Commentary

How Can Cryptic Epitopes Trigger Autoimmunity?

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T cell tolerance depends on the presentation of self-proteins to T cells and therefore can only be established to those self-determinants which, under steady-state conditions, are generated in sufficient amounts to be recognized by T cells undergoing deletion in thymus or anergy in the periphery. Thus, there is large number of self-determinants that are cryptic because they are not generated at all or are generated at subthreshold levels. T cells specific for these cryptic epitopes are present in the normal repertoire and might become activated and autoaggressive if the epitopes are presented at higher concentrations. This concept, which had been originally proposed by Sercarz and colleagues, represents today the major hypothesis for the pathogenesis of autoimmune diseases (1). The fundamental question is how epitopes that are normally cryptic may become visible to the immune system and elicit a sustained pathogenetic response.

Two reports in this issue of The Journal of Experimental Medicine describe two novel mechanisms that may be responsible for revealing cryptic determinants. It is tempting to put this new information together with previous reports to try to delineate how different mechanisms may synergize for the induction, development, and maintenance of an autoimmune response.

Processing Boosted by Receptor Down-regulation

It is well established that endogenous cellular proteins are presented on class II molecules at levels that depend on their capacity to enter the processing compartment (2). Barnaba and co-workers demonstrate that down-regulation of a cell surface protein induced by an external ligand may result in increased processing and presentation of otherwise cryptic epitopes (3). These authors have isolated from HIV-infected patients DR-restricted T cell clones specific for human CD4 determinants, which have been mapped to two distinct sites using synthetic peptides. These clones recognize EBV-B cells that take up and present engineered soluble CD4 molecules, but do not recognize T cell clones that are growing in IL-2, although the latter express both CD4 and class II molecules. Thus, the epitopes defined by these clones are cryptic since they are not generated (or at least not at sufficient levels) by constitutive processing of the endogenous CD4. Strikingly however, these epitopes are readily generated by activated T cells when surface CD4 is down-regulated by HIV-gp120 or by antibodies to CD4.

How is it that some autoreactive CD4-specific T cells can escape tolerance induction in the thymus and be activated

in the periphery? The most plausible explanation is that the epitopes recognized by these T cells may not be generated in the thymus, where CD4 peptides are presented by phagocytic cells that process endogenous CD4 molecules or phagocytose dying thymocytes. The reported evidence that these epitopes are produced only by activated T cells after CD4 down-regulation suggests that both quantitative and qualitative changes in processing may be involved. A larger number of molecules will be targeted to degradation and these molecules may be delivered to a different endocytic compartment containing different sets of proteases, such as granzymes. It is also possible that ligand binding may influence processing as discussed below. As a consequence of their differential expression, these epitopes will not induce tolerance in the thymus, but it will induce a specific response in periphery when presented by activated T cells. Indeed, activated T cells are able to present antigen (4) and to prime naive T cells (5, 6).

The findings reported by Salemi et al. (3) have important implications for HIV pathogenesis because they establish an important link between the immune response to the virus and autoimmunity. In HIV-infected patients, CD4 can be efficiently down-regulated by gp120, especially if crosslinked by anti–gp120 antibodies. Presentation of cryptic CD4 epitopes on activated T cells, which function as professional APC, may result in priming of CD4-specific T cells. These, in turn, could then attack activated T cells in which CD4 has been downregulated by gp120 (or perhaps by antigenic stimulation as discussed below). Alternatively, CD4-specific T cells could help B cells to make anti CD4 antibodies, which will further boost the response by increasing CD4 down-regulation. This HIV immunopathology could be also initiated by the production of anti-CD4 antibodies induced by CD4–gp120 complexes and gp120–specific T cells by the classical mechanism of intermolecular help (7).

In addition to the potentially important role in HIV pathogenesis, the model proposed by Barnaba and colleagues may represent a general mechanism for presentation of TCR epitopes to T cells. Indeed, antigen stimulation is known to lead to massive down-regulation of TCR and coreceptors. Thus, one can expect that cryptic epitopes may be transiently presented by T and B cells after antigenic stimulation. Presentation will be facilitated by two simultaneous events that follow T cell activation: the increase in class II synthesis and the up-regulation of adhesion and costimulatory molecules (6).

It is tempting to suggest that class II–restricted presentation of cryptic epitopes of the TCR and possibly also of the

B cell receptor complex, as well as the consequent induction of regulatory T cell responses, may be more frequent than expected. The use of recently activated T or B cells as targets or stimulators may be the key to reveal these responses in vitro.

Processing Altered by Ligand Binding

It is well known that antibodies can increase the efficiency of antigen capture in antigen-specific B cells and in FcR$^+$ professional APC, a mechanism that has been implicated in the generation of cryptic epitopes and in the consequent autoimmune responses (8). More than a decade ago, Berzofsky, Celada, and co-workers suggested that antibodies may also affect antigen processing (9, 10). Watts and co-workers now demonstrate that antibodies can modulate processing of antigen so that the production of some epitopes can be increased 10–100-fold (11).

This study is the culmination of a series of experiments carried out in this laboratory during the last several years. The starting observation was that high affinity antibody does not dissociate from antigen at the mildly acidic pH of the processing compartment and, therefore, the substrate for proteases is the antigen antibody complex rather than antigen alone. The influence of antibody binding on processing has been studied biochemically by monitoring the appearance of distinct proteolytic antigenic fragments still bound to Ig (12) and functionally by analyzing the capacity of APCs to trigger T cell clones specific for different determinants (13).

Simitsek et al. (11) have used a system that makes it possible to discriminate between the effect of antibody on antigen uptake and the effect on antigen processing. They show that antibody binding can suppress the generation of some epitopes, while at the same time boosting the generation of others. Remarkably, both boosted and suppressed epitopes are present within a protein domain that is "footprinted" by the antibody, while epitopes that lie outside this domain are not affected. The mechanisms responsible for these contrasting effects are not clear, but certainly do not involve competition for MHC binding. It is possible that the antibody, by binding and stabilizing a protein domain, may influence the accessibility of the site to proteases. Trimming of an antigen fragment bound to Ig may result in the destruction of some epitopes and simultaneous increased yield of others. An alternative possibility is that antibody may facilitate transfer of some determinants to class II molecules, which are known to be able to bind large unfolded fragments.

In this study, the epitope that is boosted by antibody is not cryptic in a strict sense, since it can be generated at a lower but stimulatory levels also in the absence of the antibody. It is interesting to consider the possibility that the induction of a T cell response to this partially cryptic epitope might have followed the activation of B cells producing the boosting antibody, which can be induced by any antigen-specific T cell. This may result in a T–B circuit in which T cells preferentially interact with B cells of the boosting specificity. Thus, this mechanism has dominant and self-sustaining properties since the boosting effect of antibody on antigen processing is likely to take place, not only in B

cells, but also in professional APC that have taken up antigen– antibody complexes (13). In this way, the effect of antibody on processing can be amplified and can exert a profound effect on the selection and diversification of the T cell responses.

Although these results have been obtained using a bacterial antigen, the model is likely to be of general relevance for presentation of self-antigens as well. Indeed, positive and negative modulation of processing appear to be the inevitable consequence of high affinity binding and will influence processing of soluble as well as cellular proteins that are bound by high affinity ligands, not necessarily antibodies.

Presentation of Cryptic Epitopes and Induction of Autoimmune Responses

There are two distinct aspects that are relevant to understand the role of cryptic epitopes in autoimmune diseases: (a) the presentation of cryptic epitopes and (b) the triggering of a T cell response.

As shown in Fig. 1, the mechanisms responsible for generation of cryptic epitopes fall into three general categories. The first is increased antigen delivery to the processing compartment. This is the case when surface receptors are downregulated by antibodies or other ligands (3). In addition, an important role may be played by membrane Ig on B cells or by soluble IgG antibodies that drive antigen capture by FcR$^+$ APC. The second general category is modulation of antigen processing, which may occur when antigen is bound to antibodies or high affinity ligands (11). Cryptic epitopes might also be generated by subtle changes in the processing machinery. It will be interesting to see whether different endosomal compartments may have a qualitatively different capacity to process antigen. APCs may also express slightly different sets of proteases (14), and proteases can be regulated by exogenous stimuli such as cytokines (15, 16). The third category is an increase in class II synthesis or in expression of adhesion and costimulatory molecules. These mechanisms may enhance the yield of cryptic epitopes and may increase the T cell stimulatory capacity.
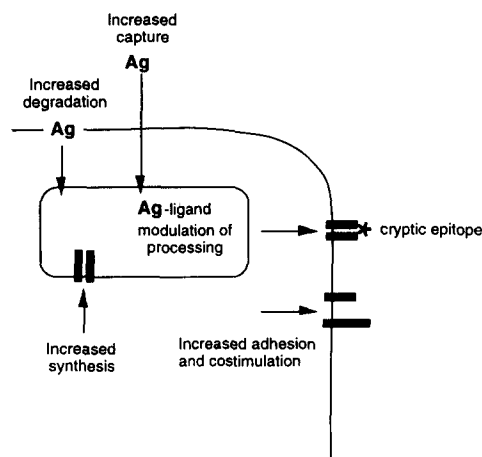

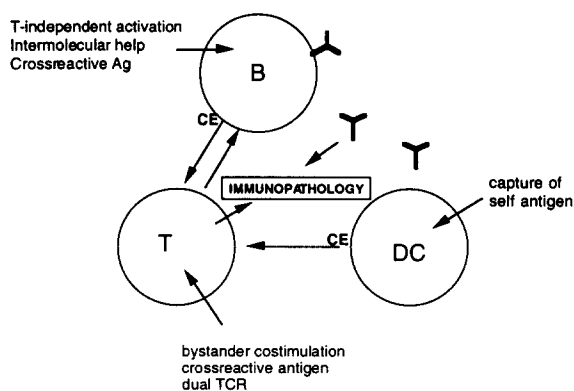Figure 1. Mechanisms that may lead to presentation of cryptic epitopes.

T-independent activation
Intermolecular help
Crossreactive Ag

IMMUNOPATHOLOGY

capture of
self antigen

bystander costimulation
crossreactive antigen
dual TCR

Figure 2. Mechanisms that may initiate and maintain an autoimmune response to cryptic epitopes (CE).

It is important to point out that all these mechanisms may act synergistically. Antibodies can increase uptake, induce down-regulation, and modify processing. T or B cell activation may induce receptor down-regulation and increased synthesis of class II and costimulatory molecules. Cytokines can increase synthesis of class II molecules and proteases. Thus, this process is catalytic in the sense that, after an initial trigger, the response may become self-sustaining through the reciprocal stimulatory effect of T cells and B cells, antibodies and cytokines, a fact that may explain the spreading of the autoimmune response (17).

If these are plausible mechanisms, what could be the initiating event that triggers this "autoimmune spiral"?

A first attractive possibility is that the induction of an autoimmune response follows the same rules as the induction of a response to foreign antigens, namely presentation on dendritic cells (Fig. 2). The function of these cells is to capture antigen at peripheral sites and migrate to T cell areas of lymphoid organs, where they trigger naive T cells (18). Growing evidence indicates that dendritic cells have specialized mechanisms for antigen capture, and that the antigen capturing

and migratory properties can be modulated by cytokines and inflammatory stimuli (19). It will be important to identify the conditions and the mechanisms that may allow dendritic cells to take up and present self antigens from dying cells.

The second possibility is that the cryptic epitopes are presented on nonprofessional APC, such as resting B cells or epithelial cells. According to the current dogma, T cells should be anergized rather than triggered (20). Nevertheless, there may be cases where T cells can be activated even if the antigen is presented on nonprofessional APC. This may occur at inflammatory sites or in the microenvironment of a lymph node where costimulation could be provided in a bystander fashion. Alternatively, T cells could be primed by recognition of a cross-reactive antigen presented by professional APC as a result of molecular mimicry (21) or of the presence of a second TCR with a different antigenic specificity (22).

The third possibility is that the initiating event is the activation of autoreactive B cells. This may well occur in the absence of T cells specific for the self antigen. For instance, autoreactive B cells could take up a self-antigen complexed with a foreign antigen and be stimulated by T cells specific for the latter (23). Alternatively, autoreactive B cells may take up a foreign antigen that cross-reacts with a self-antigen at the B cell level, but contains different T cell epitopes (8). Finally, B cells can be directly activated by highly organized self antigen (24). While in most of these cases the autoAb responses are expected to be self-limited, there is the possibility that autoreactive T cells may be primed by activated B cells that efficiently take up and present self-antigen (8), or by dendritic cells that take up self-antigen complexed to IgG antibodies.

In conclusion, independently from the mechanism that has initiated the autoimmune spiral, the unveiling of cryptic epitopes via the effect of antibody and cytokines on antigen capture or processing may result in a self-sustained immune response that is responsible for the autoimmune disease.

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