Arraying autoimmunity treatment

Eli E Sercarz

Protein and peptide arrays that reveal antigens which induce a diverse autoantibody response can be used to guide tolerizing therapy.

© 2003 Nature Publishing Group http://www.nature.com/naturebiotechnology

Protein arrays offer great promise for profiling changes in protein levels and composition to develop new biomarkers for early diagnosis of disease and to design new treatments. But can the mass of data collected in such experiments be applied to practical, clinical use? In an exciting advance, Robinson *et al.*[1] report in this issue the use of protein microarrays to predict and design a multicomponent protective DNA vaccine against an autoimmune disease. By revealing the spectrum of antibodies raised in individual mice during an autoimmune disease, microarray analysis is exploited to predict the relevant autoantigen cDNAs for inclusion in DNA vaccines. The authors demonstrate that superior protection is afforded by vaccines which raise the largest diversity of antibodies.

In contrast to vaccines against infectious disease, which have exploited components derived from the particular microbe or a related antigen responsible for pathogenesis[2], treatments for autoimmune disease have employed pharmaceutical approaches that act nonspecifically to shut off or depress general immune reactivity. To date, not one autoimmune disease has been successfully treated using a specific vaccine. The goal of Robinson *et al.* is to specifically induce 'immune tolerance,' a procedure first introduced 50 years ago in transplantation, where it was shown that specific unresponsiveness to a graft could be instituted by presentation of cellular antigens in early neonatal life[3]. Since then, several other ways of inducing tolerance have been described both in basic and clinical systems. Typically, in those autoimmune diseases driven by proinflammatory T-cell activity (*e.g.,* multiple sclerosis, diabetes or rheumatoid arthritis), a plethora of antigens appear to be involved, making it difficult to attempt to devise a single specific tolerogenic vaccine. Because appropriate experimental tolerance-inducing regimens have often failed to be chosen, the potential of a vaccine containing numerous antigens to exacerbate (rather than ameliorate) an autoimmune condition has been an understandable concern.

The involvement of multiple antigens in autoimmune disease has been a well-studied phenomenon. Using a very limited antigen stimulus (*e.g.*, a peptide from one of the participating autoantigenic molecules) to induce disease in an animal model, the specific response to other peptides in the same antigen (intramolecular determinant spreading) or in other antigens in the vicinity (intermolecular determinant spreading) diversifies through determinant (or epitope) spreading. The early descriptions of spreading considered T-cell responses directed against newly visible determinants whose processing and immunogenicity had been enhanced by events in the freshly created inflammatory focus induced by the initiating determinant. Immune responses to infectious disease, directed against many antigens occurring as a sequel to viral or microbial attack, may be attributed either to the simultaneous and conflagrational release of proinflammatory cytokines or to sequential epitope spreading following an initiating, restricted response. In either case, the initial, external stimulus is followed by priming with neo-determinants that arise from damaged tissue. Very often, in experimental determinant-spreading experiments, there is a well-defined order of expression, with T cells engaged against a hierarchy of determinants from the same antigen or from other antigens in the neighborhood[4]. Of course, among a panel of humans, predictability is not the rule, and each individual may experience a unique pattern of diversification[5].

In the present study, the authors set out with the goal of discovering the breadth of the antibody response originating from a single initiation source. Several novel ideas were explored. First, could the scope of the broadened response be exploited to predict either an optimal set of tolerogens (antigens that induce tolerance/specific unresponsiveness) or a self-reactive vaccine? Second, even though the response observed was an antibody response, would this really relate directly to tolerance induced at the T-cell level? Third, how many antigens would have to be blended into the ideal vaccine? And finally, would DNA vaccines induce tolerance or exacerbate autoimmunity?

To start with, it was not clear that the antigenic specificities necessary to shut down autoreactive T cells could be discerned from an examination of antibody responses. In their experiments, Robinson *et al.* induced experimental autoimmune encephalomyelitis (EAE), a rodent model for multiple sclerosis, either with one of two peptides, myelin basic protein 85–99 or proteolipid protein (PLP)139–151, or with spinal cord homogenate. The animals were then followed for antibody reactivity using a panel of over 200 protein and peptide neuroantigens on arrays, together with the number of disease relapses displayed.

As expected from earlier studies on both T- and B-cell determinant spreading, they detected a variety of antibody activities directed against epitopes different from, and non-cross-reactive with, the immunizing antigen. Members of the genetically homogeneous rodent population did not show an identical spreading pattern. Interestingly, those individuals showing the most diverse B-cell response also experienced the largest average number of relapses of the T-cell disease, EAE. Although the response to the spinal cord homogenate was not as diverse as some of the peptide-induced responses, these mice experienced a generally high relapse rate, probably owing to an extensive induction of T-cell reactivity. This was suggestive of the idea that determinant spreading was associated with the seriousness of the disease process, thought to be spearheaded by the T-cell response. The test panels of peptides used to examine the specificity of the antibodies induced were broadly representative of the neuropeptide spectrum. However, rather surprising by its omission was the absence of the second most prominent member of the PLP T-cell response hierarchy, PLP(178–191). Perhaps it gave little or no B-cell response, despite its prominence

Eli E Sercarz is at the Torrey Pines Institute for Molecular Studies, 3550 General Atomics Court, San Diego, California 92121, USA .
e-mail: esercarz@tpims.org

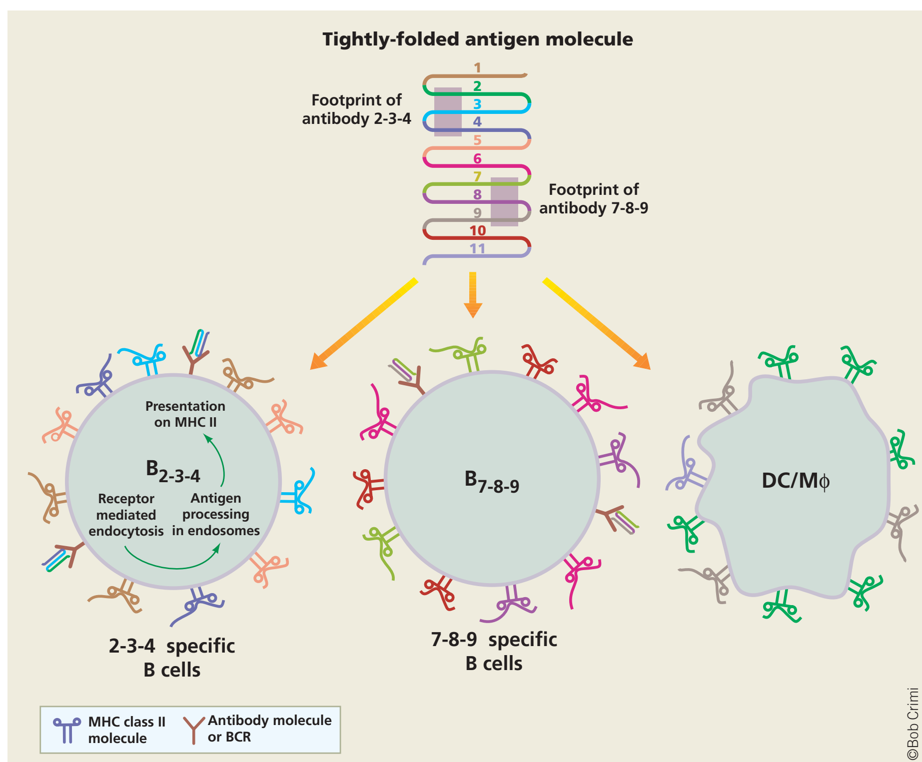

Figure 1 Presentation of linear peptides by professional antigen presenting cells. The spectrum of antigenic determinants that will be presented by unique B cells to T cells differs depending on the relationship of the bound, assembled topographic determinant to flanking regions on the bound antigen and to the types of processing enzymes that are engaged. Thus, T cells of many specificities will be able to reciprocally stimulate the same B cell. B-cell presentation from the native molecule will lead to diversification of the T-cell response. As different T cells become activated, a broader response will develop. (If a linear peptide is used as immunogen, only T cells specific for determinants within that peptide will be activated by B cells presenting that peptide-MHC). When dendritic cells or macrophages present determinants from an antigen, their array of endopeptidases, exopeptidases and reductases as well as the location of enzyme sensitive sites on the antigen will determine which determinants are the most accessible for binding to the MHC-II groove. Among the available determinants, the one with the greatest affinity for the MHC will emerge as the dominant determinant presented by that cell type, which should be generally true for all of these cells.

© 2003 Nature Publishing Group http://www.nature.com/naturebiotechnology

in the sequential orchestration following the PLP(139–151) response.

At this point, it is worthwhile to consider the contribution of antibodies and B cells to the development and pathogenicity of autoimmunity. Although antibodies to major pathogenic immunogens can be detected quite early in organ-specific autoimmune diseases where they can serve a diagnostic function, and have been shown in some instances to be directly involved in causing tissue damage[6], other experiments have demonstrated that T and not B cells can passively transfer disease, and that B-deficient animals are equivalently sensitive subjects for the induction of autoimmunity. However, B cells play a determinant-spreading function when they are wearing their antigen presentation hat. Ordinarily, the B-cell response begins by the binding of an 'assembled topographic determinant'[7] of the native macromolecule, or one of its fragments, to a B-cell receptor (BcR). The capture of a macromolecular antigen by a BcR specific for the assembled determinant 2-3-4 (see **Fig. 1**) will lead to the presentation of only some of the other determinants of that antigen[8]. Thus, a variety of determinants can be expected to be efficiently presented via this B-cell type of determinant spreading, leading to a diverse response.

Even so, when the animal is injected with a single linear peptide determinant, it will bind to some BcRs of appropriate specificity and be transferred to the antigen-presenting major histocompatibility complex (MHC) molecules in the acidic endosomal compartments of the B cell; some direct binding to MHC-II molecules on the B-cell surface will also occur. The determinant will also be presented by macrophages and dendritic cells, and specific T cells will be recruited, among them some highly proinflammatory T cells that can drive autoimmune responses (see below). Any further spreading of the response will occur via a proinflammatory mechanism dominated by T-helper (T_H)1 cells, leading to further recruitment of T cells of other specificities via cytokine engagement, enhanced antigen processing and costimulation, and the presentation of previously invisible determinants. In the experiments performed by Robinson *et al.*, the two immunogenic peptides are strong T_H1 inducers, and they would be expected to recruit a large inflammatory response by other T cells.

The choice by Robinson *et al.* of a potent determinant to induce 'driver T-cell clones'—T_H1 cells with high-affinity receptors that invade the target organ and recruit other cells[9]—is key to the initiation and propagation of an autoaggressive response. In the natural course of processing a native protein antigen, the dominant determinant will again be a matter of consequence, but it will also have a strong element of chance. The self- or foreign determinant that becomes dominantly expressed, for example, from a tightly folded protein molecule, will probably be one adjacent to the first endopeptidase cleavage of the antigen that has a reasonably strong affinity for one of the ambient MHC molecules[10]. These well-expressed determinants will generally purge the T-cell repertoire during development of all but the lowest avidity specific T cells, that should do little or no autoimmune damage. More important are those self-directed 'driver' T cells of high affinity for antigen, which have evaded negative selection and can respond to low concentrations of their specific determinants in the periphery[11]. Thus, the degree of spread is likely to be a function of the activity of driver T clones, and not merely to the overall diversity of the response.

One relevant principle that has emerged is that once a 'driver' T-cell clone (or clones) is established, it becomes the sensible choice to target in inducing tolerance. Such a driver clone usually would recruit other T_H1 clones, so controlling the driver may effectively close down the response. However, it is also likely that a dominant, small set of driver clones exists for each antigen in the target organ, so it would be an advantage to tolerize each of them. Maximum security from autoimmunity would be predicted

© 2003 Nature Publishing Group http://www.nature.com/naturebiotechnology

when the crucial drivers, as well as their major recruits (to 'spread determinants'), are all tolerized. This is the tack pursued by Robinson *et al.*, where the DNA encoding each of the antigens whose specific T cells are easily recruitable is used in a tolerance-inducing regimen to ask whether the number of disease relapses usually found in autoimmune animals can be reduced.

The last question remaining is whether DNA coding for the putatively tolerance-inducing segments of the protein antigens would actually induce tolerance, rather than exacerbating autoimmunity. Robinson *et al.* pursued the notion that the breadth of response to the initiating peptide (they tested two), after determinant spreading, would point to candidate antigenic molecules that should be employed in the attempted tolerance trial. Previous studies[12,13] had shown clearly that DNA encoding the antigen in question tended to induce deletion/anergy, or to activate regulatory helper T cells (T_H2 cells), which provide a suppressive environment for self-reactive T cells.

Robinson *et al.* attempted treatment of previously PLP(139–151)-primed mice, on day 18, with a DNA cocktail encoding several antigens to which responses had spread in their earlier array experiments. They found that this DNA cocktail treatment was successful in reducing subsequent relapses, and it was even more potent if DNA encoding the cytokine interleukin (IL)-4 were added. Treatment with DNA representing PLP(139–151) alone was a relatively poor substitute for the cocktail. Although the authors claim that the cocktail constituents were 'array determined,' the four neuroantigens chosen would have been a reasonable choice by anyone with knowledge of the EAE literature. Nevertheless, these results strongly suggest that DNA cocktail administration leads to a diversified tolerogenic presentation of the antigens in the cocktail, probably owing to the production of a T_H2 cytokine milieu, which helps to maintain the downregulated state achieved following recovery from the acute paralytic attack. Additional studies need to be undertaken to discover the most efficacious constitution of the tolerizing cocktail for different subjects and each disease condition.

By assessing the diversification from an initially narrow antibody response using a protein array, Robinson *et al.* have gained insight into the complexity of the tolerogenic DNA that would have to be used to induce and maintain a state of tolerance in each mouse. Apparently, using DNA encoding four proteins led to no obvious exacerbation of disease in the mice. Therefore, in the human situation, a sequential study of serum reactivity in patients or of T-cell reactivity *in vitro* to a large set of neuroantigenic peptides could predict the course of future tolerogenic protection studies. The approach may succeed in providing a therapeutic avenue, rather than a preventive strategy, which would fill the long-sought need for a treatment for T_H1 autoimmune diseases such as multiple sclerosis, type I diabetes and arthritis. Further mechanistic studies to elaborate the connection between determinant spreading at the antibody level, the choice of antigens and cytokines to be used in the DNA tolerization vaccine and the true extent of reduction in the self-reactive T- (or B-) cell responses should establish the generality of these exciting results, and their usefulness in devising therapy for patient populations.

1. Robinson, W.H. *et al. Nat. Biotechnol.* **21**, 1033–1039 (2003).
2. Jenner, E. *The Three Original Publications on Vaccination against Smallpox by Edward Jenner*, in *Harvard Classics*, vol. 38 (P.F. Collier & Son, New York, 1910).
3. Billingham, R.E., Brent, L. & Medawar, P.B. *Nature* **172**, 603–606 (1953).
4. Lehmann, P.V., Forsthuber, T., Miller, A. & Sercarz, E.E. *Nature* **358**, 155–157 (1992).
5. Tuohy, V.K., Yu, M., Yin, L., Kawczak, J.A. & Kinkel, R.P. *J. Exp. Med.* **189**, 1033–1042 (1999).
6. Genain, C.P., *et al. Nat. Med.* **5**, 170–175 (1999).
7. Benjamin, D.C. *et al. Annu. Rev. Immunol.* **2**, 67–101 (1984).
8. Simitsek, P.D. *et al. J. Exp. Med.* **181**, 1957–1963 (1995).
9. Sercarz, E.E. *J. Autoimmun.* **14**, 275–277 (2000).
10. Sercarz, E.E. *Nat. Immunol.* **3**, 110–112 (2002).
11. Maverakis, E. *et al. Proc. Natl. Acad. Sci. USA* **100**, 5342–5347 (2003).
12. Bot, A. *et al. J. Immunol.* **167**, 2950–2955 (2001).
13. Garren, H. *et al. Immunity* **15**, 15–22 (2001).

Two hands (or four) are better than one

Ben M Dunn & Jörg Bungert

A bacterial protease inhibitor provides a scaffold for the *in vitro* evolution of specific inhibitors of human serine proteases.

The ideal of a stable protein scaffold onto which a variable loop could be grafted to provide for specific interaction with protein targets has been a goal since the beginnings of the protein engineering discipline[1,2]. Nature has provided a number of suitable systems, but achieving the desired selectivity has been a sizable barrier. Two papers, one in a recent issue of the *Proceedings of the National Academy of Sciences USA*[3] and the other published here[4], take giant steps toward the discovery of truly selective inhibitors of peptidases. In the first report, Komiyama *et al.*[3] optimize active site interactions and randomize potential 'adventitious' contacts in eglin c from the medicinal leech, *Hirudo medicinalis*. In this issue, Stoop and Craik[4] describe a combinatorial method for achieving wide variation in four loops that contact protease targets to provide an even finer control of selectivity.

Ben M. Dunn and Jörg Bungert are in the Department of Biochemistry and Molecular Biology, University of Florida College of Medicine, P.O. Box 100245, 1600 SW Archer Road, Gainesville, Florida 32610-0245, USA. e-mail: bdunn@mail.med.ufl.edu

Protein proteinase inhibitors[5] typically provide a tightly folded protein core with at least one prominent loop that protrudes, to some degree, from the surface and that can interact with the active site of a proteolytic enzyme (see **Figs. 1a**,**b**). In most but not all cases, the loop binds through an active site cleft in a 'canonical'[6] manner, much as a substrate would. Because of subtle geometric differences, a substrate would be cleaved, whereas an inhibitor is not. In some cases, the inhibitor is slowly cleaved, but remains tightly associated with the enzyme[7]. In either case, the enzyme active site is occupied, preventing substrate processing.

Nature has provided many examples of the coevolution of peptidases and peptidase inhibitors. For a select number of these systems, we now have excellent structural details of the atomic contacts involved in the interactions between the two protein molecules. Unfortunately, for many proteases of medical importance, naturally occurring protein inhibitors have not arisen or have not been discovered. Therefore, there has been great interest, both theoretical and practical, in adapting known pro-