/or increased release from damaged hepatocytes over the course of AOSD.

**MACROPHAGES AND CD163**

Macrophages are involved in the regulation of iron homeostasis which, during inflammatory conditions, leads to increased iron uptake and suppressed iron release [10]. Indeed, in the course of inflammatory conditions “M1 macrophages” execute...
iron uptake and iron storage; on the other hand, during the resolution of inflammation, “M2 macrophages” are involved in iron release. These “M2 macrophages” usually express scavenger receptors, and CD163, involved in haptoglobin–hemoglobin complex uptake, is one of the best characterized. sCD163 represents the serum form of this molecule and it is released by shedding into the sera during inflammatory conditions. Its precise function has not yet been defined; however, different stimuli are responsible for its production including Toll-like receptor (TLR) activation [10]. This molecule was found over-expressed in several infectious conditions; nonetheless, it has been proposed as a biomarker for MAS. Over the course of MAS the sCD163 levels positively correlate with ferritin serum levels, suggesting a possible pathogenic relationship between these molecules. Thus, according to such findings, the sCD163 is considered one of the main markers of macrophage activation [10].

**CD163 AND ADULT-ONSET STILL’S DISEASE**

To determine the possible link between ferritin production and macrophage activation, sCD163 expression was recently evaluated for the first time by our group in patients with AOSD (in press). We evaluated the expression of sCD163, with the aim of defining its possible utility as a biomarker of disease activity as well as identifying a possible correlation with ferritin serum levels. Patients with sepsis and healthy subjects served as control groups. Despite the lack of specificity, sCD163 was significantly increased in active patients with AOSD when compared with non-active patients. Importantly, a positive correlation between sCD163 and ferritin serum levels supports the hypothesis of a possible role of macrophages in ferritin production.

Thus, in the unfinished puzzle of autoinflammatory diseases, new players have arrived on the scene and their exact role has still to be defined.

**References**


**Capsule**

**Reversion of advanced Ebola virus disease in non-human primates with ZMapp**

Without an approved vaccine or treatments, Ebola outbreak management has been limited to palliative care and barrier methods to prevent transmission. These approaches, however, have yet to end the outbreak of Ebola after its prolonged presence in West Africa. Qiu et al. show that a combination of monoclonal antibodies (ZMapp), optimized from two previous antibody cocktails, is able to rescue 100% of rhesus macaques when treatment is initiated up to 5 days post-challenge. High fever, viremia and abnormalities in blood count and blood chemistry were evident in many animals before ZMapp intervention. Advanced disease, as indicated by elevated liver enzymes, mucosal hemorrhages and generalized petechia could be reversed, leading to full recovery. ELISA and neutralizing antibody assays indicate that ZMapp is cross-reactive with the Guinean variant of Ebola. ZMapp exceeds the efficacy of any other therapeutics described so far, and results warrant further development of this cocktail for clinical use.

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**“In matters of style, swim with the current; in matters of principle, stand like a rock”**

Thomas Jefferson (1743-1826), American Founding Father, principal author of the Declaration of Independence (1776), and third President of the United States (1801–1809). He was a spokesman for democracy, embracing the principles of republicanism and the rights of the individual
The Hyperferritinemic Syndrome

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KEY WORDS: hyperferritinemic syndrome, autoantibodies, autoimmunity, Still’s disease, macrophage activation syndrome

Ferritin is an iron-binding molecule that stores iron in a biologically available form. It also plays a role in numerous other conditions, including inflammatory, neurodegenerative and malignant diseases. Most clinicians dealing with inflammatory diseases perceive serum ferritin levels as a non-specific marker of the acute-phase response. Despite increasing evidence that circulating ferritin levels indeed reflect an acute-phase response, how and why serum ferritin is elevated is not yet known [1]. Circulating ferritin levels also play a critical role in inflammation. The expression of ferritin is regulated not only by the cytoplasmatic amount of iron, but also by cytokines, oxidative stress, hormones and lipopolysaccharide, among others [2].

Over the last few years, accumulating data have implicated a role for ferritin as a signaling molecule and direct mediator of the immune system. This molecule can be either immunosuppressive or pro-inflammatory. The immunosuppressive effect is well known and seems to play a role in the development of autoimmune diseases. Different mechanisms may inhibit the ferritin-mediated suppression of the immune cells, and in turn, this impaired immunosuppression may favor the loss of tolerance and the development of autoimmune diseases [3]. The autoimmune diseases associated with hyperferritinemia include systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), multiple sclerosis (MS) and antiphospholipid syndrome (APS) [2,4,5]. Yet, autoantibodies against ferritin are also associated with different autoimmune diseases [1]. Hyperferritinemia is also linked to several inflammatory conditions such as sepsis, systemic inflammatory response syndrome (SIRS), multiorgan dysfunction syndrome (MODS), macrophage activation syndrome (MAS), and the catastrophic variant of the antiphospholipid syndrome (CAPS) [6,7]. Furthermore, in critically ill patients, hyperferritinemia is associated with the severity of the underlying disease [6].

On the other hand, it has been proposed that ferritin can also be a pro-inflammatory signaling molecule [8]. There seems to be a complex interaction between ferritin and cytokines in the control of pro-inflammatory and anti-inflammatory mediators. Pro-inflammatory cytokines can induce ferritin expression; in turn, ferritin may induce the expression of pro-inflammatory cytokines. Moreover, ferritin induction of anti-inflammatory cytokines (interleukin-10) is an important mechanism underlying the immunosuppressive effects of ferritin [1].

So, ferritin can be either an immunosuppressive or a pro-inflammatory molecule. These opposing effects are probably dependent on the activation of different pathways, through different receptors, possibly employing different effectors (i.e., L- versus H-ferritin), and possibly different contexts. Actually, this last idea resembles the “two-hit hypothesis”; for instance, for the high levels of ferritin to be pathogenic a second hit may be necessary, like a pro-inflammatory environment, a specific genetic background or even an infectious agent [1].

Four clinical conditions may be associated with high ferritin levels: MAS, adult-onset Still’s disease (AOSD), CAPS, and septic shock [9]. These disorders are characterized by life-threatening hyperinflammation with high levels of ferritin and a cytokinetic storm, clinically presented as multiorgan failure. They share similar clinical signs, symptoms and laboratory parameters [1]. In addition, they also respond to similar therapies. In all conditions there is a good response to treatment with corticosteroids, plasma exchange and intravenous immunoglobulin G (IVIg), supporting a common pathogenic mechanism, and ferritin may be the link between them. It was previously shown that ferritin levels decreased gradually after each plasma exchange session. Furthermore, IVIg may be relevant not only because antibodies against ferritin may be present, but IVIg may also prevent the release of pro-inflammatory cytokines. Macrophages seem to be major players in these four conditions. They are responsible for the production of cytokines and also appear to be extremely important in the production and secretion of serum ferritin [1,10]. We hypothesize that the hyperferritinemia seen in these clinical conditions is part of the pathogenic mechanism and not merely a secondary product of the inflammatory process. Probably, in an inflammatory environment as observed in these diseases, the grossly high levels of ferritin may be involved in some sort of loop mechanism where ferritin’s inflammatory proprieties are exacerbated, leading to an extreme expression of additional inflammatory mediators [1]. Still, not all patients with these clinical conditions have hyperferritinemia; indeed, in about 10% of AOSD patients the
ferritin levels are normal. Perhaps in this subgroup of patients the disease has a different etiology with a different pathogenesis. However, there are other diseases characterized by high levels of ferritin that do not have an inflammatory response, such as hyperferritinemia-cataract syndrome [1].

This concept of hyperferritinemia as a major contributor in the pathogenesis of these conditions may be extremely relevant when considering more targeted therapy. In order to recall the importance of this concept, we propose to include these clinical conditions under a single classification: “The Hyperferritinemic Syndrome.”

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References


Capable
Bring out your dead — hungry receptors await
Every day billions of cells die within the body. Specialized cells called phagocytes patrol the blood and act as cellular garbage collectors, clearing dead cells to prevent tissue damage and inflammation. Phagocytes recognize dead cells because they express molecular “eat me” signals on their surfaces. Zagórska and group examined how mouse phagocytes use different cellular protein receptors, called TAMs, during this process.

The TAM receptors Mer and Axl recognize the “eat me” signals on the surface of dead cells. Mer kept the peace by removing the dead cells that accumulate during normal wear and tear. In contrast, during inflammation, Axl protein expression increased and it took over the removal process from Mer.

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Eitan Israeli

DNA damage induced differentiation of leukemic cells as an anti-cancer barrier
Self-renewal is the hallmark feature both of normal stem cells and cancer stem cells. Since the regenerative capacity of normal hematopoietic stem cells is limited by the accumulation of reactive oxygen species and DNA double-strand breaks, scientists speculated that DNA damage might also constrain leukemic self-renewal and malignant hematopoiesis, and here Santos et al. show that the histone methyl-transferase MLL4, a suppressor of B cell lymphoma, is required for stem cell activity and an aggressive form of acute myeloid leukemia harboring the MLL-AF9 oncogene. Deletion of MLL4 enhances myelopoiesis and myeloid differentiation of leukemic blasts, which protects mice from death related to acute myeloid leukemia. MLL4 exerts its function by regulating transcriptional programs associated with the antioxidant response. Addition of reactive oxygen species scavengers or ectopic expression of FOXO3 protects MLL4−/− MLL-AF9 cells from DNA damage and inhibits myeloid maturation. Similar to MLL4 deficiency, loss of ATM or BRCA1 sensitizes transformed cells to differentiation, suggesting that myeloid differentiation is promoted by loss of genome integrity. Indeed, the authors show that restriction enzyme-induced double-strand breaks are sufficient to induce differentiation of MLL-AF9 blasts, which requires cyclin-dependent kinase inhibitor p21Cip1 (Cdkn1a) activity. In summary, they uncovered an unexpected tumor-promoting role of genome guardians in enforcing the oncogene-induced differentiation blockade in acute myeloid leukemia.

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Eitan Israeli
A Phase IB Clinical Trial with Dekavil (F8-Ill10), an Immunoregulatory ‘Armed Antibody’ for the Treatment of Rheumatoid Arthritis, Used in Combination with Methotrexate

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A therapeutic strategy based on the selective delivery of an immunoregulatory cytokine to the sites of inflammatory disease has been developed. Dekavil is an ‘armed antibody’, composed of the human F8 antibody (specific to the EDA domain of fibronectin, a marker of angiogenesis) fused to the anti-inflammatory cytokine interleukin-10 (IL-10), enabling delivery and accumulation of the cytokine at sites of disease [1,2]. A Phase Ib clinical trial is now underway, which features the administration of weekly escalating doses (6, 15, 30, 60, 110, 160, 210 and 300 µg/kg) of Dekavil in combination with a fixed dose of methotrexate (MTX) to cohorts of three to six rheumatoid arthritis (RA) patients who have previously failed at least one line of anti-tumor necrosis factor (TNF) therapy. This is not a placebo-controlled trial. The objective is to establish the maximum tolerated dose (MTD) and the recommended dose (RD) of the combined treatment, to study safety and tolerability, and to obtain preliminary therapeutic information. The treatment is given as a once-weekly subcutaneous injection for up to 8 weeks.

As of today, 24 patients have received at least one drug administration of F8IL10, from dose levels of between 6 and 300 µg/kg, in combination with MTX, and were therefore evaluable for safety. No dose limiting toxicities (DLTs), serious adverse events (SAEs), or Serious unexpected suspected adverse reactions (SUSARs) have been recorded. No MTD has been reached. The dose level of 300 µg/kg is currently being used. Twelve of 24 treated patients reported mild reactions at the injection sites. A single systemic adverse reaction, progressive anemia, was reported in one patient treated with the 160 µg/kg dose level. All adverse reactions recorded resolved after the end of treatment with little to no therapeutic interventions.

Initial signs of therapeutic benefit have been observed in the treated patients, even at the low drug dosages of the initial steps of the dose escalation. Overall, 15 of 23 patients evaluable for efficacy have experienced therapeutic benefit (in terms of American College of Rheumatology responses). Among these, 15 patients experienced ACR 20 response, 7 experienced ACR 50 response, and 3 achieved ACR 70 response (15 µg/kg, 30 µg/kg and 60 µg/kg cohorts). Variation in the duration of the response was observed. Of note, two patients in the 30 µg/kg cohort and in the 60 µg/kg cohort achieved long-lasting remission (ACR 70 maintained in excess of one year from the last study drug administration).

The promising safety data, together with preliminary positive signs of activity, suggest that the targeted delivery of IL-10 to the sites of inflammation may be beneficial to patients with RA with a possibility for a long-lasting therapeutic potential. These results warrant future clinical investigations in dedicated randomized trials.

KEYWORDS: rheumatoid arthritis (RA), Dekavil, methotrexate (MTX)

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Implantation is an event depending on several steps. The basic features of the fetal-maternal communication system of human pregnancy comprise two arms (placental and paracrine). The villous trophoblasts are the fetal tissue of the anatomic interface of the placental arm, while the fetal membranes are the fetal tissues of the anatomic interface of the paracrine arm of this system. A communication link is established by way of the placental arm: maternal blood directly bathes the villous trophoblasts; fetal blood is contained in fetal capillaries that traverse the intravillous space. At every step, there is a continuous embryo-uterus interaction. The discovery of the implantation window and emergence of the concept of uterine receptivity led to the intriguing idea that cytokines could be central to such a process [1].

During apposition/adhesion, the induction of adhesion molecules and the proper ligands are critical steps. Hence, it is essential that the expression of receptors/ligands at the cell surface of the embryo and uterus coincide. Interleukins (IL) are involved and hormonal regulation plays a major role in both the uterus and the embryo. Adhesion molecules are also modulated. Subsequently, invasion-penetration occurs, and at this step several enzymes are involved, especially matrix metalloproteases, counterbalanced by their inhibitors. In normal pregnancy, the maternal immunological response against trophoblast antigens, concomitant with a transient depression of maternal cell-mediated immunity to protect semi-allogenic embryo from rejection, is the predominant mechanism for a high live birth rate.

Recurrent spontaneous abortion (RSA) is defined as two or more consecutive spontaneous abortions, a heterogeneous condition (with numerous causes and clinical presentations) that may occur at any stage of pregnancy [2]. Mechanisms of RSA involve immune mediated pathways including the presence of a predominant T helper (Th)1-type immunity during pregnancy, a decrease in T regulatory cells and an increase in natural killer (NK) cells. These phenomena can occur locally (at the site of implantation) and are often reflected in the peripheral blood. The interaction between human leukocyte antigen molecules regulate NK cells activity at the maternal-fetal interface. Two main populations have been suggested, NK1 and NK2. The peripheral NK cells (PNKs) cytokine repertoire comprises mainly type 1 cytokines, such as interferon-gamma (IFNγ) and tumor necrosis factor-alpha (TNFα), although proper stimuli can induce production of type 2 cytokines such as IL-4, IL-5 and IL-13 [3]. Even more intriguing, NK cells have long been suspected as the cause of RSA since the original report by Aoki and colleagues [4]. In RSA, increased numbers and killing activity of NK cells in the peripheral blood predict the likelihood of another miscarriage and are considered a causal and prognostic factor for infertility, and miscarriage. Thus, NK cells may also play a key role in immunological infertility and in RSA if the concentrations are too high. In RSA, the creation of an imbalance in Th1-Th2 response, resulting in a prevalent Th1 cytokine environment in the periphery, may lead to NK cell activation and proliferation, which could result in migration of cytotoxic NK cells into the uterus and, in turn, contribute to mechanisms involved in miscarriage [5]. Alternatively, the local endometrial immune assessment may be disrupted at various levels, causing defects in homing of the proper NK population to the uterus, local production of cytokines and hormones such as IL-15 and prolactin, and impairment of more downstream events such as production of immunoregulatory factors by uterine NK cells. Disruption at any of these levels may alter the ability of the uterine NK cell population to perform its normal functions and may result in an unsuccessful pregnancy [6].

**NK and Antiphospholipid Antibodies (APL)**

APL are found in approximately 5–17% of the general population but are more frequent in patients with RSA [3]. An increased number of NK cells correlate with reduced gestational age at abortion in patients with APL-RSA. NK cells might precipitate damage initiated by APL or they might cause pathology in RSA independent of APL, contributing to RSA
in patients with APS. APS is an autoimmune disease characterized by the presence of one or more laboratory findings of APL and at least one clinical manifestation in addition to deep venous and/or arterial thrombosis and/or pregnancy disease comprising RSA, with or without thrombocytopenia. The association between APS and RSA is well known, such that RSA represents one of the clinical diagnostic criteria for APS.

The risk of pregnancy loss in women with APS is higher from the 10th gestational week (GW) onward (fetal period). The Sapporo criteria and the revised criteria for APS underline this situation by considering only patients with one or more unexplained fetal loss (of a morphologically normal fetus) beyond the 10th GW, or three or more unexplained consecutive spontaneous abortions before the 10th GW.

A number of controversies plague the current understanding of APS and RSA; thus several authors have reviewed the role of APS and specifically of APL in RSA and stressed the existence of different subclasses of clinical subsets of RSA in APS patients. Indeed, intrinsic to the definition of APS is a dichotomy regarding patients who have multiple (> 3) abortions within the first 10 GWs and those who have at least one abortion beyond the 10th GW.

Striking evidence of how NK cells behave as pathogenic effectors in RSA comes from a recent study that suggested the possible role of NK cells in the pathogenesis of abortive events in a subpopulation of APS-RSA patients, previously explained in terms of autoimmune specific reactions (APL-mediated).

aPL may cause pregnancy loss via many mechanisms such as thrombosis in decidual vessels, platelet activation, increased expression of adhesion molecules on endometrial cells, and inhibition of anticoagulants. Furthermore, aPL inhibit human chorionic gonadotropin secretion by trophoblast cells, prevent the metalloprotease urokinase from binding to receptors on the trophoblast and inhibit prostaglandin synthesis by decidual cells (decidualization), activated placental complement NK cells, and increase of TNFa, IL-1b and IL-6 [7].

**NK, THYROID AND APL**

Anti-thyroid antibodies and aPL are associated with reduced fertility, miscarriage and preterm delivery, but the precise mechanisms by which thyroid antibodies as well as those against other tissues are suppressed during pregnancy and often exacerbate after delivery remain obscure. Presumably, the rapid reduction in immune suppressor functions after delivery leads to the reestablishment and/or exacerbation of these conditions. Subclinical hypothyroidism of the mother may impair the course of pregnancy and may disturb the normal development of the fetus [8,9]. It usually originates from an underlying autoimmunity, the most common cause of thyroid dysfunction in pregnancy. Thyroid-stimulating hormone may act as a direct stimulator of the immune response, and triiodothyronine and thyroxine act on migration and proliferation of dendritic cells, NK cells and T cells. Previous studies suggested that the measure of peripheral blood NK cell percentage was a reliable predictor of pregnancy outcome in women with infertility and RSA rather than the measure of NK cell activity. All these data support the recommendation of assessing peripheral blood NK cell percentage in the context of female reproductive failure since it may enhance treatment outcome by delineating the underlying etiology [10].

**CONCLUSIONS**

The interactions between NK cell and other autoimmune factors, such as aPS and thyroid, may be associated with impaired pregnancy, and the modulation in the number of circulating NK cells is most likely a primary event rather than an active inflammation/drug administration consequence during an inflammatory/autoimmune process. Thus, NK cells are key players in the pathogenesis of RSA. The role of NK cells at different anatomic sites as well as the role of genetic factors, especially in terms of response to different stimuli from the local microenvironment to which NK cells home and become activated, should be further investigated.

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**References**


Detection of target genes of miR-125b
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Background: Mir-125b is a highly evolutionary conserved microRNA (miRNA), encoded by two different loci on the human genome: miR-125b-1 on chromosome 11 and miR-125b-2 on chromosome 21. Mir-125b is highly expressed in hematopoietic stem cells (HSC) and enhances their survival. Over-expression of miR-125b in specific subtypes of myeloid and lymphoid leukemias provides resistance to apoptosis, enhances proliferation and blocks the differentiation of malignant cells. Each miRNA controls many cellular RNAs and proteins. Although a few target genes of miR-125b were already identified, the mechanism whereby this miR-125b exerts its pro-survival phenotype is still unknown.

Objectives: To identify unknown target genes of miR-125b.

Methods: Potential target genes of miR-125b were chosen by bioinformatics analysis of experimental data obtained in our lab, utilizing proteomics and gene expression of mouse lymphoid Ba/F3 cells transduced with miR-125b versus control empty vector. These data were further cross-examined with four publicly available bioinformatics analysis databases for predicted miRNA targets: Targetscan, Miranda, Microcosm and PicTar. Four potential target genes – BBC3, MAPK14, CSNK2A1 and CASP2 – were chosen for further analysis. To examine whether these chosen genes are directly targeted by miR-125b, we performed dual luciferase assay: human embryonic kidney (HEK) 293T cells over-expressing miR-125b, mutated miR-125b or empty vector were transfected with luciferase vector containing the 3’UTR of the mRNA of each potential target gene. The luciferase activity in the cells was then measured.

Results: The luciferase activities in the cells transfected with the vector containing the 3’UTR of two target genes, BBC3 and CASP2, were significantly suppressed by 30–40% (P < 0.01) in the cells over-expressing miR-125b compared to the empty vector-expressing cells. These suppressions were abolished in the mutated miR-125b-expressing cells. These results indicate that these two genes are directly targeted by miR-125b.

Conclusions: The identification of two pro-apoptotic genes – BBC3 and CASP2 – as direct targets of miR-125b may explain the pro-survival phenotype of the cells expressing this microRNA.

Analysis of mechanisms of bacterial (Serratia marcescens) attachment and migration along fungal hyphae
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Background: Interactions between bacteria and fungi are well documented in the literature, including mutualism, commensalism and pathogenesis. An incidental finding in our laboratory showed the remarkable capacity of Serratia marcescens to migrate along the mycelium of Zygomycete molds, to the best of our knowledge a phenomenon not previously ascribed to this bacterium.

Objectives: We conducted a series of experiments to better define the nature of this phenomenon.

Methods: Two strains of S. marcescens were tested for their ability to migrate along the mycelia of several different fungi to test the specificity of the interaction. Bacterial migration over killed Mucor mycelium and over aerial hyphae of Mucor and Rhizopus was tested as well. The role of flagella, a filamentous appendage involved in bacterial locomotion, and fimbriae, hairlike appendages involved in adherence to surfaces, were tested using S. marcescens mutant strains.

Results: We found that migration of S. marcescens along fungal mycelium was not restricted to Zygomycete molds. It was noted, albeit slower, on several Basidiomycete spp. as well; interestingly, no migration was seen on any mold of the phylum Ascomycota. S. marcescens migration did not necessitate fungal viability or surrounding growth medium, as bacteria migrated along aerial hyphae as well. Flagellum-defective strains of S. marcescens were able to migrate the same distance as the wild-type, although significantly slower. Specific attachment, if it occurs, does not necessitate type 1 fimbrial adhesion since mutants defective in this adhesin migrated equally well or faster than the wild-type. Bacterial translocation along fungi is an active process, as it occurred on killed mycelia. At the molecular level, the migration mechanism seems to be multifactorial: flagella support, but are not a prerequisite for the process, and although bacterial migration probably necessitates some kind of specific interaction, suggested by the selectivity of the phenomenon, it is likely mediated by other means than type 1 fimbriae, a recognized gram-negative bacterial adhesin.

Conclusions: it has been postulated that bacterial virulence factors originally evolved to compete with eukaryotes in the environment.
A better understanding of the mechanisms allowing *S. marcescens* to attach to, and translocate along the mycelia of eukaryotic fungi may provide better ways to prevent and treat bacterial colonization of biotic and abiotic surfaces, thus decreasing the need for antibiotics.

**Gene expression biomarkers to predict the outcome of immune-modulatory treatment in patients with relapsing-remitting multiple sclerosis**

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**Background:** Several immune-modulatory treatments are known to be effective for relapsing-remitting multiple sclerosis (RRMS), in terms of reducing the number of relapses and the disease progression. However, each treatment is characterized by patients with an unsatisfactory response. Currently, there is no effective tool to predict treatment efficacy in the individual patient prior to administration.

**Objectives:** To evaluate peripheral blood predictive biomarkers for glatiramer acetate (GA) and interferon-beta (IFNβ) treatment outcome in RRMS patients.

**Methods:** We used 85 HG-U133A2 (Affymetrix) gene-expression microarrays to analyze peripheral blood samples taken prior to treatment initiation with GA (*n*=37, age 38.6±10.3 years, disease duration 6.4±1.2 years, expanded disability status scale (EDSS) 2.1±0.2) and interferon-beta (IFNβ) (*n*=48, age 37.8±10.5 years, disease duration 6.6±1.1 years, EDSS 2.0±0.2). Good treatment response at 2 years of treatment was defined as a reduction of at least one relapse compared with the 2 year rate prior to treatment combined with annual increase of up to 0.5 in the EDSS score. Statistical comparison of the baseline expression of differentiating genes between responders and non-responders was performed to identify potential markers for treatment response.

**Results:** Good clinical outcome was observed in 25/37 (67%) GA treated patients. A signature of 762 gene-transcripts differentiated between good and poor responders at baseline, significantly enriched with genes related to apoptosis (*P*= 5.27E-05) and inflammation (*P*= 6.28E-05). A three-gene classifier including *ACTR*, *WDR45* and *PPR1R13B*, all known to be related to apoptosis, showed a robust discrimination rate for treatment response. Good clinical outcome was observed in 34 of 48 (70%) IFNβ-treated patients. A signature of 627 gene transcripts differentiated between good and poor responders. This signature was significantly enriched with genes related to T cell growth and proliferation (*P*= 1.37E-03) being over-expressed in good responders at baseline. A three-gene classifier including *PRUNE*, *POUF1* and *TRD*, known to be related to cellular growth, showed a robust discrimination rate for treatment response.

**Conclusions:** Our results suggest that baseline gene expression biomarkers can predict treatment response for GA and IFNβ treatment, offering future clinical applications.

**Capsule**

**MMWR Early Release on the Ebola outbreak**

*Ebola Virus Disease Outbreak – West Africa, September 2014*

Updated data on the Ebola virus disease outbreak in West Africa indicate that, as of September 23, a total of 6574 cases had been reported from five West African countries (Guinea, Liberia, Nigeria, Senegal, and Sierra Leone). The highest reported case counts were from Liberia (3458 cases), Sierra Leone (2021) and Guinea (1074).

Incident Management System Ebola Epidemiology Team, CDC; Ministries of Health of Guinea, Sierra Leone, Liberia, Nigeria, and Senegal; Viral Special Pathogens Branch, National Center for Emerging and Zoonotic Infectious Diseases, CDC.

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*Ebola Virus Disease Outbreak – Nigeria, July-September 2014*

On July 20, an acutely ill traveler from Liberia arrived at the international airport in Lagos, Nigeria, and was confirmed to have Ebola virus disease after being admitted to a private hospital. The Federal Ministry of Health, with the Lagos State government and international partners, activated an Ebola Incident Management Center as a precursor to the current Emergency Operations Center to rapidly respond to this outbreak. The index patient died on July 25; as of September 24, there were 19 laboratory-confirmed Ebola cases and one probable case in two states, with 894 contacts identified and followed during the response.

Shuaib et al. *MMWR* 2014;63(Early Release):1-6

*Importation and Containment of Ebola Virus Disease – Senegal, August-September 2014*

On 29 August 2014, Senegal confirmed its first case of Ebola virus disease in a Guinean man, aged 21 years, who had traveled from Guinea to Dakar, Senegal, in mid-August to visit family. Senegalese medical and public health personnel were alerted about this patient after public health staff in Guinea contacted his family in Senegal on August 27. This report describes the investigation and containment measures that followed.

Mirkovic et al. *MMWR* 2014;63 (Early Release):1-2

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