Minireview

Autoreactive CD8 T Cells in Organ-Specific Autoimmunity: Emerging Targets for Therapeutic Intervention

Roland S. Liblau,¹ F. Susan Wong,²,4 Lennart T. Mars,¹ and Pere Santamaria³,4

¹INSERM U563 and Immunology Laboratory
Toulouse University Hospital
France
²Department of Pathology and Microbiology
School of Medical Sciences
University of Bristol
Bristol BS8 1TD
United Kingdom
³Department of Microbiology and Infectious Diseases
Faculty of Medicine
University of Calgary
Calgary, Alberta T2N 4N1
Canada

The importance of CD8 T cells in the pathogenesis of organ-specific autoimmune diseases has not previously been well recognized. Recent evidence, however, indicates that autoreactive CD8 T cells can contribute substantially to tissue damage in both murine and human autoimmune disorders. As such, these T cells now become an attractive target for therapeutic intervention.

CD8 T cells play a major role in immune responses, their natural function being related to protection against viral infections and tumors. They perform this function by cytotoxic damage of target cells expressing MHC class I molecules and the relevant antigenic peptide as well as by the production of effector cytokines such as IFN-γ. As almost all cells express MHC class I molecules, it is clear that there is great potential for tissue damage. The importance of CD8 T cells in autoimmune diseases such as type 1 diabetes (T1D), rheumatoid arthritis (RA), autoimmune thyroiditis, and multiple sclerosis (MS) had not previously been well recognized, perhaps in part because genetic susceptibility and resistance to these diseases are profoundly affected by polymorphisms of MHC class II genes, with little evidence for an independent role for the closely linked MHC class I genes. Technical difficulties in expanding and maintaining autoreactive CD8 T cells in vitro have also hindered their in-depth analysis. However, CD8 T cells are emerging as important contributors to tissue damage in murine and human autoimmune disorders.

Evidence for a Pathogenic Role of Autoreactive CD8 T Cells

T1D is a prototypic organ-specific autoimmune disease in which CD8 T cells play a critical role in pancreatic β cell destruction. T1D in humans is associated with certain MHC class I alleles, and CD8 T cells are abundant among the mononuclear cells that infiltrate the pancreatic islets (insulitis) at clinical onset of disease and in diabetic patients transplanted with pancreas grafts from healthy monozygotic co-twins or HLA-identical siblings (reviewed in Vizler et al., 1999). Nonobese diabetic (NOD) mice develop a disease, closely resembling human T1D, that requires both CD4 and CD8 T cells. Since β cells do not express MHC class II molecules, it has been proposed that autoreactive CD4 T cells differentiate into effectors by engaging β cell antigens (shed during the physiological remodeling of tissue or by a prior insult) on local antigen-presenting cells (APCs). Studies in β2-microglobulin-deficient NOD mice have suggested that the initial β cell insult might be effected by cytotoxic CD8 T cells, which are consistently recruited to islets in NOD mice (DiLorenzo et al., 1998; Amrani et al., 2000).

A CD8 T cell population that recognizes an insulin-derived peptide in islets of 3- to 4-week-old prediabetic NOD mice has been reported (Wong et al., 1999). This T cell population shrinks with age (Wong et al., 1999; Amrani et al., 2000) and is replaced, in part, by another population of highly diabetogenic CD8 T cells that use homologous Vα17-Jα24 TCRα chains (Verdaguer et al., 1997; DiLorenzo et al., 1998). This Vα17-Jα24 population is already a significant component in the earliest NOD islet CD8 T cell infiltrate (DiLorenzo et al., 1998). The natural self-peptide recognized by these diabetogenic T cells is unknown, but screening of combinatorial peptide libraries led to the identification of a mimotope peptide, designated NRP, which behaves as a full agonist (Anderson et al., 1999). This population expands in size as the mice age and undergoes an “avidity maturation” process that coincides with an accelerated phase of disease and contributes to the progression from benign insulitis to overt disease (Amrani et al., 2000). This phenomenon indicates that intercellular competition has a major influence on the progression of autoimmunity. The mechanisms underlying the seemingly hierarchical recruitment of CD8 T cells targeting different autoantigens into pancreatic islets are unclear. However, the earlier recruitment of insulin- versus NRP-reactive CD8 T cells cannot be accounted for by differences in the peripheral precursor frequency of the different subpopulations (P.S., unpublished data). Differences in accessibility to antigen crosspresentation pathways (see below) or in the timing or levels of expression of different autoantigens might play a role. It is also reasonable to suggest that competition for APC resources or other antigen-independent factors contribute to the dynamics of T cell recruitment in organ-specific autoimmunity.

From these and other β cell antigen-specific CD8 T cell clones, a number of TCR-transgenic NOD mice have been generated that develop diabetes. Interestingly, the development of diabetes in each of these lines is different, especially after crosses with RAG−/− or SCID mice. One study has shown that the transgenic CD8 T cells may not require CD4 T cells to cause diabetes (Graser et al., 2000), while another indicates that CD4 T cells may contribute to the recruitment to the islets (Verdaguer et al., 1997). A third transgenic TCR appears to destine thymocytes for developmental arrest or negative selection, as mature CD8 T cells only develop when a suc-
cessful endogenous rearrangement is present (Kanagawa et al., 2000).

A number of other transgenic models of T1D have been generated in which mice expressing neo-autoantigens exclusively in β cells (expression driven by the rat insulin promoter [RIP]) were mated with transgenic mice expressing neo-autoantigen-specific, MHC class I-restricted TCRs (RIP-hemagglutinin [HA] × TCR, RIP-lymphocytic choriomeningitis virus [LCMV] proteins × TCR, and RIP-ovalbumin [OVA] × TCR models). In the HA and OVA models, diabetes develops spontaneously early on in life (Vizler et al., 1999). In contrast, in the LCMV model the autoreactive CD8 T cells are ignorant, and diabetes does not occur unless mice are infected with LCMV or crossed on a genetic background favoring enhanced T cell responsiveness (Garza et al., 2002). Collectively, these studies underline the pathogenic potential of autoreactive CD8 T cells but reveal some heterogeneity. These differences have not been formally compared but probably relate to the density of MHC:self-peptide complexes recognized, the affinity of the TCR for the MHC:peptide complex, and the genetic backgrounds of the transgenic mice.

MS is a chronic human disease caused by inflammatory cell-induced demyelination in the central nervous system (CNS). Infiltrating CD8 T cells predominate over CD4 T cells, especially in regions of active demyelination, and these CD8 T cells appear to undergo local clonal expansion as assessed by analysis of TCRβ gene rearrangements at the single-cell level (Babbe et al., 2000). Autoreactive CD8 T cells responsive to myelin-derived peptides have been reported in MS patients that have the potential to kill HLA class I-matched oligodendrocytes in vitro, independent of exogenous peptide. Experimental autoimmune encephalomyelitis (EAE) is an experimental model for MS, induced in susceptible animals by immunization with myelin antigens. Although autoreactive CD4 T cells are implicated as major effectors of EAE, there is evidence pointing to a role for CD8 T cells in disease progression and severity. For example, a myelin oligodendrocyte glycoprotein-derived peptide has recently been shown to elicit encephalitogenic CD8 T cells in vivo (Sun et al., 2001). In addition, myelin basic protein (MBP) is processed and presented in vivo by the MHC class I pathway, and, in fact, responding CD8 T cell clones induce a CNS immunopathology in mice that resembles some forms of MS, implicating CD8 T cells as potential effectors of demyelination in MS (Huseby et al., 2001).

In addition, it is clear that CD8 T cells can also kill neurons in vitro (Medana et al., 2000). This mechanism might contribute to the axonal loss observed in MS and to the pathogenesis of several human autoimmune diseases of low prevalence, such as paraneoplastic neurological syndromes and Rassmussen’s encephalitis (a CNS inflammatory disease causing epilepsy). Indeed, expanded populations of HLA-A2-restricted, onconeural antigen (cdr2)-specific CD8 T cells have been detected in the blood of cancer patients presenting with paraneoplastic cerebellar degeneration as a result of selective autimmune destruction of Purkinje cells (Albert et al., 1998). Furthermore, in Rassmussen’s encephalitis lesions, CD8 T cells are found in close contact with β2-microglobulin-expressing degenerating neurons, their cytotoxic granules being oriented toward the contact zone. CD8 T cells of either autoreactive or as yet unknown specificity have been implicated in a number of other organ-specific autoimmune diseases both in animal models and humans (e.g., thyroiditis, vitiligo, polymyositis, inflammatory bowel disease, primary biliary cirrhosis).

Activation of Autoreactive CD8 T Cells

The activation of naive CD8 T cells requires triggering by MHC:peptide and costimulatory molecules expressed on the surface of a specialized APC, most likely a dendritic cell (DC) (Figure 1). How, where, and when CD8 T cells recognize MHC:self-peptide in spontaneous autoimmune disorders is not well understood. Target cells usually cannot drive CD8 T cell responses in the absence of professional APCs, as they express MHC class I molecules but not costimulatory molecules. One means of CD8 T cell priming is crosspresentation of tissue-specific self-antigen by DCs (Heath and Carbone, 2001). This process has been demonstrated for soluble and cell-associated self-antigens. DCs can process cellular antigens from apoptotic cells, and both apoptotic and necrotic cells can promote immune responses by activating DCs. It has recently been shown that the efficiency of crosspriming increases with (1) high antigen level in the target tissue, (2) induction of apoptosis in self-antigen-expressing cells, (3) presence of autoreactive CD4 T cells, (4) presence of immune complex-forming autoantibodies, and (5) high T cell precursor frequency (Heath and Carbone, 2001; Kita et al., 2002). Whether spontaneous autoimmune CD8 T cell responses are skewed toward antigenic targets that can access antigen crosspresentation pathways in specialized APCs such as CD8+ DCs remains to be determined, but it is a possibility. Priming of NRP-reactive CD8 T cells, for example, specifically occurs in the pancreatic lymph nodes, and its magnitude increases with the degree of β cell death (Zhang et al., 2002). Posttranslational modifications of intracellular proteins, such as those occurring during the process of apoptosis (i.e., phosphorylation or caspase cleavage), might create neo-antigens capable of bypassing tolerance and priming autoreactive CD8 T cell responses, as proposed for certain autoantibody responses (Utz et al., 1997).

In T1D models, the requirement for CD4 T cell help in the activation of autoreactive CD8 T cells is not universal (Vizler et al., 1999, Graser et al., 2000; Garza et al., 2002), although clearly needed in most cases. For instance, naive NRP-reactive CD8 T cells undergo activation in the pancreatic lymph nodes by engaging autoantigen on professional APCs but do not efficiently accumulate in islets in the absence of CD4 T cells (Verdaguer et al., 1997). This suggests that initiation of T1D requires a β cell insult and the rapid recruitment of autoreactive CD8 T cells, one or both steps being CD4 dependent. The role of CD4 T cells in the activation of CD8 T cells is probably not restricted to the induction of β cell damage and likely involves the activation of APCs, possibly via ligation of CD40 by CD154 (Figure 1). CD40 engagement endows APCs with the ability to costimulate CD8 T cells and to foster their differentiation. Indeed, stimuli that activate DCs, such as agonistic anti-CD40 mAb or CpG
CD8 T cell-induced autoimmunity might be inhibited at the systemic level by depletion of APC types capable of cross-presenting target cell-derived peptides, by depletion of antigen-activated T-helper cells (explosives), or by potentiating the suppressor activity of regulatory T cells (ambulance). Strategies that might be effective at the regional level include costimulatory blockade, inhibition of CD8 T cells trafficking to the target tissue or of antigen-loaded APCs to the regional lymphoid tissue, inhibition of death effector pathways, and inhibition of apoptotic cell capture by local APCs (stop sign).

DNA, uncouple the need for CD4 T cell help in the activation and recruitment of NRP-reactive CD8 T cells in vivo (Amrani et al., 2002). In the RIP–LCMV model, the activation of low-avidity CD8 T cells by LCMV infection is sufficient to induce diabetes. This may occur by activation of the local APCs, as CD80 expression in the pancreas could also prime for T1D.

The role of localized inflammation in diabetes has been studied using a double transgenic model in which RIP–CD80 is stably expressed in the islets, whereas local expression of TNFα can be rapidly repressed with tacrycline (Green and Flavell, 2000). On a C57BL/6 background, neither the presence of TNFα nor CD80 alone gives rise to diabetes. When TNFα is repressed in the RIP–CD80 transgenic mice after postnatal day 30, a CD8-dependent diabetes occurs quickly, and when it is repressed at day 25, diabetes develops with delayed kinetics. However, if TNFα is repressed at day 21, diabetes is prevented. There is apparently a critical time window in this model that determines whether autoreactive CD8 T cells differentiate into fully diabetogenic effectors. During this time window, highly potent CD4+ CD25+ regulatory T cells accumulate in the pancreatic islets and lymph nodes if TNFα is repressed at day 25 (Green et al., 2002). If this process can be generalized, it would suggest that short-term local inflammation promotes generation and activation of regulatory T cells, whereas prolonged inflammation somehow allows autoreactive CD8 T cells to overcome these regulatory events.

Effector Pathways in CD8 Autoimmunity

CTL can directly kill target cells through at least two different pathways. The differential roles of Fas–Fas ligand- and perforin-induced β cell damage in diabetes are controversial; their relative importance may be dependent on the etiology. Studies in the LCMV model of T1D suggested a major role for perforin in β cell death. Using a similar model, Seewald et al. (2000) have argued for a major role for cytokines as (direct or indirect) effectors of β cell death in virus-induced T1D. In contrast, studies of perforin− or Fas−deficient NOD mice have also yielded apparently contradictory results: perforin−deficient NOD mice develop severe insulinopenia but rarely become diabetic, and NOD/−pr mice develop neither diabetes nor insulinopenia despite expressing perforin. It is likely that in the NOD mouse both pathways are important but not necessarily involved to the same extent at all stages of disease, with evidence suggesting that Fas−Fas ligand interactions are important in the earlier phases and other mechanisms of cell damage become progressively more important. In the NRP-specific TCR transgenic NOD mice, autoreactive CD8 T cells kill β cells exclusively via Fas. The Fas−dependent lysis of β cells is significantly enhanced by a number of cytokines, including IL-1α, IL-1β, TNFα, and/or IFN-γ. Interestingly, IL-1 selectively induces Fas expression on β cells but not on other islet cells. Accordingly, 90% of the β cells but few α cells of pancreatic tissue from acutely diabetic patients express Fas (Moriwaki et al., 1999). Fas and its ligand, when
simultaneously expressed on thyroid follicular cells, have also been proposed to play a detrimental role in Hashimoto’s thyroiditis. Thus, the Fas-FasL interaction has the potential to kill target cells as innocent bystanders, a mechanism used by autoreactive CD4 T cells to kill β cells. Although CD8 T cells can use Fas-dependent lysis to damage β cells, whether CD8 T cells can also kill target cells as bystanders or whether Fas-dependent lysis by CD8 T cells requires a cognate interaction has yet to be formally demonstrated.

The molecular nature of the recognized autoantigens may influence the choice of the cytotoxicity pathway that is employed by CD8 T cells in a given autoimmune disorder. The avidity of the T cells involved at different stages of disease may be another contributing factor, since Fas-mediated cytotoxicity may be elicited at a lower threshold of avidity (of target cell-CTL interactions) than perforin-mediated cytotoxicity. Alternatively, β cells may change their susceptibility to Fas- versus perforin-mediated cytotoxicity as the disease progresses, such as by undergoing changes in their ability to bind and/or internalize perforin or granzyme B (Motyka et al., 2000) or in their ability to activate effector caspases via different signaling cascades. It is possible that diabetogenesis in NOD mice is initiated by CD4 and CD8 clones capable of lysing β cells exclusively via Fas and later amplified by clones that can kill via other death effector pathways, including perforin. Fas-FasL interactions also appear to play a critical role in the induction and progression of EAE but not in its effector phase (Suvannavejh et al., 2000). Certain cell types, such as neurons, display greater susceptibility to Fas- than to perforin-mediated lysis (Medana et al., 2000). Therefore, cytotoxicity may be involved at different stages of an autoimmune process via different mechanisms (Figure 1).

Activated CD8 T cells can produce very high levels of TNFα and IFN-γ, which may contribute directly and/or indirectly to target cell destruction in autoimmune diseases (Figure 1). When expressed in the islets of prediabetic NOD mice, TNFα enhances the presentation of β cell self-antigens to diabetogenic CD8 T cells by triggering TNFR on APCs (Green et al., 2000) and, possibly, islet cells (Pakala et al., 1999). β cells express low levels of TNFR1 constitutively, but express both TNFR1 and TNFR2 during inflammation. TNFα also plays an effector role in inflammatory bowel disease (IBD) and RA, although its cellular source is by no means restricted to CD8 T cells. Mice expressing elevated levels of TNFα develop arthritis and IBD. Furthermore, TNFR2-Ig fusion proteins or humanized anti-TNFα mAbs can ameliorate IBD and RA patients. In addition, TNFα can damage the myelin sheath and can induce oligodendrocyte apoptosis, suggesting an effector role for TNFα-TNFR1 interactions in the pathogenesis of EAE and MS. This view, however, is at odds with observations suggesting that TNFα plays an anti-inflammatory role in EAE and that MS activity is exacerbated in patients receiving TNFα-blocking therapy. TNF family members are also powerful paracrine inducers of other cytokotoxic cytokines, such as IL-1α and IL-1β, resulting in increased NO production. NO is directly responsible for inducing necrosis of β

| Table 1. Strategies to Further Investigate the Role for CD8 T Cells in Human Autoimmune Diseases |
|------------------------|----------------------------------------------------------|
| **Tools**               | **Objectives**                                           |
| Reverse immunology approach (in vitro proteasome digestion of candidate autoantigens, mass spectrometry-sequencing analysis of the generated peptides, sensitization of target cells with the digests) | Identify naturally processed immunogenic self-peptides |
| Microdissection of target tissue (laser capture dissection) | Analyze TCRβ (and α?) rearrangement of infiltrating CD8 T cells at the single-cell level |

**Strategies to Characterize the Antigen Specificity and Function of Disease-Relevant CD8 T Cells**

- Expansion/cloning of tissue-infiltrating CD8 T cells. Further characterization using candidate antigens, synthetic peptide libraries, or target tissue/cell cDNA expression libraries
- Microdissection of target tissue (laser capture dissection)

**Strategies to Characterize Autoreactive CD8 T Cells**

- HLA class I-self-peptide multimers
- Molecular analysis at the single-cell level (ELISPOT, FACS analysis, RT-PCR)
- Characterization of TCR usage by autoreactive CD8 T cells (sorting of tetramer+ or cytokine+ T cells and evaluation of TCR usage by quantitative PCR, Immunoscope, and sequencing)
cells in response to IL-1β or to combinations of TNFα, IFN-γ, and IL-1β but is not required for cytokine-induced β cell apoptosis. Thus, TNF-TNFR interactions can also indirectly lead to necrosis.

Much interest has centered on the role of IFN-γ in T1D. The importance of this cytokine depends on the model and the stage of diabetes. IFN-γ is likely to have a number of pathogenic effects, including the upregulation of MHC class I on the islets and facilitating the homing of CD8 T cells to the islets. Although IFN-γR1 knockout mice do not develop diabetes, both IFN-γR1 and IFN-γR2 knockout mice do so with only slightly decelerated kinetics, suggesting that IFN-γ is dispensable. This contrasts with the LCMV model of diabetes where IFN-γ appears to play a crucial role, probably together with other cytokines (Weiswald et al., 2000).

Future Therapeutic Prospects

The mechanisms leading to tissue destruction obviously depend on the particular disease but may also vary between patients with a given autoimmune disease. A major task is to precisely define which immunological pathways are elicited in a given patient and to establish general patterns. Easy access to tools allowing the quantification, sorting, and analysis of antigen-specific CD8 T cells should help identify immunodominant self-peptides and determine whether they are shared by patients bearing the same MHC class I alleles (Table 1). If so, a number of approaches might be applied to promote antigen-specific tolerance (Figure 1). Some of these approaches, such as systemic administration of agonist peptides or soluble MHC-peptide complexes, have been validated in animal models of autoimmune diseases and result in deletion or anergy of autoreactive CD8 T cells. The short half-life of peptides in circulation and the need for repeated administrations are but some of the limitations of peptide therapy in chronic autoimmune disorders. Another problem is the choice of both the type and the dose of peptide. It is generally assumed that the peptide affinity for MHC is the primary determinant of the tolerogenic activity, such that high affinity for MHC and high doses are preferred over low affinity and low doses. Studies employing high and low affinity mimics of NRP in NOD mice have shown that the affinity of the MHC:peptide complex for T cells is another critical variable, particularly under circumstances where the target population comprises both high- and low-avidity T cells. For example, high doses of low-affinity ligands or low doses of high-avidity ligands are effective, but low doses of low-affinity ligands accelerate disease by activating high-avidity T cells. This is akin to the activation of MBP-reactive CD4 T cells and disease exacerbation in some MS patients treated with a mutant MBP peptide (Bielekova et al., 2000). Furthermore, the effects of inactivation of a given T cell population on the size of competing autoreactive T cell subpopulations is unknown and may be counterproductive. Refinements based on the previous approaches have recently been generated, such as use of antigenic peptide covalently linked to β2-microglobulin, which may prolong the half-life of the relevant MHC:peptide complex on tolerogenic APCs. The use of multimeric MHC:peptide complexes, with or without α3 mutation to prevent CD8 ligation, is yet another alternative (Xu et al., 2001). These multimers target specific CD8 T cells for apoptosis without eliciting CTL activity. Whether these strategies are useful in vivo remains to be determined. The route of administration is clearly another important consideration, as oral tolerance with MHC class I binding self-peptides has little, if any, beneficial effect in CD8-mediated models of T1D.

In many instances, however, the antigen specificity of the pathogenic CD8 T cells is not known or the disease has already progressed to a stage where antigen-specific therapy may be inefficient. Early treatment with depleting anti-CD8 mAbs or other molecules inhibiting CD8 T cell activation or tissue transmigration might hold promise. In this regard, a potential mechanism to block CD8 T cell-mediated disease is to interfere with costimulatory or T-helper-assisted differentiation of naive CD8 T cells into effectors (Figure 1). Although direct blockade of the CD28-CD80/CD86 costimulatory pathway by soluble CTLA-4 may be effective in some instances, these strategies carry the risk of abrogating the natural immunosuppressive action of regulatory T cells (Salomon et al., 2000). These CD4+ "CD25+" regulatory T cells have the potential to control CD8 T cell activation in vitro and in vivo (Green et al., 2002). Clearly, a more precise understanding of the mechanisms underlying the collaboration between CD4 and CD8 T cells as well as the regulation of autoreactive CD8 T cell activation in autoimmune diseases is required to fully understand the implications of these different therapeutic strategies.

Blockade of inflammatory cell recruitment and neutralization of pathways used by CD8 T cells (and by other immune cell types) to effect tissue damage could also be used (Figure 1). Perhaps the best example of a T cell cytokine whose blockade has had clear therapeutic benefit in some, but not all, autoimmune inflammation is TNFα. Another non-antigen-specific strategy to interfere with CD8 T cell-mediated disease would be to inhibit their recruitment to the target tissue, such as by blocking molecules involved in chemotraction, rolling, adhesion to postcapillary endothelium, and transmigration. While a considerable amount of information has been obtained with respect to the role of CD8 T cells in experimental autoimmune diseases, much work remains to be done to define if pathogenesis of the human disease equivalents occurs in a similar manner. Combination therapy or approaches aiming at targeting both autoreactive CD4 and CD8 T cells will, in all likelihood, be more efficacious than sharply focused strategies. Investigating therapeutic options in the experimental models will hopefully ultimately provide new directions for treatment of many of these diseases, which are increasing in frequency.

Selected Reading

Babbe, H., Roers, A., Waisman, A., Lassmann, H., Goebels, N., Hohl-