The specificity of T cell regulation that enables self-nonself discrimination in the periphery

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It was recently shown that perceiving the avidity of T cell activation can be translated into peripheral T cell regulation to control autoimmune disease. This regulation is achieved by CD8+ T cells that recognize a common surrogate target structure, Qa-1/Hsp60sp, preferentially expressed by activated T cells of intermediate but not high avidity. A truncated self-reactive repertoire, devoid of high-avidity T cells, generated by thymic negative selection, allows selective down-regulation of intermediate-avidity T cells to accomplish self-nonself discrimination in the periphery. Identification of the common surrogate target structure expressed on intermediate-avidity T cells opens up a conceptual theme to understand the relationship between the specificity of peripheral immune regulation and self-nonself discrimination. Here, we investigated peptide vaccination induced cross-protection mediated by CD8+ T cells in two autoimmune disease models, experimental allergic encephalomyelitis (EAE) and type 1 diabetes (T1D). We show that Qa-1 restricted CD8+ T cells cross-protect animals from either EAE or T1D without abrogating the immune response to foreign antigens. Cross-protection occurs because potentially pathogenic self-reactive T cells included in the pool of intermediate-avidity T cells are capable of preferentially expressing common surrogate target structures on their surface to render themselves subject to the down-regulation, independent of the specificity of the antigens that they are triggered by. Thus, like in the thymus, the immune system discriminates self from nonself, during adaptive immunity in the periphery, not by recognizing the structural differences between self and foreign antigens, but rather by perceiving the avidity of T cell activation.

avidity model | Qa-1/HLA-E-restricted CD8+ T cells | Qa-1/Hsp60sp | autoimmune disease | cross-protection

The pioneering work of Burnet and Medawar suggested that the definition of “self” versus “nonself” is arbitrary to the immune system because foreign antigens presented during fetal life are thereafter considered self (1, 2). This fact indicated that the immune system discriminates self from nonself, during adaptive immunity, not by recognizing the structural differences that define self versus foreign antigens. Moreover, it is known that T cell receptors (TCRs) on all T cells are self-referential in the sense that they are positively selected for survival on self-peptides bound to MHC molecules during thymic positive selection (3–6) before thymic negative selection, in which thymocytes expressing TCRs of high avidity to the majority of self-antigens are deleted (7–9). It thus further indicates that the immune system begins to learn how to discriminate self from nonself by sensing the avidity of the thymocyte activation in the thymus.

It is generally accepted that thymic negative selection eliminates the imminent danger of pathogenic autoimmunity in the periphery and is the major mechanism of self-tolerance. However, while releasing the “innocent” self-reactive T cells with low avidity, thymic negative selection also allows a large fraction of self-reactive T cells with intermediate avidity to be released into the periphery under normal circumstances (10–12). The existence of the intermediate-avidity self-reactive T cells in the periphery represents a potential danger of pathogenic autoimmunity inherited in each individual because these T cells can often be activated when they encounter self-peptides presented at a sufficient level during the adaptive immune response and some may differentiate into potentially pathogenic effector cells to initiate an autoimmune disease (12–15). We thus postulated that self-nonself discrimination must continue in the periphery after thymic negative selection and one of the major functions of peripheral regulatory mechanisms is to selectively down-regulate immune responses to self-antigens without damaging the normal on going responses to foreign pathogens to maintain self-tolerance (16). However, how the immune system achieves self-nonself discrimination in the periphery is an enigma.

In this regard, we have proposed and tested an avidity model of peripheral T cell regulation in which self-nonself discrimination can be achieved in the periphery by the Qa-1-restricted CD8+ T cells that selectively down-regulate intermediate-avidity T cells to both self and foreign antigens (17, 18). Among T cells activated by foreign antigens, only those of intermediate avidity are targeted for down-regulation whereas the T cells with high avidity to the foreign antigens, not only escape down-regulation, but could also be indirectly promoted to survive and grow, because of less competition for resources, such as space, energy, and nutrition. However, because the self-reactive repertoire is composed mainly of intermediate- and low-avidity but devoid of high-avidity T cells the overall effect of the down-regulation of intermediate-avidity T cells in the context of self-antigens is the suppression of autoimmunity.

The concept of perceiving the avidity of T cell activation can be translated into peripheral T cell regulation and the molecular structures recognized by regulatory T cells that enable them to discriminate self from nonself in the periphery are the key issues in regulatory T cell biology. We have recently identified a surrogate target structure, Qa-1/Hsp60sp, which is preferentially expressed on intermediate-avidity T cells after antigen activation and is specifically recognized by Qa-1-restricted CD8+ T cells (19). This specific T/T interaction between regulatory and target cells allows the distinction of high-avidity T cells from intermediate-avidity T cells, and enables the immune system to selectively down-regulate any potentially pathogenic self-reactive T cells that are included in the pool of intermediate-avidity T cells.

To further understand the relationship between the specificity of peripheral immune regulation and self-nonself discrimination, we studied the mechanism of antigen-peptide vaccination induced “cross-protection” phenomenon in two distinct autoimmune disease models, experimental allergic encephalomyelitis (EAE) and type 1 diabetes (T1D). We show that Qa-1-restricted CD8+ T cells cross-protect animals from either EAE or T1D without damaging the normal ongoing immune response to foreign antigens. This

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surrogate target structure expressed regardless of which antigens the target T cells are triggered by. To test this prediction in the context of pathogenic autoimmunity, we studied an experimental protocol of cross-protection mediated by Qa-1-restricted CD8+ T cells in two autoimmune disease models of Qa-1a strains. EAE in B10PL mice and T1D in NOD mice. We vaccinated B10PL mice or NOD mice with either α-acetylated, NH2-terminal nine amino acids of myelin basic protein (1-9Nac MBP) (peptide X) or p277 (peptide Y) followed by induction of EAE with 1-9Nac MBP in B10PL mice or spontaneously developed T1D in NOD mice, which could be elicited by several self-antigens, including peptide B:9-23 derived from insulin (peptide Z) (20). If the prediction is correct, the Qa-1-restricted CD8+ T cells primed by intermediate-avidity T cells activated by peptide X or Y should be able to suppress the intermediate-avidity T cells activated by peptide X or Z, therefore protecting the animals from autoimmune disease elicited by either self-peptide X or Z (Fig. 1).

We first vaccinated B10PL mice with either 1-9Nac MBP, a MHC class II binding peptide derived from MBP, or p277, a MHC class II binding peptide derived from Hsp60. In this regard, it is well known that 1-9NacMBP is a pathogenic peptide in B10PL mice and p277 may be involved in the pathogenesis of T1D in certain mouse strains (21) but irrelevant to EAE. As shown by Fig. 2A, animals were equally effectively protected from EAE by either 1-9Nac MBP or p277 vaccination compared with control mice.

We then studied the T1D model in NOD mice. In this regard, it is known that p277, when used as a vaccine, effectively protects NOD mice from T1D, perhaps because of the induction of a shift from Th1 to Th2 response to the particular self-antigen (22). We vaccinated NOD mice with p277 peptide, which completely protected NOD mice from spontaneously developed T1D up to 35 weeks of age compared with control mice. Reciprocally, NOD mice were also vaccinated with 1-9Nac MBP, which equally protected NOD mice from spontaneously developed T1D (Fig. 2B). The effectiveness of both 1-9NacMBP and p277 in the protection of either EAE or T1D disease models is defined here as cross-protection.

Because Qa-1-restricted CD8+ T cells have been observed to protect animals from EAE (19, 23), we tested whether the induction of the regulatory CD8+ T cells in vivo accounts for the cross-protection achieved by the Qa-1-restricted CD8+ T cells, which recognize and selectively down-regulate potentially pathogenic self-reactive CD4+ T cells included in the pool of intermediate-avidity T cells, activated by any self-antigens, that preferentially express the common surrogate target structure, Qa-1/Hsp60sp, on their surface.

Thus, like in the thymus, the immune system discriminates self from nonself during adaptive immunity in the periphery, not by recognizing the structural differences between self and foreign antigens, but rather by perceiving the avidity of T cell activation.

**Results**

**Cross-Protection Between EAE and T1D Is Mediated by the CD8+ T Cells.** Identification of a common surrogate target structure expressed on intermediate-avidity T cells and recognized by the Qa-1-restricted CD8+ T cells (19) allows the prediction that the specificity of the regulation by the CD8+ T cells is not at the level of the antigens that activate the target T cells. Instead, the specificity is at the level of perceiving a particular biological consequence of intermediate-avidity T cell activation by recognizing the common

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**Fig. 1.** Cross-protection in both EAE and T1D autoimmune disease models. Animals are protected by vaccination with different antigen peptides.

**Fig. 2.** Vaccination with self-peptide 1-9NacMBP or P277 cross-protected B10PL mice from EAE and NOD mice from T1D and the protection is CD8+ T cell dependent. (A) Vaccination of B10PL mice with MBP or P277 peptide equally protected B10PL mice from the subsequent induction of EAE. The data are representative of 4 separate experiments with 4–5 mice per group. (B) Vaccination of NOD mice with MBP or P277 peptide equally protected NOD mice from spontaneously developed T1D. The data are representative of 4 separate experiments with 4–5 mice per group. (C) CD8+ T cells isolated from either MBP or P277 peptide vaccination-protected EAE mice further protected naive B10PL mice from the subsequently-induced EAE when adoptively transferred. Control: Mice induced to develop EAE without CD8+ T cell transfer. CD8T/Naive: Mice induced to develop EAE after adoptively transferred with CD8+ T cells from naive mice. CD8T/MBP: Mice induced to develop EAE but protected when adoptively transferred with CD8+ T cells from MBP vaccination-protected EAE mice. CD8T/p277: Mice induced to develop EAE but protected when adoptively transferred with CD8+ T cells from p277 vaccination-protected EAE mice. The data are representative of 4 separate experiments with 4–5 mice per group. (D) CD8+ T cells isolated from either 1-9NacMBP or P277 peptide vaccination-protected EAE mice further protected naive NOD mice from the spontaneously-developed T1D when adoptively transferred. The lines are the same as in C except the experiments were performed in NOD mice.
A significantly higher ratio of Qa-1/Hsp60sp versus Qa-1/Qdm when the CD8+ protection from spontaneously developed T1D was also obtained subsequently induced EAE when adoptively transferred. Equal control naive mice significantly protected recipient mice from determine the specificity of the CD8+ used a Qa-1a-expressing cell 3F4 as peptide-presenting cell to preferentially expressed on the intermediate-avidity T cells and susceptible target T cells, which are responsible for both EAE and recognize certain common target structures expressed by the designates transfectant 3F4 loaded with Qdm and control peptide. The lines are representative of 4 separate experiments performed in B10PL mice. (B) CD8+ T cells isolated from either 1-NacMBP or p277 peptide vaccination-protected T1D mice selectively inhibit an intermediate-avidity p277-specific T cell clone 15A6 or transfectant 3F4 loaded with Hsp60sp but not control low-avidity clone 13C4 or transfectant 3F4 loaded with Qdm and control peptide. The data are representative of 4 separate experiments performed in B10PL mice.

Qa-1/Hsp60sp Is a Common Target Structure Recognized by the CD8+ T Cells Isolated from Both Cross-Protected EAE and T1D Mice. The cross-protection observed suggests that the regulatory CD8+ T cells recognize certain common target structures expressed by the susceptible target T cells, which are responsible for both EAE and T1D in vivo. We thus considered that Qa-1/Hsp60sp, known to be preferentially expressed on the intermediate-avidity T cells and specifically recognized by the Qa-1-restricted CD8+ T cells (19), may represent one such target structure recognized by the CD8+ T cells that mediate the cross-protection. To test this hypothesis, we used a Qa-1a-expressing cell 3F4 as peptide-presenting cell to determine the specificity of the CD8+ T cells isolated from either MBP- or p277-vaccination-protected B10PL and NOD mice. In this regard, 3F4 was established as a Qa-1a-expressing cell by transfecting the human B cell line C1R with recombinant murine Qa-1a cDNA (24, 25) and has successfully served as a Qa-1 binding peptide-presenting cell to test whether Hsp60sp is a specific target for the Qa-1-restricted CD8+ T cells (19). Physiological target T cells, the intermediate-avidity 1-Nac MBP-specific clone 1AE10 from B10PL mice, and p277-specific clone 15A6 from NOD mice served as positive controls, and low-avidity MBP-specific clone 4D10 and p277-specific clone 13C4 served as negative control to assess the function and the specificity of the CD8+ T cells. In this regard, we have demonstrated that quantitative but not qualitative differences of the surface expression between Qa-1/Hsp60sp versus Qa-1/Qdm is a function of avidity of T cell activation that determines their susceptibility to the down-regulation by the CD8+ T (19). A significantly higher ratio of Qa-1/Hsp60sp versus Qa-1/Qdm in intermediate-avidity T cells, reflected by the M/H/Qa-1 protein index, is the molecular basis for the susceptibility of intermediate-avidity T cells to the down-regulation by the Qa-1-restricted CD8+ T cells (19). In the current studies, illustrated in Fig. S1, significantly higher M/H/Qa-1 protein indexes are expressed by the intermediate-avidity clones 1AE10 and 15A6, compared with the low-avidity clones 4D10 and 13C4, consistent with their susceptibility to the down-regulation by the CD8+ T cells. Furthermore, as shown, CD8+ T cells isolated from either MBP- or p277 vaccination-protected B10PL mice, which inhibited the positive control clone 1AE10, but not the negative control clone 4D10, also efficiently down-regulated the 3F4 cells sensitized with Hsp60sp but not Qdm or control irrelevant peptide (Fig. 3A). Similarly, CD8+ T cells isolated from either p277- or MBP vaccination-protected NOD mice, which inhibited the positive control clone 15A6, but not the negative control clone 13C4, efficiently down-regulated the 3F4 cells sensitized with Hsp60sp but not Qdm or control irrelevant peptide (Fig. 3B). CD8+ T cells isolated from naive B10PL or NOD mice did not have any effect on the target cells (data not shown). This set of experiments demonstrates that Qa-1/Hsp60sp represents a common target structure, which can be specifically recognized by the Qa-1-restricted CD8+ T cells that are induced during vaccination of mice with different antigenic peptides. Thus, Qa-1/Hsp60sp, expressed on the cell surface, can prime and be subject to the CD8+ T cells that account for the in vivo amelioration of both EAE and T1D. These results are consistent with the observation that Qa-1-restricted CD8+ T cells can be directly induced by Hsp60sp-loaded dendritic cells, which protect animals from subsequently induced EAE (19).
ED50, compared with mice received control CD8+ T cells. The inhibited in vivo T cell responses to pathogenic self-peptides 1-9Nac MBP and B:9-23 by the adoptively-transferred CD8+ T cells from either MBP- or p277 vaccination-protected mice is consistent with the in vivo cross-protection of the diseases observed. In contrast, the same regulatory CD8+ T cells, when adoptively transferred, enhanced the overall primary immune response to the conventional foreign antigen HEL in both B10PL (Fig. 4A) and NOD (Fig. 4B) mice, shown by a higher magnitude of T cell proliferation combined with an increased overall avidity, reflected by a lower ED50, compared with mice received control CD8+ T cells. Thus, the overall inhibitory effect of suppression observed in cross-protection, mediated by the Qa-1-restricted CD8+ T cells, is selectively confined within the immune responses to self but not to foreign antigens. This set of observations supports our hypothesis that peripheral regulatory mechanisms, such as the Qa-1-restricted CD8+ T cell-mediated pathway, that function to specifically maintain self-tolerance by discriminating self from nonself, can be induced to control autoimmune diseases.

Discussion

The central issue addressed in this article is the relationship between the specificity of peripheral immune regulation and self-nonself discrimination in the control of autoimmune diseases. The intriguing feature of the in vivo cross-protection described in these studies is the observation that vaccination with same antigenic peptides efficiently protects animals from two distinct autoimmune diseases in which the pathogenic self-antigens responsible for the diseases differ from each other and the target organs, attacked by the autoimmune process, also differ. Equally interesting is the observation that independent of whether or not the vaccine peptides are responsible for a given autoimmune disease, vaccinating animals with different antigen peptides effectively protects them from the same autoimmune disease. Moreover, the suppression that mediates the cross-protection phenomenon is confined only within the overall immune responses to self-antigens without damaging the normal ongoing immune responses to foreign antigens.

The cross-protection phenomenon strongly suggests that recognition of a common target structure on potentially pathogenic self-reactive T cells, expressed as a consequence of T cell activation during autoimmune process, by the Qa-1-restricted CD8+ T cells, accounts for the effective in vivo amelioration of autoimmune diseases observed. In light of the experimental evidence that the potentially pathogenic self-reactive T cells are included in the pool of intermediate-avidity self-reactive T cells (12–15), we have recently shown that the preferential expression of Qa-1/Hsp60sp on certain activated T cells is a function of the avidity of their TCR–ligand interactions (19). We further demonstrate in the current studies that Qa-1/Hsp60sp indeed represents a common target structure recognized by the Qa-1-restricted CD8+ T cells isolated from either cross-protected EAE or T1D mice (Fig. 3).

Cross-protection occurs because potentially pathogenic self-reactive T cells included in the pool of intermediate-avidity T cells, when activated, are capable of preferentially expressing Qa-1/Hsp60sp on their surface and become subject to the down-regulation by the CD8+ T cells, regardless of which self-antigens trigger the target T cells.

Preferential expression of a common target structure on the intermediate-avidity T cells activated by any self-antigens (19), which are responsible for a given autoimmune disease in vivo, establishes the molecular and cellular basis for the cross-protection. We envision that upon peptide vaccination the common target structure generated is essential for specifically triggering the regulatory CD8+ T cells to differentiate into effector cells in vivo (the induction phase). The primed CD8+ T cells then, in turn, down-regulate potentially pathogenic self-reactive T cells, by recognizing the common target structure preferentially expressed, as a consequence of intermediate-avidity T cell activation, by any self-antigens (the effector phase). The animals are therefore protected from the development of any given autoimmune disease. This way the regulatory CD8+ T cells primed by intermediate-avidity T cells activated by vaccine peptides during the primary responses would selectively down-regulate intermediate-avidity T cells activated by any different set of self-antigens during later immune responses, which are responsible for a given autoimmune disease in vivo (Fig. 5). As important, specific recognition of the common target structure preferentially expressed on intermediate-avidity T cells by the Qa-1-restricted CD8+ T cells also enables the immune system, at a biological system level, to distinguish high-avidity T cells from intermediate-avidity T cells, to achieve self-nonself discrimination in the periphery (Figs. 4 and 5).

In our current studies, the two self-peptides, functioning as effective vaccines, both are MHC class II binding peptides that are capable of eliciting effective MHC class II responses in both B10PL and NOD mice. Although 1-9NacMBP is a self-peptide responsible for pathogenic autoimmune EAE in B10PL mice (27) and p277 can induce T1D in certain mouse strains (21) and therefore may be involved in T1D in NOD mice, there is no evidence that p277 can induce EAE in B10PL mice or that 1-9NacMBP can induce T1D in NOD mice. Interestingly, both peptides are equally capable of...
preventing either EAE or T1D in vaccinated animals. This key observation indicates that independent of whether or not the vaccine peptides are pathogenic to a given autoimmune disease, as long as they are able to initiate an immune response to provide the common target structures, such as Qa-1/Hsp60sp, to prime the Qa-1-restricted CD8+ T cells, they are capable of cross-protection (Fig. 5). Thus, via specific recognition of the common target structures, cross-protection is accomplished by a selective down-regulation of the relevant pathogenic self-reactive T cells activated by particular self-antigens responsible for a given autoimmune disease in vivo, which could be either relevant or irrelevant to the vaccine peptides.

It is important to emphasize that, conceptually, the intermediate-avidity T cells described in the avidity model (16–18) represent a rather large pool of thymic escapees that have the avidity lower than those deleted in the thymus but that cover a wide spectrum of avidity. It could extend from a high end close to the low boundary of the threshold of thymic negative selection to a low end, which might be near the high boundary of the threshold of thymic positive selection. The exact biological threshold of the intermediate avidity has not yet been identified. It may vary to a certain extent in different experimental systems. In this regard, it has been shown in several studies that certain self-reactive T cells with low avidity, presumably at the low end of the intermediate avidity that we refer to, can be activated in the periphery to initiate autoimmune diseases (12, 13, 15), such as 1-9NacMBP-specific encephalitogenic clones in B10PL mice. However, in some autoimmune diseases, pathogenic self-reactive T cells appear to have much higher avidity to self-antigens, probably at the high end of the spectrum of the intermediate avidity, such as certain pathogenic diabetic clones in NOD mice (14). The observations that Qa-1-restricted CD8+ T cells protect animals from both EAE and T1D in our studies and others’ (23) are consistent with the notion that this regulatory pathway selectively down-regulate intermediate-avidity self-reactive T cells, which are covered by a rather wide spectrum of avidity in the periphery. Cross-protection across distinct autoimmune diseases provides biological evidence for the hypothesis that perceiving the avidity of T cell activation can be translated into peripheral T cell regulation to discriminate self from nonself in the periphery independent of the antigen specificity of the T cells regulated. In this regard, the avidity model contains an important conceptual element of ergotypic regulation in that both types of regulation recognize the consequence of T cell activation, regardless of which antigens activate the target T cells. However, it also differs from ergotypic regulation because the ergotypic regulation functions independent of the avidity of T cell activation (28, 29).

In summary, the notion that perceiving the avidity of T cell activation can be translated into peripheral T cell regulation is the essence of the avidity model that provides a conceptual framework to understand the biological inevitability that the consequence of thymic negative selection determines how the adaptive immunity is regulated in the periphery to accomplish self-nonself discrimination. The physical link between thymic negative selection and peripheral immune regulation is the fact that release of intermediate-avidity self-reactive T cells into the periphery, which contain potentially pathogenic self-reactive T cells, is a biological consequence of thymic negative selection and must be specifically dealt with by peripheral regulatory mechanisms. The Qa-1-restricted CD8+ T cell-mediated regulatory pathway represents an example of a peripheral mechanism that the immune system evolved to complete self-nonself discrimination that is achieved, “imperfectly,” by thymic negative selection, to maintain self-tolerance. This type of regulatory mechanisms differ from the intrinsic mechanisms that control the magnitude and class of immune responses, such as antigen activation-induced cell death or expression of costimulatory molecules, or the functional activation and differentiation of the CD4+ T cells into the Th1 versus Th2 or Tr1 and Tr3 regulatory cells (16, 26). They also differ from the naturally-arising CD25+Foxp3+ Tregs that negatively control the overall immune responses to both self and foreign antigens (30).
Identification of the regulatory mechanisms that function to discriminate self from nonself opens up possibilities of clinical interventions to prevent and treat autoimmune diseases without abrogating antiinfection and antimumor immunity, which is the major side effect of currently-used immuno-therapeutic drugs. In addition, the specificity of the regulations is not driven by the antigens that activate the target T cells but by the autoimmunize diseases could be achieved independently of the knowledge of the particular self-antigens involved, in any given autoimmune disease, that are largely undetermined at the present time.

**Experimental Procedures**

**Animals.** All mice used (Jackson Laboratory) were housed in the pathogen-free animal facility associated with the Columbia University Department of Comparative Medicine. The Institutional Animal Care and Use Committee at Columbia University provided approval for all animal studies.

**Reagents.** Anti-Qa-1a antisera were a kind gift from Lorraine Flaherty at the David Axelrod Institute for Public Health, Albany, NY. The staining reagents, fluorescein (FI) 53–6.72 (anti-mouse CD8) and phycoerythrin (PE)-GK1.5 (anti- mouse CD4) were purchased from PharMingen. PE-goat anti-mouse was purchased from Jackson ImmunoResearch. Peptides Hsp60p (QMRRPSVRAL and B9:23 (SHLVEALYLVCG ER) were synthesized by GeneScript. Peptides Qdmp (AMAPRTLITT), p277 (VLLGGGVARRPLDSLTIPANED), and 1-9NacMBP (ACAS-GLAPSGA) were synthesized by the protein core facility at Columbia University.

**SDS/PAGE and Western Blot Analysis.** SDS/PAGE and Western blot analysis were conducted following standard procedures. 1-9Nac MBP- or p277-specific CD4+ clones with different avidity were stimulated with irradiated splenic cells and antigen peptides (1–50 μM) for 72 h. The Abs used were anti-CD4, anti-Hsp60, anti-MHC class Ia M1/42, and anti-Qa-1a sera, followed by incubation with the secondary Ab rabbit anti-mouse HRP or rabbit anti-rat HRP. Target proteins were detected using the ECL detection kit (Amersham Biosciences). Blots were densitometrically quantitated by using ChemiDoc XR5 ImageQuant 1.4.5.0 software (Bio-Rad).

**CDB+ T Cell Inhibition Assay.** 19.A1-9Nac MBP+ or p277-specific CD4+ clones with different avidity were stimulated with irradiated splenic cells and antigen peptides (1–50 μM) for 72 h. The Abs used were anti-CD4, anti-Hsp60, anti-MHC class Ia M1/42, and anti-Qa-1a sera, followed by incubation with the secondary Ab rabbit anti-mouse HRP or rabbit anti-rat HRP. Target proteins were detected using the ECL detection kit (Amersham Biosciences). Blots were densitometrically quantitated by using ChemiDoc XR5 ImageQuant 1.4.5.0 software (Bio-Rad).

**Peptide Vaccination and Adoptive Transfer of CD8+ T Cells in EAE and T1D Models.** In the standard protocol used throughout this study female 8- to 10-week-old B10PL or 3- to 4-week-old NOD mice were immunized s.c. with 1-9Nac MBP or p277 emul-sified with incomplete Freund's Adjuvant at 100 μM per mouse as described. EAE was induced at least 1 week after peptide vaccination. CD8+ T cells were purified from spleens and draining lymph nodes of peptide vaccination-protected EAE/8B10PL mice or T1D/NOD mice by positive selection with CD8 MACS magnetic beads (Miltenyi Biotec). The purity of CD8+ T cells was routinely >95%. A total of 2.5 × 106 CD8+ T cells were adoptively transferred into naive NOD mice or B10PL mice, which were subsequently induced to develop EAE 1 week later. The effect of peptide vaccination and adoptive transfer of CD8+ T cells was evaluated by monitoring the clinical score of the animals. The disease was scored from 0 to 4: 0, no clinical signs; 1, slight posture loss; 2, gait abnormalities; 3, flaccid paralysis; 4, moribund.

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Fig. S1. The H/M/Qa-1 protein index of 1-9Nac MBP-specific intermediate-avidity clone 1AE10 and low-avidity clone 4D10 and p277-specific intermediate-avidity clone 15A6 and low-avidity clone 13C4 is shown. Three separate experiments are summarized. The H/M/Qa-1 protein index is calculated as H/M Protein expression ratio \times protein expression index of Qa-1, which represents the ratio of Hsp60 to MHC class Ia normalized to Qa-1 at the protein expression level [Chen W, et al. (2007) Perceiving the avidity of T cell activation can be translated into peripheral T cell regulation. Proc Natl Acad Sci USA 104:20472–20477]. The protein expression index is the ratio of protein expression between a given protein and \beta-actin in the same cells. The H/M protein expression ratio is the ratio of protein expression index between Hsp60 and MHC Class Ia.