Commentary

Molecular Mimicry and T Cell–mediated Autoimmune Disease

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Both T cell tolerance and productive T cell responses require the interaction of the TCR with specific MHC–peptide ligands, triggering a cascade of signals that ultimately can lead to either proliferation or cell death (1–3). These two opposing processes, which can occur during both peripheral T cell activation and/or positive/negative selection of T cells in the thymus, seem to be tuned by two different mechanisms: either by the strength of avidity/affinity of the TCR for their ligands, or by the differential changes in the conformation or orientation of the TCR after its ligation on the MHC–peptide complexes (4, 5). Recently, the outcomes of T cell activation in periphery or of thymic selection have been related to the agonist or antagonist activity of MHC peptide ligands for the TCR; the agonist ligands, having high affinity for the TCR (slow dissociation), would promote deletion in the thymus and full activation in the periphery; the antagonist ligands, having low affinity for the TCR (rapid dissociation), would have the opposite effects (6). Analysis of the intracellular events in T cells in response to antagonist/partial agonist ligands reported distinct patterns of ζ chain phosphorylation followed by a failure to activate the ZAP-70 kinase (7, 8). Costimulatory molecules were also shown to play a crucial role in the signaling events, e.g., interactions between B7.1 or B7.2 and CD28, or CD40 and CD40L can provide costimulatory signals for T cell priming in periphery or for deletion during intrathymic T cell development (9, 10). In this context, several experimental models suggest that tolerance of self-reactive T cells can only be established to those self determinants that are generated in sufficient amounts during processing to be recognized by T cells undergoing deletion in the thymus or anergy in the periphery (11). Indeed, there is ample evidence that self-reactive T cells escaping from thymic tolerance exist in the peripheral T cell pool of healthy individuals or experimental animals (12). This may occur if the self determinants are not expressed in the thymus, e.g. if they are cryptic, because they are not generated, or they are generated at subthreshold levels for acting as T cell epitopes (11, 13, 14). T cells specific for these epitopes are present in the normal repertoire in ignorance, but might be activated and become dangerous if the cryptic epitopes are made "visible" to the immune system (13–16). A critical question is how self epitopes that are normally cryptic can influence positive selection of specific T cells in the thymus and can elicit autoreactive T cells in periphery sustaining pathogenetic responses. Different mechanisms have been proposed, particularly for explaining how cryptic epitopes can be unveiled on the one hand, and how ignorant autoreactive T cells can be awakened in periphery on the other hand (11–16).

A report in this issue of *The Journal of Experimental Medicine* strongly supports the idea that molecular mimicry could account for the activation and clonal expansion of autoreactive T cells (17). Previous studies combining single amino acid substitution analysis at TCR contact residues of a self peptide, together with the knowledge that amino acid side chains required for binding to MHC are degenerate (18), led to the identification of T cell stimulatory ligands derived from microbial proteins (19). Notably, the identified epitopes did not share sequence homologies with the autoantigen in positions other than TCR. This type of approach is, however, impaired by the large number of individual peptides necessary to define a single T cell epitope. The paper by Hemmer et al. describes the use of completely random combinatorial peptide libraries to identify multiple cross-reactive ligands for an autoreactive T cell clone specific for peptide 86–96 of the myelin basic protein (MBP). The combinatorial peptide library approach that has recently been used to define the structural basis for MHC class II–restricted peptide presentation (20) has emerged as an extremely powerful way to determine which amino acid sequence interacts with a particular protein. In the Hemmer et al. study (17), the authors have analyzed the response of the MBP-specific clone to 220 11-mer peptide sublibraries. Based on the results obtained with the peptide libraries for each amino acid position of the peptide sequence, novel peptides were found that induced proliferative response at much lower concentrations than original MBP peptide. The analysis of these sequences by protein database search led to the identification of cross-reactive peptides derived from self and microbial proteins; some of them were more potent agonists than the MBP peptide for T cell activation. From these data, the authors conclude that for at least some autoreactive T cells, antigen recognition is highly degenerate. The elegant work by Hemmer et al. is reminiscent of results of Nanda et al. (21), suggesting that some TCR, as well as polyspecific Ig, could have a multisite structure capable of being stimulated by a large panel of peptides associated with a given MHC molecule. Altogether, these evidences could shed a new light on old

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data demonstrating that virtually all antigen-specific T cells are degenerate since they are capable of recognizing different allelic forms of MHC–peptide complexes (22). The recently solved three-dimensional structure of a human TCR–MHC–peptide complex provides a structural basis for the limited specificity of the TCR (23, 24). The peptide is bound very deeply in the MHC molecule, thus providing only a few atoms of peptide residues for the contact with the TCR (24).

An interesting point discussed by Hemmer et al. (17) is that since the MBP-specific T cell clone proliferates to $\sim 2 \times 10^{11}$ different peptides resulting in assay concentrations of 5×10^{-19} g/ml for each single peptide, a concentration far below the concentration of the best hypothetical T cell epitope, it is plausible to envisage that the library contains a high number of different ligands that are stimulatory for the T cell clone. In reality, however, the degeneracy of T cell recognition may be less frequent than expected. Indeed, only a few of these sequences will be generated from natural proteins, and even fewer will be generated during antigen processing. Moreover, with regard to the self epitopes generated and presented by APCs, they seem to be no more than a few thousand (25, 26). Thus, the possibility that a degenerated TCR on a given T cell can be triggered by multiple self epitopes presented on the thymic APCs is expected to be exceptional.

A number of studies have demonstrated that although the recognition by the TCR is rather flexible in that more than one type of peptide can induce a response, the quality of the T cell response evoked by such so-called altered peptide ligands (APLs) can be very different (27). Hemmer et al. (17) only consider those peptides with agonist function resulting in cross-reactive responses by the MBP(86-96)–specific T cell clone. It is reasonable to assume that the response by the same clone could be dramatically different in response to APLs, which should be equally represented either in peptide libraries used by the Martin's group (17), or among peptides presented by APCs in vivo. Some of the APLs could induce different stimulatory functions, whereas others could switch off the T cell activation and thus influence the establishment of positive/negative selection in the thymus or of clonal activation, partial activation, or anergy in periphery.

In conclusion, the findings reported by Hemmer et al., as well as that by other laboratories (17, 19), have important implications for the pathogenesis of autoimmune disease because they establish an important link between immune responses to infectious agents and autoimmunity. With respect to the mechanisms by which the molecular mimicry at the T cell level could lead to disease, the following scenario could be envisaged. Although the number of self peptides presented by living cells is much more restricted than that shown with artificial peptide repertoires, negative or positive selection in the thymus for those T cells having a high degree of TCR degeneracy may be determined by the quality of the stimulation which results in the recognition of the different ligands presented by thymic APCs (27, 28). Thus, in addition to known parameters, such as the affinity/avidity of TCR for MHC–peptide complexes, the stability of the MHC–peptide complexes and the kinetics of TCR–MHC–peptide interaction, the degeneracy of antigen recognition by TCR seems to play a major role in influencing T cell selection. In particular, thymocytes engaging multiple agonist ligands will undergo negative selection (low off-rate interaction). In contrast, T cells triggered by a larger amount of different APLs, which could overwhelm the function of agonist peptides simultaneously present on APCs, will be positively selected, and therefore, will become part of the peripheral T cell pool (high off-rate interaction). Hemmer et al. suggest that these cells presumably would remain harmless in the periphery in case they encounter the same self peptides which positively selected the T cells in the thymus (17). It is tempting to hypothesize that if a peripheral T cell is fully activated by a given microbial epitope, the pool of cross-reactive self peptides, if acting as APLs, could switch off T cell activation. This mechanism could be advantageous for controlling autoimmunity, but disadvantageous for clearing those pathogens which have not been promptly eliminated during the early phases of the infections. Alternatively, a more adverse picture could be depicted: the T cell, activated by a microbial peptide, could also cross-react with multiple agonist self epitopes which, being cryptic, have not induced negative selection in the thymus, thus leading to priming and the establishment of autoimmune processes. This, however, should be a remote possibility in view of the different "protective" mechanisms that can take place. (*a*) Self epitopes are generally sequestered in privileged organs and are thus inaccessible to the immune system, or are cryptic because they are not generated or generated in insufficient amounts during constitutive processing by APCs. This means that they have to be unveiled, for instance, by inflammatory processes after viral infections to be efficiently processed and presented by professional APCs (14). (*b*) Cryptic epitopes may be presented by nonprofessional APCs, such as resting B cells or epithelial cells, which could tolerize the autoreactive T cells (29). (*c*) The cross-reactive self epitopes, once unveiled, will not necessarily behave as agonists, but may act either as an antagonist or as a partial agonist for TCR, leading to either death, anergy, or a change in the T cell function (27). These mechanisms could account for the limited occurrence of autoimmune phenomena despite the TCR degeneracy.

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