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SYMPOSIUM

The Use of Peptide–Major-Histocompatibility-Complex Multimers in Type 1 Diabetes Mellitus

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Abstract

Major histocompatibility complex (MHC) class I and MHC class II molecules present short peptides that are derived from endogenous and exogenous proteins, respectively, to cognate T-cell receptors (TCRs) on the surface of T cells. The exquisite specificity with which T cells recognize particular peptide–major-histocompatibility-complex (pMHC) combinations has permitted development of soluble pMHC multimers that bind exclusively to selected T-cell populations. Because the pathogenesis of type 1 diabetes mellitus (T1DM) is driven largely by islet-reactive T-cell activity that causes β-cell death, these reagents are useful tools for studying and, potentially, for treating this disease. When coupled to fluorophores or paramagnetic nanoparticles, pMHC multimers have been used to visualize the expansion and islet invasion of T-cell effectors during diabetogenesis. Administration of pMHC multimers to mice has been shown to modulate T-cell responses by signaling through the TCR or by delivering a toxic moiety that deletes the targeted T cell. In the nonobese diabetic mouse model of T1DM, a pMHC-I tetramer coupled to a potent ribosome-inactivating toxin caused long-term elimination of a specific diabetogenic cluster of differentiation 8+ T-cell population from the pancreatic islets and delayed the onset of diabetes. This review will provide an overview of the development and use of pMHC multimers, particularly in T1DM, and describe the therapeutic promise these reagents have as an antigen-specific means of ameliorating deleterious T-cell responses in this autoimmune disease.

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Abbreviations: (APC) antigen-presenting cell, (CD) cluster of differentiation, (CTL) cytotoxic T lymphocyte, (GAD65) glutamic acid decarboxylase 65 kD isoform, (HA) hemagglutinin, (IFN-γ) interferon gamma, (IGRP) islet-specific glucose-6-phosphatase catalytic subunit-related protein, (IL) interleukin, (MAB) monoclonal antibody, (MHC) major histocompatibility complex, (NOD) nonobese diabetic, (NRP) NOD-related peptide, (pMHC) peptide–major-histocompatibility-complex, (SAP) saporin, (TCR) T-cell receptor, (Tg) transgenic, (T_H) T-helper, (T1DM) type 1 diabetes mellitus

Keywords: antigen-specific T cells, autoimmunity, immunomodulation, major histocompatibility complex, multimers, type 1 diabetes mellitus

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Introduction

Type 1 diabetes mellitus (T1DM) is a chronic disease that is caused by the irreversible destruction of pancreatic beta (β) cells by the immune system, leading to a loss of insulin production and dysregulation of carbohydrate, lipid, and protein metabolism. While insulin administration can allow a relatively normal lifestyle, maintaining euglycemia requires intensive management, and despite treatment, a substantial fraction of T1DM patients ultimately develop complications, such as hypoglycemic episodes and vascular disease.[1,2] Ideally, destroyed β cells could be replaced by transplantation, but this strategy is limited by lack of readily available donor tissue, as well as the need for potentially life-long immunosuppressive therapy to prevent rejection by allo- and islet-reactive responses and, thus, is not yet a cure for T1DM.[3,4] Clearly, novel treatment approaches that can rescue and protect remaining β cells from destruction early in the disease process are critically needed.

While the inciting cause of T1DM is unknown, the principal effectors are lymphocytes, mainly autoreactive T cells—that is, those that recognize "self." To prevent autoimmunity, such T cells normally are eliminated from the body during their development or rendered quiescent by tolerance mechanisms. In T1DM, however, these T cells are inappropriately activated, leading to islet destruction. A rational treatment strategy, therefore, would be to halt autoreactive T-cell responses at the diagnosis of T1DM, when as much as 30% of β cell mass may still remain, or, with long-standing T1DM, in preparation for islet transplantation. A notable example of an anti-T-cell therapy is the administration of monoclonal antibodies (MABs) that bind to cluster of differentiation (CD)-3, which is a component of the surface T-cell receptor (TCR). In nonobese diabetic (NOD) mice, the most widely used spontaneous model of T1DM, injection of an anti-CD-3 MAB induced apoptosis of activated T cells, durable remission of overt diabetes, and long-term acceptance of syngeneic islet grafts.[5] In humans with recent-onset T1DM, single treatment courses with anti-CD-3 MABs slow—but do not prevent—the loss of β-cell function for periods greater than 1 year.[6] While these clinical results are encouraging, adverse effects, such as viral reactivation, also can occur as a consequence of disabling protective T-cell responses.[7] Hence, an immunodulatory therapy that selectively targets β-cell-destructive T cells would be ideal but has proved elusive.

Fortunately, specific T cells may be discriminated from their peers by the antigen that they recognize, a characteristic that can be exploited to both monitor and manipulate diabetogenic T-cell populations. What constitutes this antigen? For classical T cells, such as those involved in the pathogenesis of T1DM, TCRs are triggered by short peptides that are displayed ("presented") in the binding groove of major histocompatibility complex (MHC) molecules on the surface of cells with which the T cells are interacting. Specifically, CD-4+ T cells generally respond to peptides that are derived from phagocytosed proteins and presented by MHC class II molecules, while CD-8+ T cells respond to peptides that are derived from cytosolic proteins and presented by MHC class I molecules. It should be appreciated that, within a very large population of naïve T cells—for example, all those contained within an individual mouse or human—there are millions of different specificities, with most T cells responding exclusively to a single antigen (so-called "cognate" antigen); therefore, a T cell of any given specificity is exceedingly rare.

Not surprisingly, a great deal of effort has been spent to identify the peptides that are recognized by islet-infiltrating autoreactive T cells, and these appear largely to be derived from β-cell-specific proteins. Importantly, this work has yielded a catalog of peptides that are recognized *across* NOD mice or human patients—a crucial discovery—as dominant, shared T-cell responses are thought to be the significant forces driving T1DM pathogenesis and therefore constitute the most appropriate targets for manipulation. Some examples of these peptides are listed in **Table 1**. With this information in hand, as well as the ability to generate soluble MHC molecules—either by affinity purification or recombinant techniques—it has become possible to produce reagents that can differentiate rare, islet-specific T cells in complex polyclonal mixtures of lymphocytes. Because the binding affinity of a single peptide–major-histocompatibility-complex (pMHC) complex for its corresponding TCR is weak, in the micromolar range, a common characteristic of these reagents is their assembly as multimers of identical pMHC units, which confers much higher avidity (nanomolar) for the cognate T cell. Such multimers can be constructed from MHC-I molecules to target CD-8+ T cells and from MHC-II molecules to target CD-4+ T cells. This article will review the use of pMHC multimers to measure or

Table 1.
Selected Islet-Specific Peptides Recognized by CD-4+ and CD-8+ T Cells in Mice and Humans[a]

Species	MHC	Peptide designation	Amino acid sequence	Antigen
Mouse	Class I	$IGRP_{206–214}$	VYLKTNVFL	IGRP
	Class I	$InsB_{15–23}$	LYLVCGERG	Insulin1/2
	Class II	$GAD65_{206–220}$	TYEIAPVFVLLEYVT	GAD65
	Class II	$IA\text{-}2\beta_{755–777}$	QREENAPKNRSLAVLTYDHASRI	IA-2
Human	Class I	$InsB_{10–18}$	HLVEALYLV	Insulin
	Class I	$InsB_{25}–C_1$	FYTPKTRRE	Insulin
	Class II	$GAD65_{339–352}$	TVYGAFDPLLAVAD	GAD65
	Class II	$IA\text{-}2_{831–850}$	LYHVYEVNLVSEHIWCEDFL	IA-2

[a] IA-2, insulinoma-associated protein 2.

modulate antigen-specific T-cell responses *in vitro* and *in vivo*, focusing on how these novel reagents have been used to study, and alter, the pathogenesis of T1DM.

T Cells in Type 1 Diabetes Mellitus

While multiple immune cell populations are involved in the development of T1DM (reviewed elsewhere[8]), T cells are indispensable for progression to overt diabetes.[9–12] Other cell types infiltrating the islets—B cells, dendritic cells, and macrophages—serve as antigen-presenting cells (APCs), providing a combination of stimuli capable of activating autoreactive CD-4+ and CD-8+ T cells: antigen (pMHC; so-called signal one), co-stimulation (signal two), and an inflammatory cytokine microenvironment (signal three). Initially, this set of signals is delivered in the pancreatic lymph nodes, "priming" the naïve T cell for action; later, these stimuli are also provided in the islets (**Figure 1**), amplifying the responses of previously activated T cells. Signals inhibitory to T-cell activation may be given at the same time by certain APCs or T-regulatory cells, but in T1DM-susceptible individuals, these "braking forces" usually are overwhelmed by positive stimuli, resulting in clinical autoimmunity.

Activated CD-4+ T cells characteristically function as T-helper (T_H) cells, secreting specific cytokines that direct the immune response. In T1DM, islet-reactive T_H cells—primarily those that produce interferon gamma (IFN-γ) and interleukin (IL)-2—infiltrate the endocrine pancreas, where they interact with APCs via MHC-II. This interaction results in cytokine production that supports CD-8+ T-cell proliferation and reinforces T_H pro-inflammatory activity via a positive feedback loop (IFN-γ induces IL-12 secretion from APCs, which, in turn, promotes IFN-γ production by T_H cells). Therefore, T_H cells mainly help to stimulate and perpetuate autoreactive T-cell responses in the islets during diabetogenesis, although, in later stages of the disease, CD-4+ T cells have also been shown to exert direct cytotoxic activity.[13]

Primed CD-8+ T cells—generally referred to as cytotoxic T lymphocytes (CTLs)—enter and are retained within insulitis lesions in significant numbers only if they are helped by CD-4+ T cells and recognize islet antigens.[10,14] After infiltration, the TCR of the autoreactive CTL binds to its corresponding pMHC on β cells, creating an "immunologic synapse" (**Figures 1B and 1C**). The formation and stabilization of multiple immunological synapses triggers the CTL to release IFN-γ, perforin, and granzyme B, ultimately killing the β cell. Some diabetogenic CTL also express Fas ligand, which turns on the Fas-mediated apoptosis pathway within β cells after the synapse has formed.[15] Through these mechanisms, islet-specific CTLs mediate destruction of native β cells[16] as well as transplanted islet tissues.[12,17]

It seems clear that both subsets of T cells are involved in the pathogenesis of T1DM in largely nonoverlapping roles: T_H cells help prime and potentiate CTLs, which kill β cells. Which one of these populations would be best to disable in order to halt or reverse diabetes is likely an empirical question, and it should not be surprising if the most effective treatment ultimately targets both helper and cytotoxic T cells.

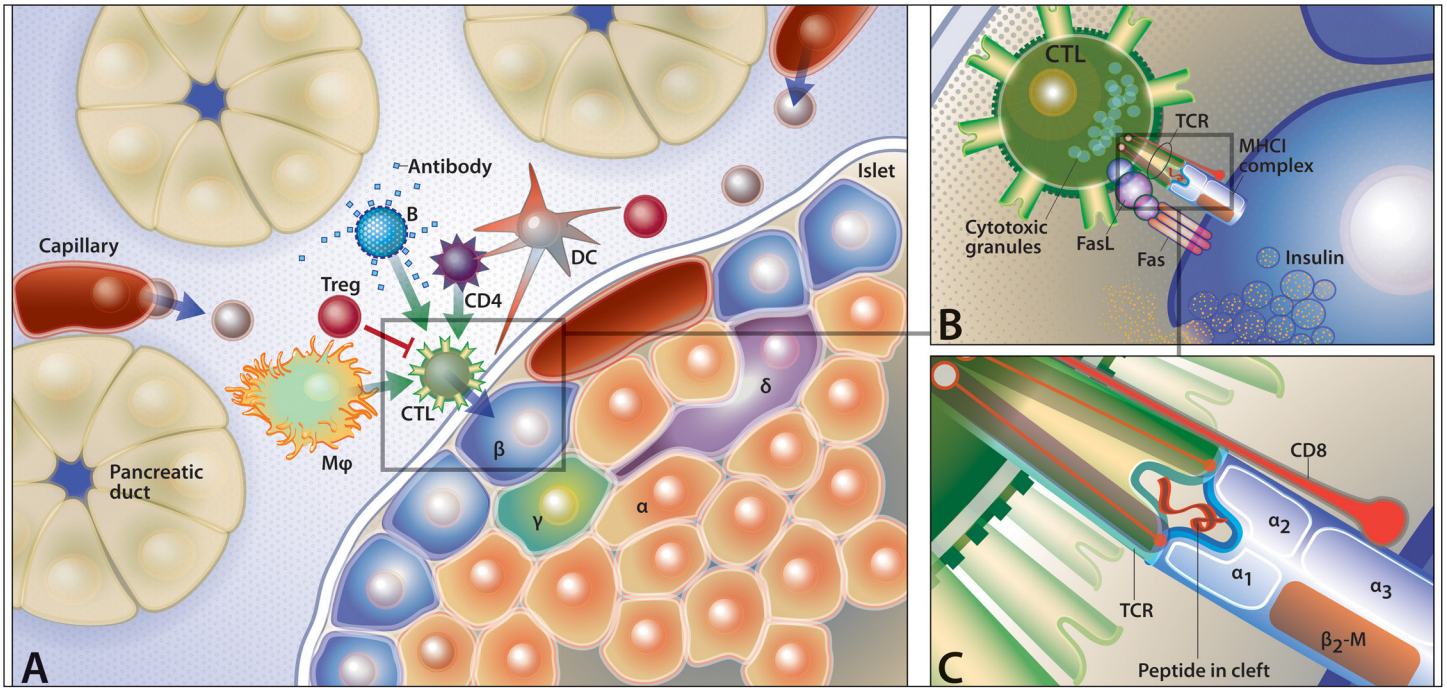

Figure 1. Immune cell interactions in T1DM. The pancreatic islets contain insulin-producing β cells, which are the targets of autoreactive T cells. Antigen-presenting cells (macrophages, dendritic cells, and β cells) stimulate diabetogenic CD-4+ and CD-8+ T cells, which infiltrate the islets and generate an inflammatory microenvironment in which β cells are destroyed. The inhibitory signals (red bar) given by T-regulatory cells to CTLs typically are overwhelmed by positive stimuli (green arrows). (**Zoom 1**) T-cell receptors of diabetogenic CTL bind to pMHC-I complexes on the surface of β cells, creating an immunological synapse, which triggers the release of cytotoxic granules and increased expression of Fas ligand, inducing apoptosis of target cells. (**Zoom 2**) The antigen that drives the proliferation and cytotoxic function of CTL through TCR signaling is a combination of a particular peptide and proper MHC-1 molecule, and it is this specific interaction that forms the basis of class I multimer technology. In analogous fashion, the TCR of CD-4+ T cells binds to surface MHC-II molecules, which are α and β chain heterodimers that present slightly longer peptides, 12–20 amino acids in length (not pictured). In both instances, the CD-4 and CD-8 co-receptors also bind to their respective MHC molecules, supplying added stability to these trimolecular interactions. Mφ, macrophages; DC, dendritic cells; Treg, T-regulatory cells.

Use of Peptide–Major-Histocompatibility-Complex Multimers

Presently, one of the most commonly used forms of pMHC multimer is as a tetramer, prepared by reacting biotinylated pMHC molecules with streptavidin, usually coupled to a fluorophore (**Figure 2A**). In a manner identical to the more familiar technique of labeling cells with a fluorescent MAB, tetramer "staining" can be used to identify or sort antigen-specific T cells via flow cytometry. This technology has excellent resolving power; for example, when used in sequential cell separation steps, tetramers can discriminate extremely rare T cells at frequencies less than 10^{-5}.[18,19] Less conventionally, pMHC multimers are sometimes used as soluble ligands for their cognate TCR, providing signal one stimulation alone, which, depending on a number of factors, can alternately result in full priming, partial activation, unresponsiveness, or apoptosis of the T cell.[20–23] Such effects are readily demonstrated with cultured T cells and can be observed after parenteral administration of pMHC tetramers. Additionally, pMHC multimers can be used to deliver a toxic moiety to a specific T cell *in vitro* or *in vivo*, resulting in rapid deletion.[24–27] Killing of a CTL by a pMHC-I toxic tetramer is depicted in **Figure 2B**. These reagents have several notable advantages over conventional MAB-based immunotoxins. First, many different antigenic specificities can be generated simply by "swapping out" different peptides while using the same MHC-heavy chain.[28] Secondly, because the pMHC is the actual TCR ligand, repeated use of such an immunotoxin cannot select for pathogenic T cells that resist multimer binding but remain capable of recognizing their targets. On the other hand, a possible unwelcome effect of pMHC multimer administration is T-cell stimulation, which could exacerbate autoimmune responses. However, this effect appears largely attributable to the transfer of peptides from multimers to cell surface MHC molecules and can be minimized by covalent linkage of the peptide to the pMHC complex.[22,29] After TCR engagement, T cells may also become transiently refractory to pMHC binding, requiring proper delay between multimer doses.[30] Lastly,

another limitation of multimers in their present form is immunogenicity; that is, antimultimer antibodies may form after repeated administration (our unpublished data), which could lead to hypersensitivity reactions or neutralization of the agent.

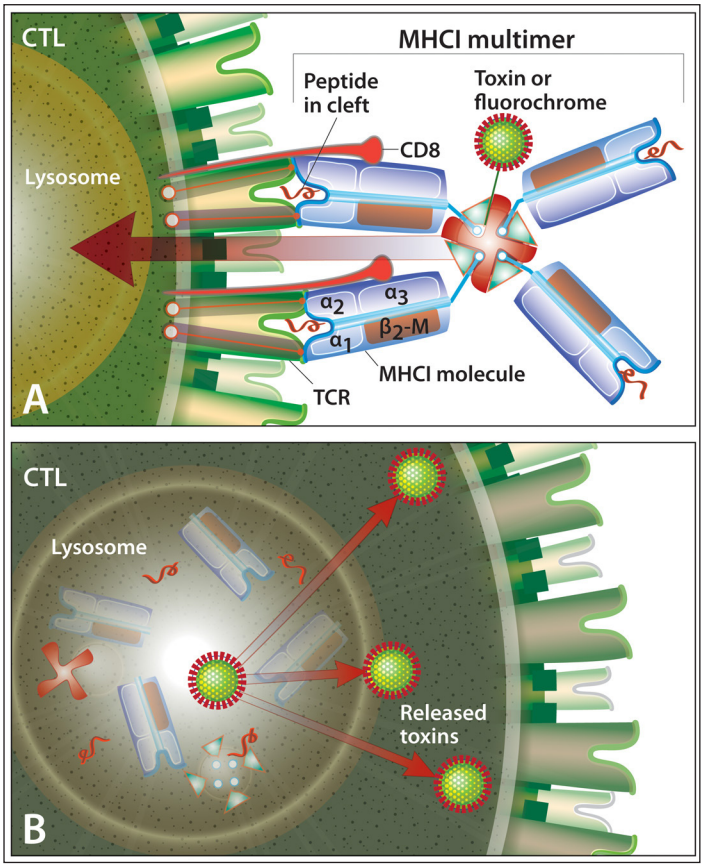

Figure 2. Multimers of pMHC molecules are soluble reagents capable of binding cognate T cells. By coupling to fluorophores or toxins, these multimers can quantify or delete antigen-specific T-cell populations, respectively. **(A)** Four identical biotinylated pMHC-I molecules are linked to streptavidin to form a tetramer. By binding to two or more TCRs simultaneously, multimer avidity for the T cell is increased sufficiently to permit practical use of these reagents. After binding to the TCR, pMHC-I multimers are internalized by CD-8+ T cells. **(B)** The toxin-streptavidin linkage is cleaved during lysosomal degradation of the tetramer, allowing the free toxin to enter the cytosol, killing the CTL.

Peptide–Major-Histocompatibility-Complex Multimers in Type 1 Diabetes

Because the pathogenesis of T1DM is marked by the antigen-driven expansion of islet-reactive T cells, as well as their infiltration into the endocrine pancreas, pMHC multimers can serve as a useful tool to visualize the steps of this process. In NOD mice, Trudeau and colleagues[31] reported that, by using pMHC-I tetramers prepared with a dominant islet peptide [NOD-related peptide (NRP), a synthetic analog of islet-specific glucose-6-phosphatase catalytic subunit-related protein $(IGRP)_{206–214}$], the cognate diabetogenic CTL could be detected in blood before hyperglycemia began, and their peripheral frequency correlated strongly with the degree of insulitis, allowing the onset of diabetes to be predicted in individual mice. Similarly, in humans, a sensitive panel of pMHC-I multimers specific for six β-cell-derived peptides found circulating CTL frequently in new-onset diabetes patients but rarely in healthy control subjects.[32] An elegant study reported the discovery of four novel preproinsulin peptides that are recognized by T cells using a repertoire of human MHC-I multimers generated by peptide "swapping" technology; impressively, the presence or absence of circulating, multimer-positive CTL was able to predict clinical outcome (insulin independence) in seven of eight islet graft recipients.[33] Multimers of pMHC-I molecules have also been used to investigate the trafficking of T cells into the islets of NOD mice. For example, TCR-transgenic (Tg) CD-8+ T cells labeled *ex vivo* with NRP–MHC-I-coated magnetic nanoparticles could be observed entering the pancreas in real time by high-resolution magnetic resonance imaging.[34] In a follow-up study, direct injection of NRP–MHC-I nanoparticles resulted in signal accumulation in the pancreas that correlated with the number of infiltrating specific T cells,[35] suggesting that it may be eventually possible to noninvasively detect insulitis in prediabetic, at risk individuals. With the development of a wider array of antigen specificities, pMHC multimers may ultimately pinpoint the critical effector population(s) during T1DM progression and subsequently be used as therapeutic tools to dampen or eliminate this pathogenic activity.

Cluster of Differentiation-4+ T Cells and Peptide–Major-Histocompatibility-Complex-II Multimers

Liu and associates[36] first described the use of pMHC-II multimers to detect CD-4+ T cells reactive to islet autoantigens. Using tetramers constructed from a murine MHC-II allele, $I\text{-}A^{g7}$, T cells specific for glutamic acid decarboxylase 65 kD isoform (GAD65)-derived peptides were identified in the lymph nodes and spleen of NOD mice. Similarly, Reijonen and coworkers[37] found circulating GAD65-reactive CD-4+ T cells in human T1DM patients.

Given the importance of CD-4+ T cells to T1DM pathogenesis, it is not unexpected that investigators have evaluated pMHC-II multimers as immunomodulatory agents in this disease. Casares and colleagues[38] created a double-Tg model of autoimmune diabetes by

crossing mice whose β cells expressed influenza virus hemagglutinin (HA) with mice whose CD-4+ T cells exclusively recognized the $HA_{110–120}$ peptide presented by the MHC-II allele, I-E^d; these mice typically developed diabetes within 10 weeks of age. Administration of an $HA_{110–120}$-I-E^d dimer induced anergy (hyporesponsiveness) of cognate T cells in the spleen and, in the pancreas, generated a population of T regulatory cells that secreted the immunosuppressive cytokine, IL-10. This immune deviation was associated with diabetes prevention when dimers were administered to prediabetic mice and with a restoration of euglycemia when given to animals with new-onset diabetes. Unfortunately, frequent injections—every 4 to 5 days—were required to maintain this state of self-tolerance. In the same model, it was possible to double this period of disease protection and reversal by using an octameric form of $HA_{110–120}$-I-E^d, which caused activation-induced cell death of cognate T cells.[39]

The ability of a pMHC-II multimer to alter the course of T1DM was also evaluated in a different model, in which diabetes is induced by adoptive transfer of TCR–Tg (BDC2.5) CD-4+ T cells into T-cell-deficient NOD recipients.[40] Weekly administration of the cognate pMHC-II multimer protected recipients from T1DM. This effect was mediated by the generation of IL-10-secreting BDC2.5 T cells, as tolerance was nullified by injection of an anti-IL-10 receptor-blocking antibody. Unfortunately, administration of the protective pMHC-II dimer to wild-type NOD mice neither prevented nor reversed T1DM, suggesting perhaps the need to target other specificities in the complex natural disease setting. Indeed, a short course of pMHC-II dimers containing covalently linked GAD65-derived peptides injected into late-stage, preclinical NOD mice blocked insulitis progression and diabetes by inducing the development of an IL-10-secreting, islet-specific T-regulatory population.[41]

Deletion of specific CD-4+ T cells *in vitro* and *in vivo* by affinity-purified or recombinant pMHC-II multimers coupled to toxins, most notably doxorubicin, has been demonstrated,[26,27] but the effects onT1DM or other autoimmune diseases have not been investigated to date.

Cluster of Differentiation-8+ T Cells and Peptide–Major-Histocompatibility-Complex Multimers

Work on class I multimers has paralleled the development of their class II counterparts. Garboczi and coworkers[42] were the first to create recombinant human pMHC-I molecules. Subsequently, tetramers of pMHC-I molecules were shown to be capable of detecting rare virus-reactive CTLs *ex vivo*, giving immunologists the quintessential tool for studying antigen-specific CD-8+ T-cell population dynamics.[43] For example, Wong and associates[44] employed a diabetogenic CD-8+ T cell clone isolated from NOD islets to screen a pancreatic complementary deoxyribonucleic acid library, ultimately identifying $InsB_{15–23}$ (**Table 1**) as the relevant peptide. Using a pMHC tetramer prepared with this peptide, they subsequently determined that $InsB_{15–23}$-reactive CTL dominated insulitis lesions at 4 weeks of age, comprising >80% of T cells, with a subsequent decline to <20% by 9 weeks. Since that work, other investigators have used pMHC-I tetramers of several different specificities simultaneously to probe the CD-8+ T-cell composition of NOD mouse islets, finding unique patterns of CTL reactivity in individual age-matched mice.[45] Similar observations have been made for human T1DM patients.[46] The lack of a dominant reactivity implies the idiosyncratic recruitment of T-cell effectors by the underlying forces that drive T1DM, which potentially could mandate identification and individualized targeting of pathogenic T cells, an onerous requirement for therapy development. On the other hand, other studies have found that CTL reactive to IGRP accumulate substantially in NOD islets with increasing age.[47] Interestingly, by using tetramers to measure the strength of TCR–NRP–K^d binding, these investigators have found that the avidity of this T-cell population for its cognate ligand increases over time. When this "avidity maturation" was slowed down by deletion of only those T cells bearing the highest affinity TCRs, progression to diabetes was halted, although insulitis lesions remained similar. These data suggest that the preservation of low avidity, β-cell-reactive CTL in the islets may have a beneficial effect, a notion confirmed in a follow-up study.[48] Together, these results imply that, even in heterogeneous pools of infiltrating T cells, targeting one or, at most, a few of the highest avidity, most pathogenic T-cell populations may be sufficient to arrest disease progression. Moreover, all of these studies illustrate the power of pMHC multimers in dissecting, in fine detail, the changes in CD-8+ T cell populations that occur in T1DM.

Like class II multimers, pMHC-I multimers mimic the natural ligands for their corresponding TCRs, and accordingly, several groups have investigated whether these reagents could alter the behavior of cognate CD-8+ T cells. For example, in an alloantigen model, exposure of TCR–Tg CD-8+ T cells to pMHC dimer-suppressed cytolytic activity *in vitro* and *in vivo*.[49] In a different alloantigen (H–Y) system, repeated injections of a pMHC tetramer into naïve recipient mice permitted prolonged survival of minor histocompatibility-mismatched skin

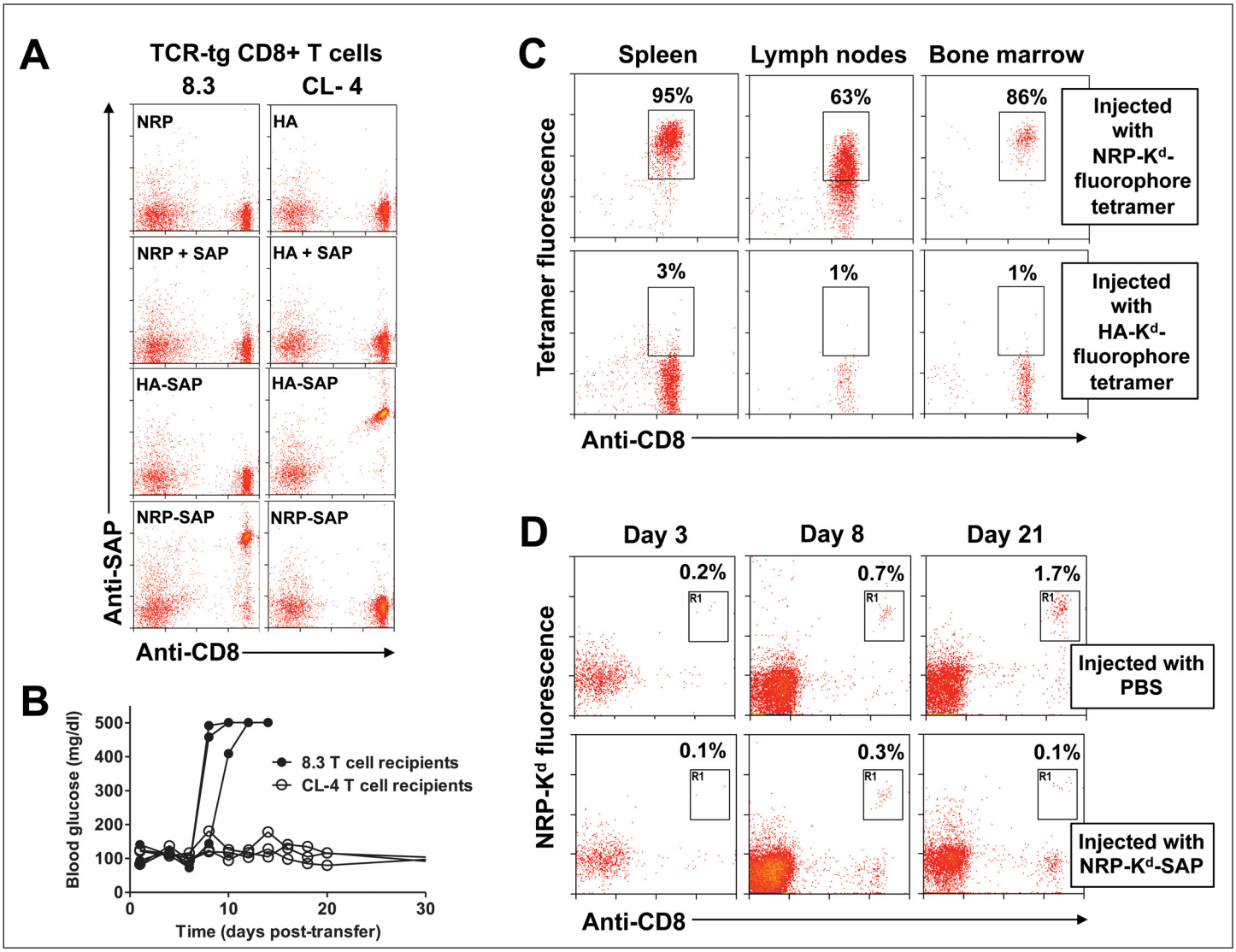

Figure 3. Nonribosomal peptide–K^d tetramers target diabetogenic CD-8+ T cells *in vitro* and *in vivo* in a model system. (**A**) Peptide–K^d tetramers conjugated to the toxin SAP retain their binding specificity for cognate T cells. Peripheral blood lymphocytes from TCR–Tg 8.3 (which recognize $IGRP_{206–214}$ and NRP peptides) and CL4 (which recognize a peptide from influenza HA) mice were incubated with the indicated tetramers and subsequently probed with a fluorophore-labeled anti-SAP Ab. Free SAP ("+ SAP") mixed with tetramers did not bind to CD-8+ T cells. (**B**) Adoptive transfer of 8.3 T cells rapidly causes diabetes in NOD.*scid* (T-cell-deficient) mice. As expected, control CL-4 T cells are not diabetogenic. (**C**) NRP–K^d tetramers can access cognate T cells *in vivo*. NOD.*scid* recipients of 8.3 T cells were injected intravenously with fluorophore-coupled NRP– K^d (top panels) or HA–K^d (bottom panels) tetramers, and the indicated tissues were harvested 4 h later. Sufficient residual islet tissues were not available for analysis from these diabetic mice. (**D**) A single treatment with NRP–K^d–SAP prevents expansion of adoptively transferred diabetogenic T cells in NOD.*scid* mice. Dot plots show a lack of expansion of 8.3 T cells in the blood of mice injected with the toxic NRP–K^d tetramer three days after receiving purified T cells. The mouse treated with phosphate-buffered saline (top panels) was diabetic by day 21; the mouse treated with NRP–K^d–SAP remained euglycemic at the end of the study, >60 days after tetramer treatment. Results in (**A**), (**C**), and (**D**) were obtained by flow cytometry; the percentages shown on the dot plots in (**C**) and (**D**) indicate the number of cells that are contained within the gated regions (boxed areas) as a percentage of the total cells analyzed. Reprinted with permission from *The Journal of Immunology*.[53]

grafts.[50] Tolerance was associated with production of unresponsive CTL with a CD-8^{lo} phenotype, which were capable of regulating their naïve allospecific peers through production of the immunosuppressive cytokine, transforming growth factor β.[21] These and other studies [22,23,51,52] have encouraged the notion that pMHC-I multimers could be used to inhibit the cytotoxic activity of β-cell-specific CD-8+ T-cell populations.

When tested in NOD mice, the effects of administering pMHC-I (NRP–K^d) tetramers on T1DM development were unfortunately quite variable across cohorts of animals, alternately delaying, accelerating, or having little appreciable impact on the onset of diabetes (personal communication with Jeff Frelinger). To overcome this limitation, we sought to determine whether these same pMHC multimers could be used to deliver a toxin to islet-

reactive T cells, efficiently eliminating the diabetogenic population and thereby halting disease progression.[53] In 2004, Yuan and colleagues[24] described the production of "suicide" tetramers: pMHC-I molecules coupled to the alpha emitter actinum-225, which were capable of killing cognate T-cell clones *in vitro*. We subsequently reported that pMHC-I tetramers conjugated to the ribosome-inactivating toxin, saporin (SAP), could selectively delete TCR–Tg T cells *in vivo*, with minimal bystander toxicity.[25] Similar results were described by Penaloza-MacMaster and associates.[54] We first established that coupling SAP to NRP–K^d did not alter binding specificity, using cognate (8.3) and noncognate (CL-4) TCR–Tg T cells (**Figure 3A**). We then found that the NRP–K^d–SAP tetramer selectively killed most 8.3 T cells within 72 h.[53] When transferred into T cell-deficient NOD.*scid* recipients, both sets of T cells undergo rapid expansion and activation; as expected, only IGRP-reactive 8.3 T cells cause diabetes (**Figure 3B**). To ensure that pMHC-I tetramers could reach their target cells *in vivo*, we demonstrated that a single intravenous injection of a fluorophore-coupled NRP–K^d tetramer labeled 8.3 T cells in diabetic NOD.*scid* recipients (**Figure 3C**). Lastly, we showed that administration of the NRP–K^d–SAP tetramer suppressed the expansion of 8.3 T cells (**Figure 3D**). Once validated, we then tested the effects of the NRP–K^d–SAP tetramer in unmanipulated female NOD mice, giving three injections, 6 days apart, beginning at 8 weeks of age. Administration of the tetramer was associated with a significant delay in the median onset of diabetes (24.5 versus 16.5 weeks for controls), with 30% of treated mice remaining euglycemic at the end of the study.[53] Importantly, toxic tetramer administration was associated with long-term eradication of cognate CTL from the islets. While preliminary, these observations encourage the idea that CTL deletion via toxin-coupled pMHC multimers may play a role in the treatment of T1DM.

It should be noted that pMHC-I multimers may also blunt diabetogenesis by nondeletional means, as illustrated by one study.[55] In this impressive work, NRP– and IGRP–K^d-coated iron oxide nanoparticles were found to protect NOD mice from T1DM in a dose-dependent manner by paradoxically expanding the antigen-experienced (autoreactive) cognate low-avidity T cell population, which subsequently exhibited a nonspecific regulatory function.[55] Further, administration of nanoparticles (but not their tetramer counterparts) led to functional recovery of β cells and restoration of euglycemia in newly diabetic wild-type and "humanized" (i.e., expressing a human MHC-I molecule) NOD mice.

Conclusions

By offering an expedient means to identify, measure, and target antigen-specific T cells, pMHC multimers appear to be extremely useful tools for studying and treating T1DM. For example, rising levels of diabetogenic CTL in peripheral blood could signal the onset of diabetes in an at-risk individual or impending graft rejection in an islet cell recipient. Alternatively, it may become possible to directly monitor the expansion and homing of these T cells *in vivo* by advanced imaging techniques. In such scenarios, the potential ability of pMHC multimers to reverse the negative clinical outcome by specifically deleting islet-destructive T cells or by inducing regulatory CD-4+ or CD-8+ T cells is an exciting development. Ideally, future studies of pMHC multimers will begin to address several important issues, such as the optimal parameters for administration, the use of pMHC multimers of different specificities simultaneously, concurrent administration of pMHC-I and pMHC-II multimers, the utility of combining pMHC multimers with other targeting agents such as MABs against B cells, and the effect of using modified pMHC molecules with diminished TCR stimulation properties. While a number of questions therefore remain unanswered, studies to date suggest that pMHC multimer technology offers promise as a rational, potent therapy—either by itself or, more likely, in conjunction with other treatments—for this devastating and far-too-common autoimmune disease.

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