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Conference Report

Silencing Autoimmunity in Multiple Sclerosis, Lupus, and Rheumatoid Arthritis

Highlights From the 90th Meeting of the American Association of Immunologists; May 6-10, 2003; Denver, Colorado

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Posted 06/18/2003

Introduction

At the symposium of the Clinical Immunology Society, held during the 90th Annual Meeting of the American Association of Immunologists (AAI), emerging targets and therapies for autoimmune diseases took central stage. Although significant progress has been made in the diagnosis and staging of diseases such as multiple sclerosis (MS), lupus erythematosus, and rheumatoid arthritis, many questions still surround their ethiopathogenesis, thus casting shadows on which should be considered the most effective treatment strategies.

Heightened and therefore pathologic responses by the immune system are thought, if not to promote, at least to maintain these syndromes and, thus, they represent a high-priority target for intervention. Owing to the complexity of the immune system, however, it is still not clear, after years of dedicated research, which arm(s) of the immune system -- T cells, autoantibodies, and/or cytokines -- represent the critical factor(s) in each of them. Thus, the debate is still ongoing on which are the key effectors that need to be silenced to achieve remission or prevent new flare-ups.

Immunosuppressive treatments (eg, corticosteroids, cyclosporin A, methotrexate) are currently used to keep these recurrent and progressive syndromes at bay, particularly in the more severe stages. Their efficacy, however, is limited and, in addition, year- or decade-long treatments are associated with significant toxicities. As pointed out by the session's speakers, more needs to be done to identify the immune circuits that have gone awry in each disease and devise selective "immunosilencing" treatments.

Statins and Multiple Sclerosis: A New Therapeutic Strategy?

The uncertainty surrounding the pathogenetic mechanisms of MS has prompted researchers to investigate a number of different therapeutic options in the past 10 years. As inflammatory processes and possibly autoimmune reactions are believed to be responsible for MS and certainly contribute to this disease, most of these new strategies are focused on finding new ways to manipulate the immune system. Achieving a more selective downregulation of autoreactive components than usually obtained with conventional anti-inflammatory steroid drugs would offer clinical benefit to MS patients, while avoiding the unwanted toxic effects associated with high-dose or prolonged use of these compounds.

The new agents studied so far, however, including interferons (IFNs), mitoxantrone, and glatiramer acetate, still require injection, are quite expensive on a long-term basis, and administration may be accompanied by undesired effects such as pain, swelling, and flu-like symptoms, or, as in the case of mitoxantrone, by potentially severe toxicity including a higher risk of infections, secondary tumors, and organ damage to heart and liver. Clinical efficacy of these agents has proven variable among patients, with an overall reduction in the number of relapses of approximately 30%.

A recent presentation at the 55th Annual Meeting of the American Academy of Neurology raised considerable interest. Timothy Vollmer and colleagues reported that simvastatin, an HMG-CoA reductase inhibitor and one of the compounds already in clinical use to reduce cholesterol levels, had beneficial effects in MS patients enrolled in an open-label single-arm study.[1]

In this phase 1 clinical trial,[1] a total of 45 patients with relapsing-remitting MS (RR-MS) received a daily oral dose of 80 mg of simvastatin for 6 months, and 41 were evaluable at the end of the study. Comparison of 3 monthly cranial MRIs with 3 MRIs performed at months 4, 5, and 6 following initiation of treatment showed a significant reduction (approximately 40%) in the mean number ($P < .0001$) and in the mean volume ($P < .0016$) of Gd-enhanced lesions (mean number pretreatment, 2.35 vs posttreatment, 1.31; mean volume pretreatment, 238 vs posttreatment, 142). No toxic effects were observed during the study. Treatment of more patients in a randomized setting is necessary to confirm these preliminary results,[2] evaluate the effects on symptoms and clinical history of the disease, and confirm the low toxicity reported so far (elevation of liver markers in 2 patients).[1] In fact, statins, although showing a good safety profile in patients with high cholesterol levels and/or cardiovascular disease, may be potentially associated with development of hepatic damage, myalgias, or polyneuropathy.[3,4]

Dissecting the Molecular Mechanisms

The rationale for this study was previous results obtained in animal models of experimental autoimmune encephalomyelitis, showing that simvastatin decreased the activity of type 1 CD4 cells (associated with cytotoxic responses) and increased function of type 2 CD4 cells (with regulatory/helper phenotype). In addition, administration of the

statin led to a reduced accumulation of tumor necrosis factor (TNF) alpha in the central nervous system lesions of affected animals. Other in vitro studies showed that simvastatin, more than lovastatin and mevastatin, inhibited proliferation of mitogen-stimulated peripheral blood lymphoid cells and downregulated expression of activation-induced adhesion molecules, membrane metalloproteases, and chemokine receptors on both T and B cells.[5]

Scott Zamvil,[6] of the University of California, San Francisco, and colleagues presented new results at the annual meeting of the AAI obtained with atorvastatin in experimental autoimmune models of MS and in vitro experiments.[7,8] As outlined, oral administration of this statin was found to:

- Prevent or attenuate chronic and relapsing paralysis in mice affected by demyelinating disease (experimental autoimmune encephalomyelitis);

- Reduce cellular infiltrates in the central nervous system;

- Block production of type 1 lymphokines (interleukin [IL]-2, IL-12, IFN-gamma and TNF alpha);

- Promote differentiation of CD4 cells toward a type 2 regulatory response; and

- Inhibit upregulation of MHC Class II antigens on microglial cells and of other costimulatory molecules involved in antigen presentation.

Overall, atorvastatin blocked activation of immune cells, both by preincubation with antigen presenting cells or with T cells (Table 1).

Of note, adoptive transfer of CD3 T cells from atorvastatin-treated mice conferred protection from experimental autoimmune encephalitis to naive mice, and protection was mediated by CD4 T cells. Specificity was shown by reversing its inhibitory effects with mevalonate, the downstream product of HMG-CoA reductase, the enzyme targeted by statins. Other researchers have confirmed these results,[9,10] adding that treatment with atorvastatin inhibited T-cell proliferation by negatively regulating cell cycle progression without interfering with calcium influxes in activated immune cells or apoptosis.[9]

Thus, statins seem to be endowed with immune regulatory functions that go beyond the well-known effects on metabolic pathways. Of note, Waehre and colleagues[11] reported that in patients with coronary heart disease, treatment with atorvastatin (80 mg/day) and simvastatin (20 mg/day) was associated with downregulation of the spontaneous release of interleukin-8 and MIP-1 alfa by peripheral blood mononuclear cells.

More investigations will reveal whether treatment with statins is an effective therapeutic strategy for patients with MS, and define the extent of immunosuppressive selectivity achieved (silencing of autoimmunity vs responses to infections and tumor growth). As

mentioned by Dr. Zamvil,[6] a double-blind, placebo-controlled, phase 2 clinical trial, involving 12 centers, is in progress in 91 patients with clinically isolated MS syndrome. Clinical responses will be evaluated alongside with antigen-based proliferation indexes, Th1/Th2 ratios, CIITA activation, as well as other activation markers and functions.

Manipulating Interferon in Systemic Lupus Erythematosus

Immune T cells, thought to play an important, if not causative, role in many autoimmune diseases, may do so directly, as effector T cells (eg, cytotoxic CD8 cells and CD4/Th1 T cells) or indirectly by sustaining and amplifying a B cell-driven, antibody-mediated immune response (eg, CD4/Th2 cells). Insulin-dependent diabetes mellitus would be an example of a disease with predominant Th1 involvement, while systemic lupus erythematosus (SLE) has been considered, so far, an example of a Th2-dependent autoimmune disease. But, is it indeed so?

Type 1 and Type 2 cells

CD4/Th1 cells are generated from naive T-cell precursors following interaction with the antigen and antigen presenting cells (APCs) in the presence of interleukin-12 (IL-12) and IL-18 produced by the APCs. Immune competent Th1 cells, in turn, produce IFN-gamma, IL-2, and TNF-beta that further amplify a Th1 response and sustain cytotoxic effector functions. CD4/Th2 cells, on the other hand, are generated in the presence of IL-4 and IFN-gamma, and following activation, they release IL-4, IL-5, IL-10, and IL-13 and sustain antibody-mediated immune responses initiated by B cells and antigen-presenting cells. Of note, IL-10 has a negative effect on the generation of Th1 cells, thus helping in the polarization of a helper T-cell response toward the Th2 type.[12,13]

Thus, both type-1 and type-2 phenotypes are cytokine driven (IL-12/IFN-gamma vs IL-4); and CD4/Th1 cells are involved in T cell-mediated immune responses, while CD4/Th2 cells contribute to humoral immune responses. A similar polarization has been described also in B cells (eg, Be1 cells produce IFN-gamma and Be2 cells secrete IL-4).[12,13]

SLE, a Type 1 or Type 2 Disorder?

SLE has been initially considered a type-2 disease since there is evidence of B cell activation and patients develop autoimmune antibodies, with pathologic deposition in different organs (eg, kidney). Ari Theofilopoulos of the Scripps Research Institute, La Jolla, California, and colleagues, however, propose that the role played by immune cells in this disease is different, with more involvement of type 1 cells than expected, and a critical role for a type 1-linked cytokine, IFN gamma.[14-17]

Several lines of evidence seem to support this hypothesis:

- Increased production of IFN-gamma and IL-12 has been found at the mRNA and

protein level in mice that spontaneously develop SLE (MRL/lpr) vs normal animals.

- Mice overexpressing IFN-gamma in the skin developed inflammation and a lupus-like syndrome, with production of antinuclear antibodies and deposits of immune complexes in the kidneys.

- Serum levels of IFN-gamma and IL-12 are increased in SLE patients, particularly those with active disease and diffuse proliferative nephritis.

- Microarray global gene expression signature of peripheral lymphocytes in SLE patients is characterized by higher expression of IFN-regulated genes.

- Administration of IFN-gamma in patients with autoimmune disease or myeloproliferative disorders was found to induce, unexpectedly, a severe form of SLE.

- Administration of IFN-gamma in New Zealand mice, which spontaneously develop a lupus-like syndrome, accelerated onset of the disease.

- Prophylactic treatment of New Zealand mice with an antagonistic IFN-gamma receptor or anti-IFN-gamma antibodies delayed onset of the disease and improved overall survival.

- Deletion of the IFN-gamma gene or of its receptor prevented development of SLE. This was observed also in mice heterozygous for the IFN-gamma deletion, suggesting a threshold effect.

Selective Inhibition of IFN-gamma

The finding that only a 50% reduction in the quantity of IFN-gamma produced was enough to prevent the disease in mice spontaneously developing SLE leads to hope that, even in humans, a partial reduction in IFN-gamma might be effective. This is important because it would allow selective manipulation of the immune system without generalized immunosuppression and potential preservation of other critical immune responses perhaps occurring at lower IFN-gamma thresholds, such as responses to infections and immune surveillance of cancer cell growth.

Theofilopoulos and colleagues thus designed a bivalent hybrid molecule consisting of the IFN-gamma receptor linked to the constant region of an immunoglobulin (Ig)G1 to confer a longer half-life in vivo (approximately 40 hours). MRL/lpr injected prophylactically with a nonviral vector expressing this IFN-gamma inhibitor did not develop the disease.[14,18] Some beneficial effects were seen also when animals were treated at a more advanced disease stage (Table 2).

As noted by the authors, owing to the high number of processes influenced by IFN-gamma (eg, antigen presentation, MHC expression on kidney cells, etc.) it is very

difficult to pinpoint exactly the step, if any, affected by inhibition of the IFN-gamma system, that is critical for development of maintenance of lupus. Treatment was, however, associated with a reduction in the severity of the glomerulonephritis and in the deposition of immunocomplexes in the kidneys. The significant decrease in titer of polyclonal IgG and antichromatin IgG provides evidence that IFN-gamma plays a key role in breaking tolerance to chromatin.

At the symposium held during the AAI meeting, Dr. Theophilopoulos presented more data obtained in NZB mice lacking the IFN locus (IFN alfa,beta knock-out mice).[19] As shown in Table 3, absence of IFN-alfa and -beta had a significant effect on these mice, with reduction in the autoimmune process and reduced mortality. Both IFNs seemed to favor development of autoimmunity not only by activating the antigen-presenting cell compartment, but also by promoting survival and differentiation of B and T cells, as well as granulocyte activation and enzyme release.

Alternative Mediators

Other cytokines and effectors might, however, still have a significant role in SLE, and thus represent alternative or additional targets in a therapeutic setting. In fact, antibodies to IL-6 and IL-10 reduced the severity of SLE in New Zealand mice. Conversely, the results obtained by interfering with the IL-4 system have been more contradictory.[15] IL-4 antagonists in MRL/lpr mice reduced mortality and severity of the disease, but in New Zealand mice, overexpression of IL-4 under the control of unrelated immunoglobulin promoters had a protective effect.

Thus, more data are needed to choose optimal target(s) for treatment of autoimmune diseases such as SLE. Yet, according to Theofilopoulos, the evidence accumulated suggests that an extreme polarization of type 1 vs type 2 diseases might not be appropriate from a pathogenetic point of view and hence not useful from therapeutic standpoint. Even a disease dominated by the presence of autoantibodies such as SLE, and thus apparently a type 2 disease, may well benefit from strategies interfering with cytokines, such as IFNs, typically associated with a type 1 response. Selection of potentially sensitive patients might be possible thanks to the recent identification of genomic signatures of IFN-gamma-inducible proteins in the serum of patients with SLE.[17]

Gene Therapy for Rheumatoid Arthritis?

Clinical researchers view rheumatoid arthritis (RA), an inflammatory disease of the joints that affects mostly, but not only, adults with a relapsing, progressively invalidating course, as a disease amenable to gene therapy.[20,21] The tissues involved are, in fact, easily reachable by arthroscopy for inspection, biopsy, or collection of target cells, as well as intra-articular injection of vectors or therapeutic agents. In addition, the only treatments available are associated with some toxicity and are palliative in nature, being aimed at containment and suppression of the inflammatory reactions associated with the

autoimmune process.

Two classes of vectors have been considered for gene therapy of RA, adenoviruses and retroviral vectors. Advantages intrinsic to the use of adenoviruses for local in vivo treatment is their ability to transform nonreplicating cells, yield high-titer amplification, display a promiscuous range of susceptible cell types (both type A and B synoviocytes), and minimize risks associated with genomic integration of viral sequences. On the other hand, the transient nature of their expression and the difficulties associated with retreatment (due to adenovirus-specific immune responses) still significantly limit the therapeutic usefulness of these vectors.[22] Molecular manipulations of wild-type adenoviruses are in progress to generate new variants ("gutted" vectors) that do not express gene products with high immunogenic and antigenic potential. Addition of new sequences may also lead, in the future, to the generation of chimeric adenoviral vectors more suitable for human use.

Retroviral vectors, on the other hand, by achieving integration in the genome of the host cells, yield better expression levels, but carry along the risk of mutagenesis by insertion, as recently seen in gene therapy trials in children with severe combined immunodeficiency.[23] A second critical limitation to their use is that they are not suitable for transformation of nonproliferating cells and they mainly transduce type B synoviocytes.[24] More than 100 nonviral vectors have also been tested, but, so far, they have yielded only very low and transient expression levels of the recombinant protein of interest.

At the annual meeting of the AAI, Christopher Evans,[25] of Harvard Medical School, Boston, Massachusetts, discussed the results obtained in experimental studies aimed at blocking activity of IL-1, one of the cytokines believed to play a critical role in RA. This molecule has proven safe, although not efficacious, in phase 3 clinical trials for sepsis, and new ways are being explored for its delivery. Administration of IL-1 antagonist is very difficult as it has a very short half-life in vivo and undergoes very large diurnal variations in concentrations. A serum level of 1 mcg/mL has been associated with antierosive activity and a serum level of 5 mcg/mL with anti-inflammatory activity in rabbit models. After administration in humans, however, only a concentration of 1.5 mcg/mL was achieved for 3-4 hours/day, insufficient to yield significant anti-inflammatory activity in vivo.

Clinical Trials

Delivery of the IL-1 antagonist gene using an amphotropic retrovirus (MFG-IRAP) derived from the Moloney murine leukemia virus to synovial cells ex vivo, was found to be safe and feasible in rabbits, rats, dogs, and mice, yielding serum levels high enough to achieve inhibitory concentrations in inflammatory diseases such as adjuvant arthritis and collagen-induced arthritis.[25] In a phase 1 clinical trial in 9 postmenopausal women affected by RA refractory to other treatments, synoviocytes obtained after palliative surgery were transduced ex vivo with the IL-1 antagonist recombinant retrovirus and reinjected in multiple joints.[26,27] Patients were divided into 3 groups, receiving 1, 5, or 10 million

cells. Gene expression was found in all joints that had received the transgene, with islands of cells embedded in the synovium producing the inhibitor. No toxic effects were observed and the patients seemed to accept the procedure well.

More investigations, however, need to be undertaken as the level of IL-1 antagonist expression achieved in vivo long-term was not high enough to ensure anti-inflammatory levels.[25,26] Immune-mediated events might be involved in loss of expression since longer in vivo production of recombinant proteins were seen in nude vs immunocompetent Wistar rats.[25] A new lentiviral HIV-1 based vector (VSV-G) is being considered as a possible alternative for gene delivery, as it can transduce nondividing cells, undergo integration in the host cell genome, and efficiently transduce rat synoviocytes in vitro.

More experimental work is also progress in animal models to evaluate whether interference with other cytokines may yield other therapeutic options for the management of RA. In vivo transformation of synovial tissues with adenoviruses encoding anti-inflammatory mediators has been evaluated in a rabbit model of antigen-induced arthritis. Transduction of a gene encoding a bivalent, soluble IL-1 type 1 receptor or a soluble TNF receptor showed only limited efficacy in reducing the synovitic process.[28] A combination of the two, however, not only protected the cartilage and reduced infiltration by inflammatory cells, but it also inhibited swelling of the joint and strongly reduced the synovitis. A gene encoding viral IL-10 showed an even higher anti-inflammatory activity.[29] Of note, treatment of only 1 knee joint was associated with beneficial effects in the contralateral knee that had received only saline, possibly due to interarticular trafficking of transduced leukocytes expressing vIL-10 or the IL-1/TNF antagonists.[29]

As discussed by Dr. Evans, a number of questions still surround gene therapy approaches for RA:

- Why target the joints if RA is a systemic disease?

- Will patients on gene therapy be transformed into "pin cushions"?

- Is there any spill out of IL-1 antagonist in the periphery?

- Are the benefits higher than the risks associated with gene therapy?

Although a systemic disease, RA is still viewed as a good candidate for gene therapy since the joints represent one of the most involved sites and they are easily accessible. Of note, a contralateral effect has been seen in experimental models suggesting the existence of transsynovial communication.[28,29] Thus, only a limited number of injections with an efficient recombinant vector would be required to achieve expression in affected joints. No IL-1 antagonist spill out has been observed so far in animal models where expression levels were higher that those achieved in humans. Risks associated

with gene therapy, as recently seen in experimental trials of patients with severe combined immunodeficiencies,[23] are still not well understood, and they will probably vary depending on the expression systems and delivery methods selected for treatment. Caution and thorough investigations will allow a better evaluation of the benefit/risk ratio for each patient, and a judicious selection of vectors and promoters[30] might help in improving this ratio.

Tables

Table 1. Effects of Atorvastatin on T Cells and Antigen Presenting Cells

Cell Type	Effect
Antigen-presenting cells	Reduction of infiltration in the CNS
	Suppression of interferon gamma-inducible expression of MHC Class II in microglial cells
	Suppression of interferon gamma-inducible expression of CIITA in microglial cells
	Suppression of interferon gamma-inducible expression of CD40, CD80, and CD86 in microglial cells
	Inhibition of STAT1
T cells	Suppression of T-cell proliferation
	Suppression of Th1 cytokines interleukin 2 and interferon gamma
	Upregulation of Th2 cytokines interleukin 4 and 10
	Differentiation of naive Th0 cells to Th2 cells
	Dose-dependent activation of STAT6

Table 2. Effects of IFN-gamma Downregulation on Survival of Autoimmune Mice

	Treatment Protocol	Survival at Month 10

Treatment at month 2	Control	0%
	Plasmid injection	60%
	Plasmid plus local electroporation	90%
Late-stage treatment at month 4	Control	0%
	Plasmid plus local electroporation	100%

Table 3. Changes Observed in Interferon-alfa and -beta Knock-out NZB Mice

Reduced mortality
Reduced hemolytic anemia
Reduced kidney disease
Reduced Ig deposits in kidneys
Reduced B1 cells in spleen and peritoneum
Reduced B2 cellularity
Reduced total IgG and IgM
Reduced anti-ds DNA IgG and IgM in spleen
Reduced proliferation of B2 cells
Reduced response of B2 cells to LPS stimulation
B-cell hematopoiesis not affected
No difference in susceptibility to apoptosis
Reduced proliferation of CD8 T cells
Reduced maturation of dendritic cells
Reduced activity of antigen-presenting cells in T-cell allostimulation
Reduced Th1 activity

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