Low-Affinity Major Histocompatibility Complex–Binding Peptides in Type 1 Diabetes

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Type 1 diabetes is characterized by T-cell–mediated destruction of insulin-producing β-cells. The strong association between autoimmune diabetes and certain susceptible major histocompatibility complex (MHC) class II alleles suggests that T-cell activation by self-peptides presented via these MHC class II alleles plays a critical role in the disorder's pathogenesis. A diverse repertoire of T-cells is generated in the thymus, first through positive selection on MHC and self-peptide within the thymic cortex. This process requires adequate peptide presentation through interactions with MHC and sufficient T-cell receptor (TCR) signaling through the TCR/MHC/self-peptide complex (Fig. 1). As such, all T-cells in normal physiology are intrinsically self-reactive. However, subsequent negative selection of self-reactive T-cells in the thymic medulla should lead to clonal deletion for TCRs that recognize self-peptide/MHC with high affinity. Although some self-reactive, high-avidity T-cells do escape into peripheral circulation, suboptimal recognition of MHC/self-peptide by the TCRs may be required for T-cells to escape tolerance mechanisms. This idea is supported by experimental observations that the affinity of TCRs for MHC/self-peptide is generally lower than that for MHC/foreign peptide and that the interactions of autoreactive TCRs to MHC/self-peptide appear to be less extensive than to foreign peptide (1,2). In light of the opposing mechanisms of positive and negative selection, fundamental questions remain regarding the affinity of TCR/MHC/self-peptide interactions that give rise to autoreactive T-cell responses.

There is increasing evidence that insulin may be the primary autoantigen in the nonobese diabetic (NOD) mouse model (3). Several studies have emphasized the insulin B9–23 epitope. This peptide binds to I-A^{g7}, the MHC class II molecule in NOD mice, with moderate affinity (4). Wegmann et al. (5) reported that the majority of CD4+ T-cells isolated from pancreatic islets of NOD mice recognized this epitope. Furthermore, an amino acid substitution within this peptide has also been shown to abrogate diabetes development in a transgenic mouse line expressing this modified insulin transgene, implying an important role for this epitope in disease development (6). In humans, HLA-DR0401-DQ0302 is a major disease susceptibility haplotype. Considerable effort has been devoted to identifying the relevant T-cell epitopes in human type 1 diabetes because this knowledge is essential for the study of autoreactive T-cells. In addition to insulin, multiple autoantigens have been reported, and many class II epitopes within each antigen have been identified (7,8). These antigenic peptides have generally demonstrated moderate-to-high affinities when tested for their MHC binding. Thus, there has been no compelling evidence that autoreactive T-cells recognize unusually low-affinity peptides. However, there is also no conclusive evidence that T-cells recognizing these moderate- and high-affinity peptides are the primary pathogenic cells in humans with type 1 diabetes.

In this issue of *Diabetes*, Levisetti et al. (9) report their study of the proinsulin 1 47–64 (PI-$1_{47–64}$) epitope in NOD mice. This peptide is located in the C-peptide region and has been shown to be naturally processed and presented (10). In contrast to insulin B9–23, $PI_{47–64}$ binds to I-A^{g7} with a very weak affinity (~30-fold lower). In spite of this, PI-$1_{47–64}$–specific T-cell lines could be isolated from NOD mice and expanded in vitro. More notably, these cells induced diabetes when transferred into NOD.scid recipient mice with an incidence rate of 50%. These results demonstrate that high-affinity peptide binding to MHC is not required to elicit a pathogenic T-cell response, which, in turn, raises the possibility that low-affinity peptides, previously ignored in some methodologies, could also play an important role in type 1 diabetes. It might be expected that these low-affinity peptides provide a means for escap-

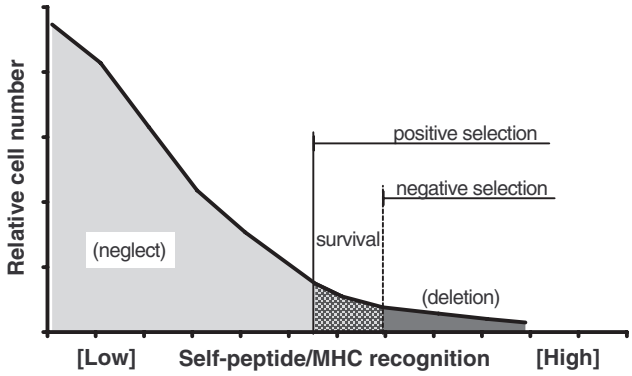

FIG. 1. The T-cell repertoire is determined by positive and negative selection. T-cells with sufficient recognition of self-peptide/MHC are preserved through positive selection (indicated by solid vertical boundary line); remaining T-cells in the thymic cortex die by neglect (light-gray shaded region). Positively selected T-cells migrate to the thymic medulla, where T-cells with excessive recognition of self-peptide/MHC are deleted through negative selection (indicated by dashed vertical boundary line). T-cells with intermediate recognition of self-peptide/MHC survive (checkered region) and migrate to the periphery.

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ing central tolerance. This would agree with previous studies in which the unstable myelin basic protein 1–11/I-A^u complexes are targets for autoreactive T-cells in the experimental autoimmune encephalomyelitis mouse model (11).

The study by Levisetti et al. persuasively demonstrates that PI-$1_{47–64}$–responsive T-cells can independently cause autoimmune diabetes. It is also significant that these cells were observed in pre-diabetic mice. The authors assert that this implies a role for these cells in early pathogenic processes. However, only 5 of 246 T-cell lines recovered following two cycles of peptide stimulation were specific for this peptide. Therefore, it is possible that T-cells that target this unstable MHC/peptide complex represent only a very small percentage of the pathogenic T-cell repertoire and, consequently, play a minor role in the autoimmune process underlying type 1 diabetes in this model. It is interesting, however, given the low affinity of $PI_{47–64}$ for I-A^{g7}, that high-avidity T-cell lines were isolated from immunized mice (i.e., half-maximal stimulation was achieved at submicromolar concentrations of peptide). It has been reported that both the I-A^{g7} and DQ0302 molecules form unstable MHC dimers (12,13). In contrast, the protective DQ0602 allele forms stable dimers (14). Since peptide binding is essential for stability, weak peptide MHC complexes (such as I-A^{g7} and PI-$1_{47–64}$) could provide a means for high-avidity T-cells to escape tolerance mechanisms, particularly for unstable alleles. In fact, it is plausible that low-affinity peptides may actually select for higher-avidity T-cells than high-affinity peptides.

Despite the strengths of their study, Levisetti et al. leave some questions unanswered. For example, they do not address the significance of PI-$1_{47–64}$–specific T-cells in spontaneous disease. It is possible that the presentations of low- and high-affinity self-peptides by MHC play different roles at varying times during disease progression. For example, low-affinity peptides could be prominent early in the disease process, whereas higher-affinity peptides become more important in later stages. Alternatively, it is possible that low-affinity peptides become more important later in the disease process as autoimmune damage and metabolic stress lead to increased or altered antigen presentation and costimulation in the inflamed tissue. It may be crucial to assess the role of regulatory T-cells in controlling PI-$1_{47–64}$–specific and polyclonal responses. It is possible that low-affinity peptides such as PI-$1_{47–64}$ are incapable of generating regulatory T-cells, whereas high-affinity peptides are involved in activated regulatory T-cells. Alternatively, low-affinity peptides might generate T-cells that are less susceptible to regulation. In any case, it will be wise for future studies to consider the potential role of low-affinity peptides in the autoimmune process.

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