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### Commentary

Suppressing unwanted immune responses without compromising host immunity against pathogens is considered the holy grail of immunology. Lack of responsiveness to self-antigens is normally maintained by multiple mechanisms, including the suppressive activities of several T cell subsets. In this issue of the *JCI*, Jiang and colleagues define a CD8<sup>+</sup> suppressor T cell subset in humans that recapitulates a regulatory pathway previously described in mice. These investigators further show that patients with type 1 diabetes have defects in their CD8<sup>+</sup> suppressor T cells, thus identifying these cells as potential therapeutic targets in human disease.

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# Comeback kids: CD8<sup>+</sup> suppressor T cells are back in the game

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**Suppressing unwanted immune responses without compromising host immunity against pathogens is considered the holy grail of immunology. Lack of responsiveness to self-antigens is normally maintained by multiple mechanisms, including the suppressive activities of several T cell subsets. In this issue of the *JCI*, Jiang and colleagues define a CD8<sup>+</sup> suppressor T cell subset in humans that recapitulates a regulatory pathway previously described in mice. These investigators further show that patients with type 1 diabetes have defects in their CD8<sup>+</sup> suppressor T cells, thus identifying these cells as potential therapeutic targets in human disease.**

A cardinal feature of the immune system is its capacity to distinguish self from nonself, a property referred to as immunological self-tolerance. Breakdown of the immune mechanisms that normally maintain self-tolerance is a factor contributing to the etiology of many human diseases, including type 1 diabetes (T1D) and MS. Devising means to promote self-tolerance while maintaining effective immune defenses against infectious agents and cancer has been a goal of immunologists ever since the pioneering studies by Medawar and colleagues on immune tolerance (1).

Immune tolerance is normally maintained by both central mechanisms, which are active during lymphocyte development in central lymphoid organs (2), and peripheral mechanisms, which are active after lymphocyte emigration to peripheral lymphoid organs (3). Developing T cells in the thymus undergo a rigorous selection process that purges potentially harmful T cells through a process known as negative selection. Through positive selection, the thymus promotes survival of T cells that recognize antigens in the context of the MHC molecules expressed by the individual, a phenomenon referred to as self-MHC restriction. However, due to the delicate balance between the need for self-MHC restriction and elimination of potentially harmful T cells, a substantial proportion of T cells with low or intermediate affinity for self-antigens manage to escape to the

periphery. These potentially harmful T cells are normally kept in check by a variety of peripheral tolerance mechanisms, including the induction of unresponsiveness, regulation by immunosuppressive cytokines, and suppression by Tregs (3). Although early studies on T cell suppression focused on cells expressing CD8, studies in recent years have centered on CD4<sup>+</sup>CD25<sup>+</sup> Tregs and NKT cells (4). However, in this issue of the *JCI*, Jiang and colleagues now show that CD8<sup>+</sup> suppressor T cells have a role in maintaining self-tolerance in humans and provide a potential means to harness these cells for therapeutic purposes (5).

## CD8<sup>+</sup> T cells with immune suppressive activities

The existence of T cells with suppressive capacity was first proposed in the early 1970s by Gershon and colleagues (6). These cells, most of which expressed CD8, were extensively investigated during the next decade, but subsequent studies failed to identify the molecular basis of suppression, which brought the field to an abrupt halt in the mid-1980s. The field was eventually revived in the mid-1990s by the identification of natural CD4<sup>+</sup>CD25<sup>+</sup> T cells as potent suppressors of autoimmunity (7). Since then, CD4<sup>+</sup> Tregs have taken center stage, and CD8<sup>+</sup> Tregs have fallen by the wayside. Nevertheless, elegant studies from die-hard proponents of the CD8<sup>+</sup> T cell suppressor hypothesis have provided strong evidence that CD8<sup>+</sup> Tregs have a role that is complementary to that of CD4<sup>+</sup> Tregs (8–10). A key finding was that vaccination with irradiated, antigen-activated autoreactive T cell clones provided protection against the sub-

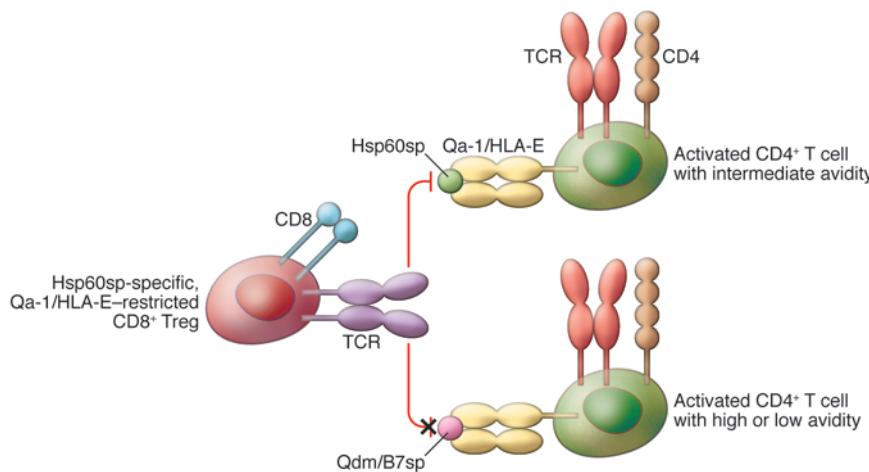
sequent induction of experimental autoimmune disease in a manner that involved suppression mediated by CD8<sup>+</sup> T cells (11, 12). These studies suggested that a subset of CD8<sup>+</sup> T cells with suppressive activity reacted with antigenic determinants on the vaccinating cells and subsequently recognized these same determinants on the pathogenic T cells. Studies using the EAE animal model of MS further demonstrated a critical role of CD8<sup>+</sup> T cells in suppressing disease following secondary challenge with autoantigens and during disease relapse (13, 14). The regulatory functions of CD8<sup>+</sup> T cells were also observed in other models of autoimmune diseases, including collagen-induced arthritis, autoimmune myocarditis, and herpes simplex virus-induced stromal keratitis (15). CD8<sup>+</sup> T cells with suppressor activities have also been implicated in human autoimmune diseases, including MS (16) and inflammatory bowel disease (17). Nevertheless, acceptance of the CD8<sup>+</sup> T cell suppressor hypothesis required demonstration of the molecular mechanisms involved.

## The Qa-1/HLA-E system

Early studies suggested a role for the Qa-1 molecule in the mechanisms underlying suppression of CD4<sup>+</sup> T cells by CD8<sup>+</sup> Tregs (18). Mouse Qa-1 and its human homolog HLA-E belong to a large group of unconventional MHC class Ib molecules that are related to conventional MHC class Ia molecules (19). Qa-1 and HLA-E are best known for their capacity to bind the inhibitory receptor CD94/NKG2A on NK cells (20). These proteins are transiently upregulated on activated T cells and bind hydrophobic peptides derived from self or foreign proteins. They are predominantly occupied by peptides, Qdm in mice and B7sp in humans, derived from the signal sequences of other MHC class I molecules. The association of CD8<sup>+</sup> Tregs with Qa-1 was most clearly demonstrated using Qa-1-deficient mice, which develop exaggerated immune responses to self-antigens (21). Furthermore, it was shown that vaccination with irradiated, antigen-activated

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**Figure 1**

Signal strength model for the ability of Qa-1/HLA-E-restricted CD8<sup>+</sup> T cells to suppress activated CD4<sup>+</sup> T cells. Activated CD4<sup>+</sup> T cells with intermediate avidity for their cognate MHC class II/peptide complexes express Qa-1/HLA-E molecules that are predominantly occupied by Hsp60sp. These T cells are effectively suppressed by Hsp60sp-specific CD8<sup>+</sup> Tregs. In contrast, activated CD4<sup>+</sup> T cells with high or low avidity for their cognate MHC class II/peptide complexes express Qa-1/HLA-E molecules that are predominantly occupied by Qdm/B7sp peptides. These T cells are not under the control of Hsp60sp-specific CD8<sup>+</sup> Tregs. The ultimate outcome of these interactions is inhibition of autoreactive T cell responses and enrichment of high-avidity responses against foreign antigens.

CD4<sup>+</sup> T cells expressing Qa-1 induced the generation of Qa-1-restricted CD8<sup>+</sup> T cells that suppressed autoimmunity (12). The new studies by Jiang and colleagues (5) provide strong evidence that this suppressor T cell pathway is also active in humans, as clearly demonstrated by the ability of HLA-E-restricted CD8<sup>+</sup> T cell lines to suppress the activities of autoreactive CD4<sup>+</sup> T cells *in vitro*.

### Sensing the signal strength of CD4<sup>+</sup> T cells

Studies with Qa-1-restricted CD8<sup>+</sup> Tregs showed that these cells do not suppress CD4<sup>+</sup> T cell responses indiscriminately. CD8<sup>+</sup> Tregs can sense the strength (or avidity) of target CD4<sup>+</sup> T cells for their cognate MHC/peptide complexes (22). Thus, Qa-1-restricted CD8<sup>+</sup> T cells suppress activated CD4<sup>+</sup> T cells exhibiting intermediate but not high or low avidity for cognate antigen (Figure 1). Qa-1-restricted T cells accomplish this feat by sensing the self peptides that are displayed by Qa-1 at the surface of intermediate-avidity but not high- or low-avidity CD4<sup>+</sup> T cells. In molecular terms, the CD8<sup>+</sup> Tregs react with complexes between Qa-1 molecules and peptides derived from the signal sequence of heat shock protein 60 (Hsp60sp), which are enriched on intermediate-avidity CD4<sup>+</sup>

T cells and thus outnumber Qa-1/Qdm complexes (23) (Figure 1). Again, the article by Jiang and colleagues (5) provides strong evidence that this molecular mechanism of suppression characterized in mice is conserved in humans. Specifically, they showed that Hsp60sp-specific, but not B7sp-specific, HLA-E-restricted CD8<sup>+</sup> T cells were able to suppress the reactivity of CD4<sup>+</sup> T cells against self-antigens (the T1D-related self-antigen glutamic acid decarboxylase [GAD] and the MS-related self-antigen myelin basic protein [MBP]) *in vitro*.

### Comparison with other Treg subsets

Although Qa-1/HLA-E-restricted CD8<sup>+</sup> Tregs exhibit many similarities to other Treg subsets, there are important differences as well (4). Most notably, CD4<sup>+</sup> Tregs suppress immune responses indiscriminately, whereas CD8<sup>+</sup> Tregs can distinguish self from nonself. For example, using an ex vivo coculture system, Jiang and colleagues showed that Hsp60sp-specific, HLA-E-restricted CD8<sup>+</sup> T cells suppressed CD4<sup>+</sup> T cell responses against the self-antigens GAD and MBP but enhanced responses against the foreign antigens tetanus toxoid and purified protein derivative (5). This was not observed for B7sp-specific CD8<sup>+</sup> T cells, which left both types of CD4<sup>+</sup> T cells undisturbed. Autoreactive T cells predominantly

exhibit intermediate or low avidity for self-antigens, whereas T cells reactive against foreign antigens exhibit a wide range of avidities. Because the CD8<sup>+</sup> Tregs generated by Jiang and colleagues recognized HLA-E/Hsp60sp on intermediate-avidity T cells, they suppressed T cell responses against self-antigens but enriched for high-avidity responses against foreign antigens (5). Although incompletely understood, this suppression likely involves both lytic and cytokine-mediated mechanisms (5, 10).

Another important difference is that CD4<sup>+</sup>CD25<sup>+</sup> Tregs and NKT cells manifest their natural suppressive activities during the early phases of an immune response, whereas the suppressive activities of CD8<sup>+</sup> Tregs are only observed during the late phases of an immune response. Thus, development of CD8<sup>+</sup> T cell suppression requires priming, something that was clearly illustrated by the vaccination studies with irradiated, self-reactive CD4<sup>+</sup> T cell clones (12). Furthermore, CD8<sup>+</sup> Tregs could be induced in mice by vaccination with DCs loaded with Hsp60sp but not Qdm, and this protected against autoimmunity (23).

### Relevance to human autoimmunity and its therapy

The studies by Jiang and colleagues show that individuals with recent-onset T1D display defects in the ability of CD8<sup>+</sup> T cells to suppress autoreactive CD4<sup>+</sup> T cells *in vitro* (5). However, for most of the patients, this deficit could be restored by an *in vitro* stimulation with Hsp60sp-loaded DCs. Therefore, these findings identify HLA-E-restricted CD8<sup>+</sup> Tregs as potential new targets for immunotherapy of T1D and other autoimmune diseases. One way to harness the therapeutic activities of these cells would be to inject *in vitro* expanded CD8<sup>+</sup> suppressor T cells back into the patients. Alternatively, patients could be vaccinated with their own DCs loaded *ex vivo* with Hsp60sp, a method that has proven effective in animal studies (23). A major advantage of these proposed therapeutic modalities, as compared with antigen-specific tolerance strategies, is that they could be performed independently of detailed knowledge of the self-antigens that are targeted by the autoimmune process.

### Perspectives

Many advances in the field of CD8<sup>+</sup> Treg biology are needed if the therapeutic potential of these cells, suggested by the studies of Jiang and colleagues (5), is to be fully



exploited. For example, it will be necessary to develop better means to identify these cells with specific markers; to improve understanding of the mechanisms that induce these cells, either naturally or during therapy; to enhance understanding of the mechanism(s) of suppression; and to improve insight into the signaling events that underlie the selective induction of Hsp60 in intermediate-avidity CD4<sup>+</sup> T cells. It will also be important to better define the role of CD94/NKG2A, the receptor on NK cells and a subset of CD8<sup>+</sup> T cells that is inhibited by Qa-1/Qdm or HLA-E/B7sp complexes (20), in conferring self/nonself tolerance. Other outstanding questions are whether Qa-1/HLA-E-restricted CD8<sup>+</sup> Tregs represent a separate lineage of T lymphocytes and whether HLA-E polymorphisms are associated with human disease, as has been suggested for T1D (24). Finally, it will be important to determine how these cells impact immune responses during different immunological situations such as infection, allograft rejection, cancer, and allergies. With more studies like those performed by Jiang and colleagues (5), answers to many of these questions should be within reach, bringing us closer to attaining the holy grail of immunology.

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## Location, location, regulation: a novel role for $\beta$ -spectrin in the heart

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**Voltage-gated Na<sup>+</sup> channels (VGSCs) are responsible for the rising phase of the action potential in excitable cells, including neurons and skeletal and cardiac myocytes. Small alterations in gating properties can lead to severe changes in cellular excitability, as evidenced by the plethora of heritable conditions attributed to mutations in VGSCs highlighting the need to better understand VGSC regulation. In this issue of the *JCI*, Hund et al. identify the ability of a key structural protein,  $\beta$ <sub>IV</sub>-spectrin, to bind and recruit Ca<sup>2+</sup>/calmodulin kinase II to the channel at a cellular location key to successful action potential initiation and propagation, where it can mediate function and excitability.**

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#### The Na<sup>+</sup> channel and disease

In excitable tissues, such as muscle, heart, and nerve, action potential (AP) initiation is most often accomplished by the opening

of voltage-gated Na<sup>+</sup> channels (VGSCs). VGSC activity is critical for normal impulse conduction and contributes to control of the duration and morphology of the cellular AP. VGSCs have a primary pore-forming  $\alpha$  subunit that is a protein with 4 homologous domains, each with 6 transmembrane segments (Figure 1).

Inward Na<sup>+</sup> current is typically the largest membrane current in excitable cells and must quickly inactivate to allow the cell to begin to repolarize. In myocytes, Na<sub>v</sub>1.5 (which is encoded by *SCN5A*) is the principal VGSC  $\alpha$  subunit expressed. It is predominantly localized in the inter-