

Immunomodulators: A brave new world

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In the future annals of the history of immunology, the last 2 decades of the 20th century will probably be identified as the dawn of the brave new world of therapeutic immunomodulation. An intersection of scientific and economic developments allowed the mushrooming of nascent new therapeutics. The advent of recombinant DNA technology allowed the preparation of pure native and engineered compounds in large quantities. The economy at the time marked the willingness of venture capitalists to take a risk on small startup biotechnology companies. This combination of brain and brawn led to the birth of therapeutic immunomodulation.

As our understanding of basic immune mechanisms increased, our ability to develop or engineer immunomodulators increased as well. This, however, turned out to be a 2-edged sword. Most of the basic immunology concepts are developed in mice, whereas the therapeutic immunomodulators are, obviously, targeted at underlying mechanisms of human disease. For example, although it was known that TNF- α is important in the defense against mycobacterial infection (resulting in the *development* of granulomas), it was not until TNF antagonists were used in human subjects that it became clear that TNF plays a pivotal role in the *maintenance* of a granuloma.^{1,2} The reactivation of tuberculosis by TNF antagonists was an unanticipated finding. Indeed, the brief history of therapeutic immunomodulation is replete with examples of unintended consequences. The use of anti- $\alpha 4$ integrin antibody resulted in the unanticipated development of progressive multifocal leukoencephalopathy in some patients, prompting one to rethink the role of adhesion molecules in the control of the JC polyomavirus infection.³ The use of granulocyte colony-stimulating factor in neutropenic patients has been associated with an increased incidence of myelodysplastic syndromes. It is now apparent that those at highest risk of this complication are patients with mutations in one of the granulocyte colony-stimulating factor receptors (CSF3R).⁴ It is clear that one cannot, as of yet, predict the effect of an inactivation or a deletion of a single molecule. Hence one should be vigilant for unusual clinical events, even if they do not make sense, during the use of any immunomodulator.

Examples of consequences of selective inactivation of an immune molecule or pathway have been accumulating in mice and in human subjects. For example, abnormalities in IL-12 or

IFN- γ pathways (in mice and in human subjects) lead to a selective susceptibility to mycobacteria and salmonella infections.^{5,6} Toll-like receptor 3 mutations were found in patients with herpes encephalitis.⁷ CCR5 knockout mice have a selective susceptibility to *Cryptococcus neoformans* infection of the central nervous system but not an increased susceptibility to pulmonary infection with this organism.⁸ IL-6 knockout mice appear to have a selectively increased susceptibility to infection with *Escherichia coli* and *Listeria monocytogenes*.^{9,10} In addition to infections, inactivation of a single molecule might be associated with specific diseases. IL-2 and IL-10 knockout mice have inflammatory bowel disease, whereas GM-CSF knockout mice have alveolar proteinosis.^{11,12} It is thus apparent that selective inactivation of a single immunologic molecule might have unanticipated consequences that do not become apparent until after a certain infection or a certain biologic event. The allergist/clinical immunologist is uniquely situated to be able to recognize and perhaps anticipate some of these complications.

The above caveats notwithstanding, therapeutic immunomodulation is here to stay and will affect all subspecialties of medicine, not just allergic and immunologic diseases. The January and February issues of the Journal are dedicated to examining the various therapeutic immunomodulators currently available or under development for use in immune disorders. It is readily apparent that varied and disparate strategies have been adopted to affect the immune response. In addition to targeting the T-cell receptor, strategies have been developed to target cytokines, cytokine receptors, cell-surface molecules, T_H subset balance, signaling sequences, and gene activation.

Casale and Stokes¹³ present a comprehensive summary of the various immunomodulators used for asthma and allergic diseases and give valuable insights into the pros and cons of each approach. Their review should serve as a handy user's guide to immunomodulators that effect the field of allergy/immunology. As is evident from the review by Casale and Stokes and as is evident from the dedicated articles in these 2 issues, therapeutics have been developed that target specific events across the spectrum of the various events involved in an immune response.

The immune response is an ordered sequence of events, which can be simplified as follows: antigen is processed and presented to lymphocytes; lymphocytes need to recognize the antigen by their receptor but also need to engage a costimulation molecule. This is followed by activation of signaling molecules, which leads to engagement of nuclear factor κ B, gene activation, and mRNA transcription, followed by the synthesis and secretion of various cytokines. The secreted cytokines bind their appropriate receptors, leading to the clinical manifestations of various diseases. Modulation of each of the above steps is discussed in these 2 issues of the Journal, with the exception of dendritic cells, the manipulation of which remains in its infancy.

Levesque and St Clair¹⁴ review the results of B cell-directed therapies using rituximab, an mAb directed against CD20 that

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is a surface marker on mature B cells. Rituximab was initially approved for the therapy of B-cell lymphoma. It became quickly apparent, however, that depleting B cells with this antibody might be beneficial in autoantibody-mediated diseases. The idea is that depleting autoreactive B cells will lead to depletion of autoreactive antibodies, which should lead to remission. Indeed, the response to rituximab in some autoimmune diseases (immune thrombocytopenic purpura, Graves' disease, and pemphigus vulgaris) correlated with the decrease in the autoantibody titer. In other diseases (eg, systemic lupus erythematosus and rheumatoid arthritis), however, a significant clinical response did not necessarily correlate with the decrease in the autoantibody titer, suggesting that B cells contribute to the disease by mechanisms other than (or in addition to) autoantibody production. This, obviously, will prompt going back to the bench and trying to figure out the various roles of B cells in such diseases. Although rituximab has not been used in allergic diseases, one wonders whether it might be beneficial in those diseases that are associated with autoantibodies (eg, a subset of chronic urticaria patients). Interestingly, in the January issue of the Journal, Simon et al,¹⁵ in an open-label pilot study, used rituximab in 6 patients with atopic eczema. Using the eczema area and severity index, all patients had a significant reduction in skin symptoms. Allergen-specific IgE levels were not significantly decreased, but cutaneous B cells were reduced by 50%. Similar to the results with rheumatologic diseases, it appears that B cells might play a significant role in atopic dermatitis apart from secreting IgE, although confirmatory studies are still needed.

Vincenti¹⁶ discusses the manipulation of T-cell coreceptors in autoimmune diseases and transplantation. Processed antigen is recognized by T cells through the T-cell receptor. The result of this interaction is regulated either positively or negatively, depending on the simultaneous engagement of CD28 or cytotoxic T lymphocyte-associated antigen 4, respectively. This property has been used to develop a fusion protein (abatacept) that blocks CD28 engagement, thus resulting in silencing of T-cell activation. Abatacept proved effective in rheumatoid arthritis but was disappointing in solid organ transplantation. In a first of sorts, abatacept was rationally re-engineered to increase its affinity to CD80 and CD86 (the ligands for CD28); the new antibody is called belatacept and appears to be very promising in transplantation. Perhaps this is the first major example whereby quantitative considerations entered the field of therapeutic immunomodulation. One anticipates more developments along the concept of varying affinity/avidity of an immunomodulator in the coming decade. The importance of the degree of stimulation or blockade is illustrated by the cautionary tale of another antibody. TGN1412 was developed to bypass the T-cell receptor and directly stimulate CD28, resulting in activation of T cells; this can be useful in several diseases, including cancer. Unfortunately, this antibody turned out to be the latest example of unintended consequences because it resulted in massive activation of lymphocytes, resulting in a cytokine "storm" and landing recipients in the intensive care unit.^{17,18} One can, theoretically, anticipate that abatacept might be beneficial in atopy because it might, if given simultaneously with immunotherapy, abrogate T-cell activation, perhaps leading to tolerance.

Immunoglobulin binding to Fc receptors can lead either to activation (eg, Fc ϵ RI) or inhibition (eg, Fc γ RIIb). In a very clever approach to molecular engineering, Saxon et al¹⁹ report the use of GE2, a fusion protein that binds simultaneously to Fc ϵ RI and Fc γ RIIb, resulting in inhibition of mast cell activation. The basic

immunology lesson from this approach is that an inhibitory signal can, in some instances, override an activating signal. The translational effect of this molecule is that one can use an allergen-nonspecific approach to attenuate allergic responses. As is the case with chimeric antibodies, patients have mounted an antibody response against this fusion protein, and it remains to be seen whether this will negate GE2's therapeutic potential. A second platform developed by Saxon et al is allergen specific and relies on the linking of Fel d 1 to a portion of IgG that binds the inhibitory Fc receptor. It is hoped that this approach should be the prototype of future immunotherapy. Trials with this molecule are not far enough along to assess efficacy.

Love et al²⁰ report another innovative approach designed to get at the heart of an immune response. Synthesis and elaboration of inflammatory mediators requires DNA transcription and RNA translation. This very process is targeted by RNA interference. This is the closest that one can come to a molecular scalpel because one can target a specific sequence for inhibition. Interfering RNAs can be single stranded (called microRNA) or double stranded (called small interfering RNA). Small interfering RNA silences a single gene, whereas microRNA can silence multiple genes at once. Several such potential therapeutics have been developed and are summarized in the article by Love et al. This approach has been investigated in macular degeneration, some infections, and tumors, but it does hold great promise for allergic and immunologic diseases. One can, for example, use these molecules for selective inactivation of the synthesis of T_H2 cytokines in atopic disease or one can use this approach for the inactivation of certain chemokines necessary for the trafficking of inflammatory cells to the bronchial wall. Indeed, chemokines, by virtue of the degeneracy of their receptors (same receptor used by several chemokines and one chemokine binding to more than one receptor),²¹ are uniquely suited for such an intervention.

If all of the above strategies fail and the immune system succeeds in releasing inflammatory cytokines, then one can neutralize these cytokines or their receptors. This, indeed, is the strategy behind the use of TNF antagonists. TNF antagonists fall in 2 major classes: mAbs directed against TNF itself (infliximab and adalimumab) and soluble TNF receptors that bind TNF (etanercept). There is a difference between these 2 classes in that although either one is effective in rheumatoid arthritis, etanercept is not effective in Crohn's disease.^{22,23} Because TNF antagonists proved useful in inflammatory diseases, it was natural to try them in asthma. These studies are summarized by Brightling et al.²⁴ Initial studies were promising, although the design was far from perfect. Subsequent studies did not reveal a major therapeutic benefit in asthma and, as indicated in the review by Casale and Stokes,¹³ it appears that most clinical trials with TNF antagonists are currently on hold. This might be premature because, as indicated by Brightling et al,²⁴ there could be a subset of asthmatic patients who might benefit from TNF neutralization. This would be an example whereby an immunomodulator might force us to rethink the classification of asthma subsets based on molecular markers. It also remains to be seen whether therapeutic benefits might differ if one compares anti-TNF antibodies with etanercept.

One other immunomodulator not covered in these 2 issues of the Journal that affects the allergist/clinical immunologist is the soluble IL-1ra antagonist (anakinra). Familial cold urticaria is now firmly established to be part of the autoinflammatory syndromes. Hoffman et al²⁵ reported a dramatic response of such patients to the daily injection of anakinra.

As allergists know very well, any medication has the potential of inducing an adverse or allergic reaction. In an upcoming issue of the Journal, Patel²⁶ will address some of the adverse reactions, allergic reactions, or both to the various immunomodulators. This should bring home the message that the allergist/immunologist needs to be conversant with the new language of immunomodulation, if not as therapeutics, then as potential allergens.

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