

Pivotal roles of CD8⁺ T cells restricted by MHC class I–like molecules in autoimmune diseases

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Unlike T cells restricted by major histocompatibility complex (MHC) class Ia or class II molecules, T cells restricted by MHC class I–like molecules demonstrate properties of both innate and adaptive immunity and are therefore considered innate–like lymphocytes (ILLs). ILLs are believed to have immunoregulatory functions, but their roles in autoimmunity and defense against infections remain elusive. To study the properties of ILLs, we generated mice expressing only MHC class I–like molecules by crossing *CIITA*^{−/−} with *K^b−/−D^b−/−* mice. Surprisingly, these mice developed a lymphoproliferative syndrome and autoimmunity, most notably inflammatory bowel disease (IBD) and insulinitis. The CD8⁺ ILLs in these mice exhibit a constitutively activated phenotype, and depletion of these cells abolished the autoimmune disorders. In addition, adoptive transfer of CD8⁺ ILLs from *K^b−/−D^b−/−CIITA*^{−/−} mice to *Rag-1*^{−/−pfn}^{−/−} mice also resulted in IBD and insulinitis. These findings provide direct evidence that CD8⁺ ILLs are sufficient to initiate and mediate autoimmune diseases.

MHC class Ia–restricted CD8⁺ T cells are the classic effector cells involved in cytotoxicity during adaptive immune responses. In addition to MHC class Ia, the mammalian genome encodes proteins with homology to the α chain of MHC class Ia molecules, which are often referred to as MHC class I–like molecules. These proteins are involved in the selection and maintenance of innate–like lymphocytes (ILLs), including NKT cells and MHC class Ib–restricted CD8⁺ T cells, which exhibit an activated memory phenotype and are thought to have critical immunomodulatory functions (1–5). By using genetically modified mice, recent studies have shown that NKT cells, restricted by the MHC class I–like molecule CD1d, play a central role in the pathogenesis of Th2 cell–driven immune disorders such as ulcerative colitis (6) and asthma (7). Importantly, NKT cells rapidly produce large amounts of IL-4 and IL-13 upon activation.

CD8⁺ T cells restricted by MHC class Ib molecules have an activated phenotype (8) and promptly produce cytokines upon activation (2), which are typical characteristics of ILLs. These cells also have a variety of immune regulatory functions, such as suppressing the activity of CD4⁺ T cells (9, 10), promoting differentiation of Th1 cells (2), and up–regulating immunity to infections (11–14) and tumors (15). Thus, like NKT cells, these MHC class Ib–restricted CD8⁺ T cells have the characteristics of ILLs. These cells may serve as a bridge between innate and adaptive immunity, especially in helping the differentiation of Th1 cells by producing IFN- γ at the early stage of an immune response. Because CD8⁺ ILLs exhibit an activated phenotype immediately after their maturation in the thymus (8), these cells are likely primed by and specific to self–antigens, suggesting that these cells might play a role in the pathogenesis of autoimmunity. Under normal physiological conditions, the number of ILLs is limited, and these cells are relatively inert, probably because the activity of these cells

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Abbreviations used: CFSE, carboxyfluorescein diacetate succinimidyl ester; H&E, hematoxylin and eosin; HLA, histocompatibility leukocyte antigen; IBD, inflammatory bowel disease; ILL, innate–like lymphocyte; Tg, transgenic.

The online version of this article contains supplemental material.

is controlled by regulatory mechanisms. We therefore hypothesized that, upon release from immune regulation, ILLs can initiate inflammation and autoimmunity. This possibility is supported by earlier studies, which showed that MHC class II-deficient mice exhibit autoimmune-like syndromes (16, 17). The pathogenesis of autoimmunity in these animals is likely initiated by MHC class I-restricted T cells that are normally suppressed by CD4⁺ T cells. The identity of the CD8⁺ T cells that cause inflammation in these MHC class II-deficient mice is unclear.

Experiments regarding the immunological functions of ILLs have been hampered by the lack of specific markers for these cells. To circumvent this problem, we have used a genetic approach to generate mice that exclusively express MHC class I-like molecules. However, generating mice that are deficient in K^b, D^b, and IA^b genes (C57BL/6 mice do not express IE and L molecules) cannot be easily accomplished, because these genes are clustered on the H-2 locus of chromosome 17. We therefore used CIITA^{-/-} mice, which fail to express MHC class II molecules and produce few mature

CD4⁺ T cells (18), except for CD1d-restricted CD4⁺ NKT cells. Thus, we crossed K^{b-/-}D^{b-/-} with CIITA^{-/-} mice to generate mice lacking both MHC class II and Ia molecules (K^{b-/-}D^{b-/-}CIITA^{-/-}), so that they only express MHC class I-like molecules. To ensure that the T cells that developed in K^{b-/-}D^{b-/-}CIITA^{-/-} mice are indeed restricted by MHC class I-like molecules, we also generated β₂m^{-/-}CIITA^{-/-} mice, which are devoid of all MHC class I, class I-like, and class II molecules. We found that K^{b-/-}D^{b-/-}CIITA^{-/-} mice, but not β₂m^{-/-}CIITA^{-/-} mice, exhibited lymphoproliferative disease with an abnormal expansion of activated CD8⁺ T cells, spontaneous inflammatory bowel disease (IBD), and insulinitis. Adoptive transfer of CD8⁺ T cells from K^{b-/-}D^{b-/-}CIITA^{-/-} mice to syngeneic RAG^{-/-} pfn^{-/-} mice induced intestinal inflammation and insulinitis. Furthermore, depletion of CD8⁺ T cells from K^{b-/-}D^{b-/-}CIITA^{-/-} mice failed to show symptoms of IBD or insulinitis, suggesting that T cells selected by MHC class I-like molecules are self-reactive, pathogenic, and capable of initiating and sustaining autoimmune inflammation.

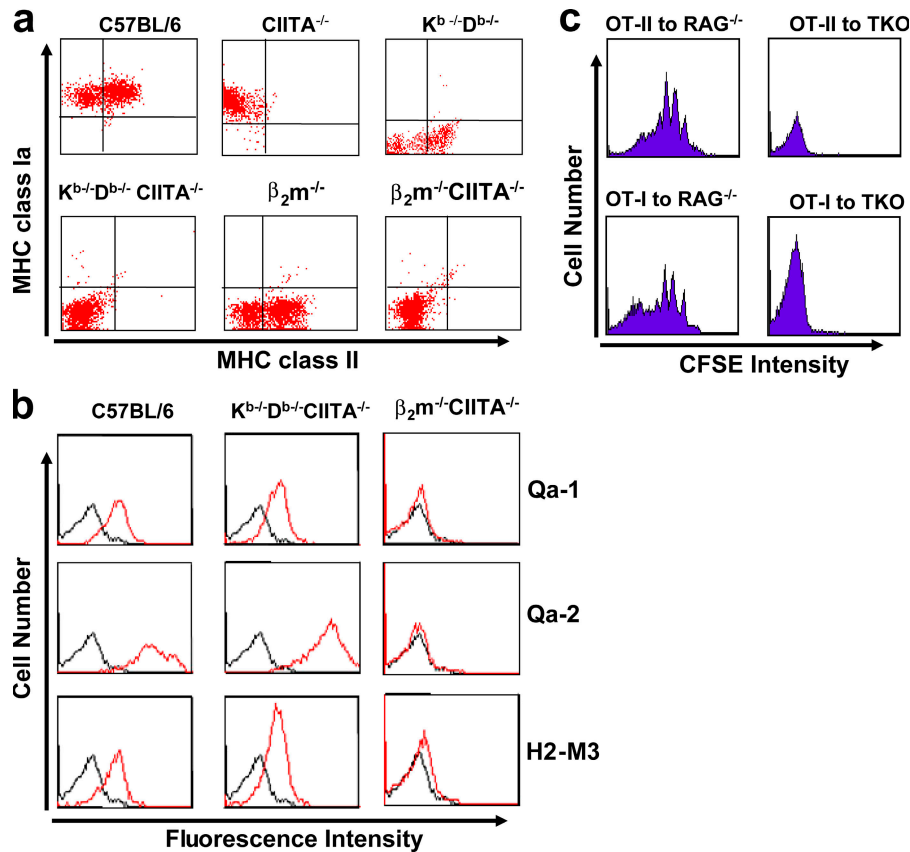


Figure 1. Expression of distinct MHC molecules on splenocytes. (a) Splenocytes from the indicated mouse strains were stained with 28-8-6 and 25-9-17 antibodies. K^{b-/-}D^{b-/-}CIITA^{-/-} and β₂m^{-/-}CIITA^{-/-} mice did not express MHC class Ia or MHC class II molecules. (b) Expression of Qa-1^b, Qa-2, and H2-M3 on splenocytes as revealed by staining with 6A8.6F10.1A, 1-1-2, and H2-M3.130 antibodies, respectively. Black lines represent isotype

control and red lines show expression of class I-like molecules. (c) MHC class II-restricted CD4⁺ T cells or MHC class Ia-restricted CD8⁺ T cells cannot survive in K^{b-/-}D^{b-/-}CIITA^{-/-} (TKO) mice. TCR Tg CD4⁺ T cells (OT-II) or CD8⁺ T cells (OT-I) were purified, CFSE stained, and adoptively transferred to RAG-2^{-/-} or K^{b-/-}D^{b-/-}CIITA^{-/-} mice. 1 wk later, spleens of these mice were evaluated for the presence of CFSE-labeled T cells.

RESULTS

 K^b -/- D^b -/- $CIITA$ -/- mice express MHC class I-like molecules but do not support the survival of MHC class Ia- or class II-restricted T cells

Double staining with 28-8-6 and 25-9-17 antibodies, which recognize MHC class Ia (both K^b and D^b) and class II IA^b proteins, respectively, showed that splenocytes from K^b -/- D^b -/- $CIITA$ -/- and β_2m -/- $CIITA$ -/- mice lack surface expression of MHC class Ia and class II IA^b molecules (Fig. 1 a). Expression of MHC class I-like molecules, including $Qa-1^b$, $Qa-2$, and H2-M3, was examined by staining with 6A8.6F10.1A, 1-1-2, and H2-M3.130 antibodies, respectively. K^b -/- D^b -/- $CIITA$ -/- mice expressed these MHC class I-like molecules at similar levels as wild-type B6 mice, whereas these molecules were undetectable in β_2m -/- $CIITA$ -/- mice (Fig. 1 b). To demonstrate that these mice were functionally devoid of MHC class Ia and class II molecules, we adoptively transferred K^b -/- D^b -/- $CIITA$ -/- mice with carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled transgenic (Tg) OT-II $CD4^+$ or OT-I $CD8^+$ T cells and investigated the survival of these cells 1 wk later. By tracking CFSE-labeled cells, or by using clonotypic antibodies specific for OT-II or OT-I Tg T cells, we failed to detect any of these cells in K^b -/- D^b -/- $CIITA$ -/- mice 1 wk after adoptive transfer. However, both OT-II and OT-I cells survived and exhibited cell division in RAG -/- pfk -/- mice (Fig. 1 c). Therefore, K^b -/- D^b -/- $CIITA$ -/- mice are devoid of functional MHC class Ia and class II molecules and T cells restricted by these molecules. These mice express only MHC class I-like molecules and thus only harbor T cells restricted by MHC class I-like molecules, providing a unique opportunity to study the function of ILLs.

 K^b -/- D^b -/- $CIITA$ -/- mice exhibit inflammation in the gut and pancreas

At 12 wk of age, all K^b -/- D^b -/- $CIITA$ -/- mice ($n = 32$) exhibited enlarged mesenteric lymph nodes, distended and highly visible Peyer's patches, and swollen intestines, as depicted in Fig. 2 a, closely resembling IBD. Histologically, there was moderate to severe inflammation characterized by infiltration of mononuclear cells (Fig. 2 b). In contrast, K^b -/- D^b -/-, $CIITA$ -/-, and β_2m -/- $CIITA$ -/- mice ($n = 24$), showed no signs of intestinal inflammation, both by gross examination and by histological analysis.

In humans, the histocompatibility leukocyte antigen (HLA)-E locus is associated with susceptibility to type I diabetes (19). In mice, pancreatic β cells have been found to express the HLA-E homologue $Qa-1^b$, presenting a nonameric peptide derived from the preproinsulin leader sequence. This complex is recognized by $Qa-1^b$ -restricted $CD8^+$ T cells and lyses pancreatic β cells (20). Thus, $CD8^+$ T cells restricted by MHC class I-like molecules may contribute to the inflammation in diabetes. We therefore examined K^b -/- D^b -/- $CIITA$ -/- mice for the development of insulinitis. Gross examination revealed enlarged and fragile pancreata (not depicted), and histological sections revealed characteristic lymphocyte

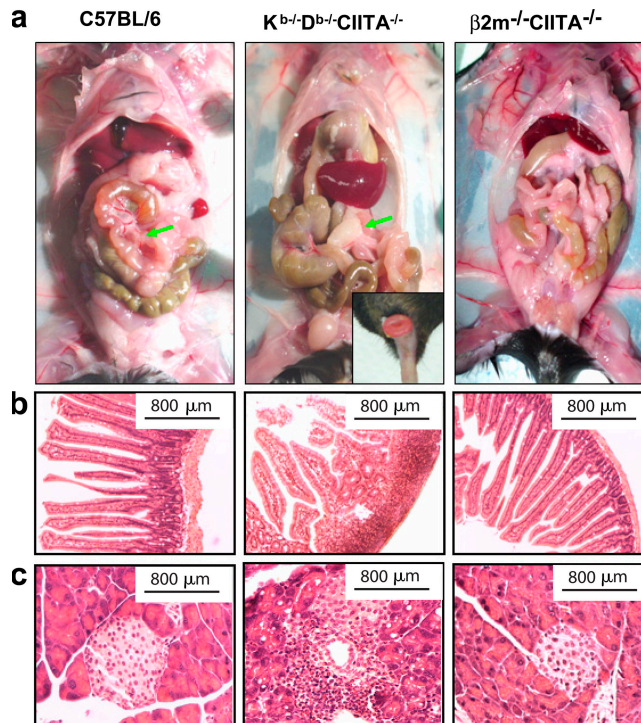


Figure 2. K^b -/- D^b -/- $CIITA$ -/- mice show signs of intestinal inflammation and insulinitis. (a) K^b -/- D^b -/- $CIITA$ -/- mice showed swollen intestines and enlarged mesenteric lymph nodes (green arrow). (middle, inset) Rectal prolapse is shown. (b) Small intestine cross sections. H&E staining shows moderate to severe inflammation in K^b -/- D^b -/- $CIITA$ -/- mice, which was absent in control mice. Bar, 800 μ m. (c) H&E-stained pancreatic sections show insulinitis in K^b -/- D^b -/- $CIITA$ -/- mice but not in control mice. Bar, 800 μ m.

infiltration in the islets, indicative of the onset of autoimmune type I diabetes (Fig. 2 c).

 K^b -/- D^b -/- $CIITA$ -/- mice exhibit lymphoproliferative disease

Lymphoid organs such as spleens and lymph nodes of K^b -/- D^b -/- $CIITA$ -/- mice were dramatically enlarged, although thymi were normal in size (unpublished data). We found that K^b -/- D^b -/- $CIITA$ -/- mice produced few $CD4^+$ T cells but a large number of $CD8^+$ T cells in their spleens. In contrast, β_2m -/- $CIITA$ -/- mice had virtually no mature $CD4^+$ or $CD8^+$ T cells (Fig. 3, a and b). Because T cells are required for most autoimmune disorders, we analyzed T cell subsets in the spleens of K^b -/- D^b -/- $CIITA$ -/- mice. The numbers of $CD44^{hi}CD8^+$ T cells were dramatically increased, whereas $CD8^+$ T cells with a naive phenotype were virtually absent (Fig. 3 b). As MHC class II molecules are absent in these mice, we determined the phenotype of the few $CD4^+$ T cells in K^b -/- D^b -/- $CIITA$ -/- mice. Staining with anti-DX5, a marker for NK and NKT cells, suggested that most $CD4^+$ cells in K^b -/- D^b -/- $CIITA$ -/- mice are NKT cells (Fig. 3 c). Therefore, K^b -/- D^b -/- $CIITA$ -/- mice lack mature MHC class II-restricted $CD4^+$ T cells. Collectively, these data suggest that the large number of $CD8^+$ T cells in

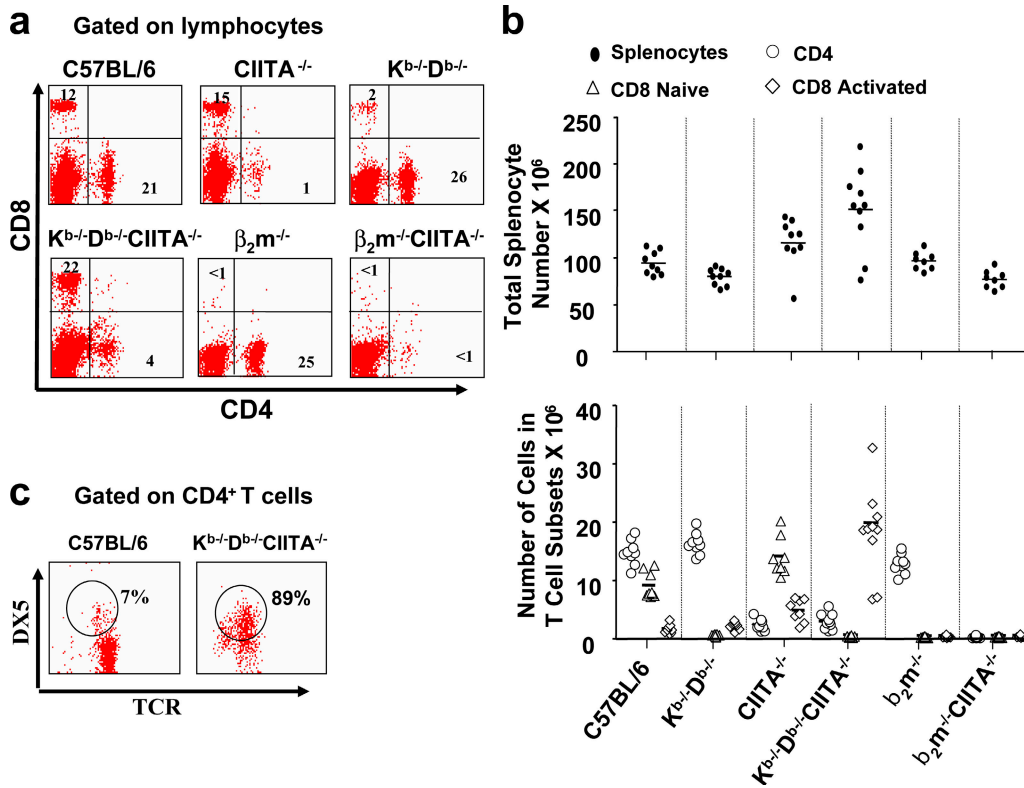


Figure 3. Increased numbers of CD8⁺ T cells in secondary lymphoid organs of K^{b-/-}D^{b-/-}CIITA^{-/-} mice. (a) Spleen cells from the indicated mouse strains were stained for anti-CD4 and anti-CD8 antibodies. Relative percentages of CD4 and CD8 cells are analyzed on the basis of forward/side scatter-based lymphocyte gating. (b) Total number of

spleen cells was counted using a hemocytometer (Bright-Line, Hauser Scientific). Different subsets of T cells are calculated from the percentage multiplied by the total number of cells. Horizontal lines represent means. (c) Spleen cells were stained with anti-CD4, anti-DX5, and anti-TCRαβ antibodies. Most CD4⁺ T cells in K^{b-/-}D^{b-/-}CIITA^{-/-} mice are NKT cells.

K^{b-/-}D^{b-/-}CIITA^{-/-} mice are selected and maintained by MHC class I-like molecules.

CD8⁺ T cells in K^{b-/-}D^{b-/-}CIITA^{-/-} mice are activated spontaneously

Because CD8⁺ T cells restricted by MHC class I-like molecules are ILLs (21), we investigated activation markers on CD8⁺ T cells of K^{b-/-}D^{b-/-}CIITA^{-/-} mice. The majority of CD8⁺ T cells have an activated phenotype (CD44^{hi}, β7integrin^{low}, CD122^{hi}, and Ly6C^{hi}; Fig. 4 a), in agreement with previous studies indicating that CD8⁺ T cells are constitutively activated in naive K^{b-/-}D^{b-/-} animals (8, 22). Therefore, CD8⁺ T cells in K^{b-/-}D^{b-/-}CIITA^{-/-} mice appear to be activated without exposure to exogenous antigens, indicating that these T cells are likely primed by self-antigens. To determine the organs where these cells are primed, we examined the thymus and found that a large proportion of the thymic single-positive CD8⁺ T cells already exhibited an activated phenotype (Fig. 4 a). However, no abnormal expansion of thymic single-positive CD8⁺ T cells was found in K^{b-/-}D^{b-/-}CIITA^{-/-} mice (Fig. 4 b). The absolute numbers of single-positive CD8 T cells in the thymus in wild-type and K^{b-/-}D^{b-/-}CIITA^{-/-} mice are not increased (Table S1, available at <http://www.jem.org/cgi/content/>

full/jem.20060936/DC1). This suggests that the elevated CD8⁺ T cell number found in the periphery is not a result of enhanced thymic selection. Instead, it is likely that CD8⁺ T cells selected on MHC class I-like molecules in the thymus are activated by self-antigens and migrate to the peripheral lymphoid organs, where they expand by hyperlymphoproliferation. Interestingly, we observed that a large proportion of these cells also expresses NKG2c (Fig. 4 c), an activating NK cell receptor. Expression of NKG2c has recently been associated with inflammation and production of inflammatory cytokines by HLA-E-restricted ILLs in human celiac disease (23). Although this finding is consistent with the innate-like property of pathogenic CD8⁺ T cells in K^{b-/-}D^{b-/-}CIITA^{-/-} mice, NKG2c is not a marker for ILLs because it is acquired only during the progression of inflammation (23). Nevertheless, expression of NKG2c by CD8⁺ T cells in K^{b-/-}D^{b-/-}CIITA^{-/-} mice again confirmed the activated phenotype of these cells.

To examine the intrinsic proliferation of CD8⁺ T cells in thymus and peripheral lymphoid organs, we performed a BrdU incorporation experiment in vivo. We found that an increased number of CD8⁺ T cells in the thymus and spleen in K^{b-/-}D^{b-/-}CIITA^{-/-} mice, as compared with wild-type C57BL/6 mice, incorporated BrdU (Fig. 4 d). Therefore, the

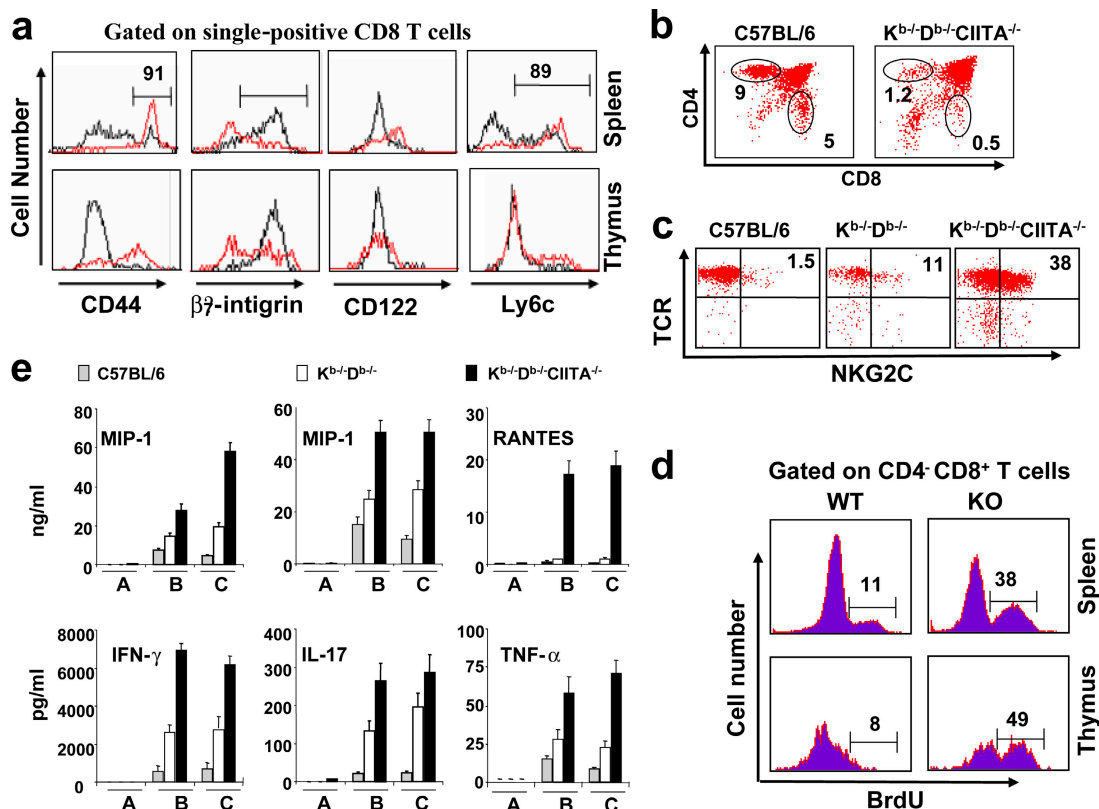


Figure 4. Characteristics of CD8⁺ T cells in *K^b-/-D^b-/-CIITA^{-/-}* mice. (a) Cells from spleen (top) and thymus (bottom) of C57BL/6 (black line) and *K^b-/-D^b-/-CIITA^{-/-}* (red line) mice were stained with anti-CD8 antibodies and antibodies directed against various activation markers as indicated. CD8⁺ T cells in *K^b-/-D^b-/-CIITA^{-/-}* mice are activated in both spleen and thymus. (b) Staining with anti-CD4 and anti-CD8 antibodies shows no abnormal expansion of single-positive cells in thymus. (c) Spleen cells from wild-type, *K^b-/-D^b-/-*, and *K^b-/-D^b-/-CIITA^{-/-}* mice were stained with anti-CD8 α , anti-TCR β , and anti-NKG2A/B/C antibodies. The cells shown are gated on CD8 α ⁺ cells. (d) Spleen cells or thymocytes

from wild-type or *K^b-/-D^b-/-CIITA^{-/-}* mice that were fed BrdU for 9 d were stained with anti-CD4, anti-CD8 α , and anti-BrdU. Incorporation of BrdU in CD8⁺ T cells was determined by gating on CD4⁺CD8⁺ cells. The numbers in a–d represent the percentages of cells in the total displayed populations. (e) Purified CD8⁺ T cells from wild-type, *K^b-/-D^b-/-*, and *K^b-/-D^b-/-CIITA^{-/-}* mice were activated overnight on plate-bound anti-CD3 and anti-CD28 antibodies in the absence (B) and presence of IL-2 (C) and compared with unstimulated cells (A). Cytokines in the supernatants were measured by the Luminex multiplex cytokine assay. Error bars represent means \pm SEM.

hyperproliferation of CD8⁺ T cells in these animals is already initiated in the thymus and continues in the periphery.

Cytokines and chemokines produced by CD8⁺ T cells of *K^b-/-D^b-/-CIITA^{-/-}* mice

To investigate cytokine and chemokine production by CD8⁺ T cells in *K^b-/-D^b-/-CIITA^{-/-}* mice, we activated sorted CD8⁺ T cells from B6, *K^b-/-D^b-/-*, and *K^b-/-D^b-/-CIITA^{-/-}* mice with plate-bound anti-CD3 and anti-CD28 antibodies. We found a considerable increase in IFN- γ , TNF- α , IL-17, macrophage inflammatory protein 1 α and 1 β , and RANTES for CD8⁺ T cells from *K^b-/-D^b-/-CIITA^{-/-}* mice (Fig. 4 e) but no change in IL-4, IL-6, and IL-10 (unpublished data). Interestingly, although the levels of inflammatory cytokines produced by CD8⁺ T cells isolated from *K^b-/-D^b-/-* mice are higher than C57BL/6 mice, they remain substantially lower than *K^b-/-D^b-/-CIITA^{-/-}* mice. It is possible that the CD8⁺ T cells in *K^b-/-D^b-/-* mice are

regulated by CD4⁺ T cells and that their activation potential is influenced by the presence of CD4⁺ T cells. Supplementation of IL-2 to the culture medium did not change the cytokine production pattern of CD8⁺ T cells from *K^b-/-D^b-/-* mice. This observation suggests that abrogation of acquired tolerance in CD8⁺ T cells of *K^b-/-D^b-/-* mice is independent of IL-2. The mechanisms controlling the tolerance of these pathogenic T cells are currently being investigated.

Surprisingly, we found that splenocytes from *K^b-/-D^b-/-CIITA^{-/-}* mice activated with anti-CD3 antibodies produced large amounts of IL-12 (unpublished data). T cells generally do not produce IL-12. However, it is conceivable that high levels of IFN- γ , produced by the CD8⁺ T cells restricted by MHC class I-like molecules, are responsible for activating IL-12-producing cells such as macrophages. To test this hypothesis, we activated splenocytes with anti-CD3 overnight and performed intracellular staining of IL-12 in combination with surface marker staining. We found that

CD11b⁺ cells, but not CD8⁺ T cells, showed strong intracellular IL-12 expression (Fig. S1, available at <http://www.jem.org/cgi/content/full/jem.20060936/DC1>). Neutralization of IFN- γ with a specific antibody inhibited IL-12 production by CD11b⁺ cells. This is in agreement with our earlier study showing that anti-CD3-induced IL-12 production in vivo is mediated by the early burst of IFN- γ from CD8⁺ T cells restricted by MHC class I-like molecules (2). These findings provide direct evidence that, in the absence of conventional T cells, CD8⁺ T lymphocytes restricted by MHC class I-like molecules are pathogenic and sufficient to mediate autoimmune diseases. To determine whether the accumulation of peripheral CD8⁺ T cell is caused by clonal expansion, we tested the V β usage of CD8⁺ T cells in K^b-/-D^b-/-CIITA^{-/-} and corresponding wild-type mice. Although these cells expressed a variety of TCR β chains, we were unable to detect T cells expressing V β 9, V β 10, and V β 14. We observed a substantial increase in the usage of V β 2, V β 6, V β 8.1,2, and V β 13 (Table S2, available at <http://www.jem.org/cgi/content/full/jem.20060936/DC1>). Thus, CD8⁺ T cells restricted by MHC class I-like molecules bear activated phenotypes, produce increased amounts of inflammatory cytokines, and have a biased V β repertoire.

CD8⁺ T cells in K^b-/-D^b-/-CIITA^{-/-} mice are pathogenic

To verify the role of CD8⁺ T cells in the pathological changes in K^b-/-D^b-/-CIITA^{-/-} mice, we adoptively transferred CD8⁺ T cells from K^b-/-D^b-/-CIITA^{-/-} mice to RAG^{-/-}pfn^{-/-} mice. Within 3 wk, recipient RAG^{-/-}pfn^{-/-} mice developed intestinal and pancreatic inflammation (Fig. 5, a and b), whereas adoptive transfer of CD8⁺ T cells from normal mice did not induce such changes. Furthermore, we depleted CD8⁺ T cells from K^b-/-D^b-/-CIITA^{-/-} mice using monoclonal anti-CD8 antibodies (Fig. 5, c and d). These mice also failed to exhibit intestinal or pancreatic inflammation.

DISCUSSION

Our studies have revealed that mice bearing only MHC class I-like molecules produce predominantly activated CD8⁺ T cells that secrete considerably more inflammatory cytokines than CD8⁺ T cells from wild-type mice. Therefore, it is likely that CD8⁺ T cells restricted by MHC class I-like molecules are pathogenic and mediate the observed autoimmunity in K^b-/-D^b-/-CIITA^{-/-} mice. Adoptive transfer of CD8⁺ T cells from K^b-/-D^b-/-CIITA^{-/-} mice induced colonic and pancreatic inflammation, whereas

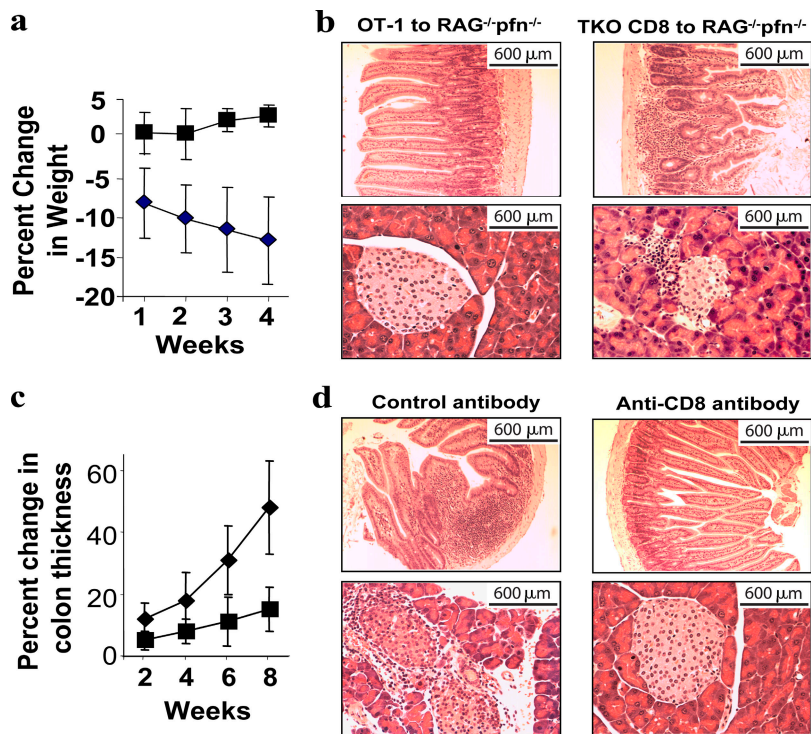


Figure 5. CD8⁺ T cells from K^b-/-D^b-/-CIITA^{-/-} mice induce intestinal inflammation upon adoptive transfer. (a) Sorted CD8⁺ T cells from K^b-/-D^b-/-CIITA^{-/-} (◆) or OT-1 TCR Tg (■) mice were adoptively transferred to RAG^{-/-}pfn^{-/-} mice. Recipients of CD8⁺ T cells from K^b-/-D^b-/-CIITA^{-/-} mice showed progressive weight loss. Error bars represent means \pm SEM. (b) Intestinal (top) and pancreatic (bottom) cross sections revealed ongoing inflammation in mice that received cells from K^b-/-D^b-/-CIITA^{-/-} mice. Bar, 600 μ m. (c) Depletion of

CD8⁺ T cells rescues K^b-/-D^b-/-CIITA^{-/-} mice from colonic inflammation. 8-wk-old mice were depleted of CD8⁺ T cells by injecting anti-CD8 antibodies. Every 2 wk a group of mice was killed, and the thickness of the colon was measured. The CD8 T cell-depleted (■) and control (◆) groups are shown. Error bars represent means \pm SEM. (d) Histological analysis of cross sections of intestine (top) and pancreata (bottom) show the reduction of inflammation in CD8⁺ T cell-depleted animals. Bar, 600 μ m.

$K^b\text{-}/\text{-}D^b\text{-}/\text{-}CIITA\text{-}/\text{-}$ mice depleted of $CD8^+$ T cells failed to develop inflammation in the gut and pancreas, suggesting that $CD8^+$ T cells restricted by MHC class I-like molecules play a critical role in the observed inflammation in $K^b\text{-}/\text{-}D^b\text{-}/\text{-}CIITA\text{-}/\text{-}$ mice. Thus, our results provide strong evidence that $CD8^+$ T cells restricted by MHC class I-like molecules, in the absence of MHC class Ia-restricted $CD8^+$ T cells and class II-restricted $CD4^+$ T cells, become pathogenic, resulting in the development of the observed autoimmune diseases. The early spontaneous onset of autoimmune disease in an otherwise resistant mouse strain clearly indicates that these cells are major initiators and mediators of autoimmunity. The lack of autoimmunity in $K^b\text{-}/\text{-}D^b\text{-}/\text{-}$ and $CIITA\text{-}/\text{-}$ mice strongly argues that $CD4^+$ T cells and MHC class Ia-restricted $CD8^+$ T cells are both capable of suppressing the activity of the $CD8^+$ T cells restricted by MHC class I-like molecules. Because MHC class I-like molecules are nonpolymorphic in nature and either fail to present antigen (e.g., thymus leukemia), glycolipid (e.g., CD1d), or a very restricted set of self-antigens (e.g., Qa-1^b presents a peptide derived from Hsp60), autoimmune disease in $K^b\text{-}/\text{-}D^b\text{-}/\text{-}CIITA\text{-}/\text{-}$ mice might be initiated by a small population of oligoclonal $CD8^+$ T cells that are activated by a restricted set of self-antigens presented by MHC class I-like molecules. Although our data clearly show that T cells restricted by MHC class I-like molecules are important for the autoimmune phenotype, we do not know which antigen is responsible for eliciting the autoimmune responses.

Breaking tolerance of $CD8^+$ T cells restricted by MHC class I-like molecules may represent a key step in the pathogenesis of autoimmune disorders. A previous report has established an etiological link between infection by gram-negative bacteria and autoimmune diseases (24). It is possible that, in mice or humans with genetic susceptibility to autoimmunity, microbial products can activate innate immune components that alter the immune tolerant status, thus allowing pathogenic $CD8^+$ T cells restricted by MHC class I-like molecules to expand and initiate autoimmune disease. In fact, a recent report showed that a Qa-1^b-restricted $CD8^+$ T cell clone can kill macrophages that are stressed or infected with *Salmonella* (24). Interestingly, the killing of infected cells in this system was not guided by the harbored organism but depended on host stress proteins such as Hsp60. Furthermore, these authors showed that Qa-1 binds with an Hsp60-derived peptide, which can be recognized by a Qa-1^b-restricted $CD8^+$ T cell clone (24). Interestingly, Qa-1^b can also bind a peptide derived from preproinsulin and activate the same Qa-1^b-restricted $CD8^+$ T cell clone that recognizes Hsp60. This finding suggests that self-peptides presented by Qa-1 can mediate the development of autoimmunity. Nevertheless, the contribution of Qa-1-presented peptides to autoimmunity in our model system, in which all T cells are restricted by MHC class I-like molecules, remains to be established.

$CD8^+$ T cells restricted by MHC class I-like molecules are selected and activated in the thymus. Under normal con-

ditions, these cells are tolerant, but when tolerance is broken these T cells rapidly expand in the peripheral lymphoid organs and initiate autoimmune disease. Our results indicate that these $CD8^+$ T cells are highly autoreactive. Furthermore, these cells are fully capable of initiating and propagating autoimmune diseases on their own. MHC class I-like molecules are nonpolymorphic and bind to a limited set of self-antigens, resulting in a small population of selected T cells with limited diversity. As $K^b\text{-}/\text{-}D^b\text{-}/\text{-}CIITA\text{-}/\text{-}$ mice do not bear conventional $CD4^+CD25^+$ or $CD8^+$ T reg cells, their $CD8^+$ T cells are not subject to T reg cell-mediated tolerance and are free to initiate and propagate autoimmune diseases. Therefore, both MHC class II-restricted $CD4^+$ T cells and MHC class Ia-restricted $CD8^+$ T cells are required to suppress autoimmunity.

In summary, innate-like $CD8^+$ T cells are primed and activated by self-antigens in the thymus and transported to the periphery, where they can expand exponentially and induce autoimmune diseases. These cells can induce inflammation in multiple organs. Therefore, innate-like $CD8^+$ T cells may contribute to the generation of a variety of autoimmune diseases. These innate-like $CD8^+$ T cells are restricted by MHC class I-like molecules, which are nonpolymorphic in nature and thus present a limited set of self and/or foreign antigens to T cells. Uncontrolled reactivity of innate-like $CD8^+$ T cells to self or foreign antigens may play a critical role in many autoimmune diseases.

MATERIALS AND METHODS

Mice and antibodies. $CIITA$ and β_2m knockout mice on the C57BL/6 (H-2^b) background were purchased from the Jackson Laboratory. Mice with phenotypes $RAG\text{-}2\text{-}/\text{-}$, $OT\text{-}I$ in $RAG\text{-}2\text{-}/\text{-}$ background, $OT\text{-}II$ in $RAG\text{-}2\text{-}/\text{-}$ background, and $K^b\text{-}/\text{-}D^b\text{-}/\text{-}$ double knockout were obtained from Taconic Farms. $K^b\text{-}/\text{-}D^b\text{-}/\text{-}$ double knockout mice were described previously (25). $K^b\text{-}/\text{-}D^b\text{-}/\text{-}CIITA\text{-}/\text{-}$ triple knockout and $\beta_2m\text{-}/\text{-}CIITA\text{-}/\text{-}$ double knockout mice were generated by breeding $CIITA\text{-}/\text{-}$ with $K^b\text{-}/\text{-}D^b\text{-}/\text{-}$ mice or $CIITA\text{-}/\text{-}$ with $\beta_2m\text{-}/\text{-}$ mice, respectively. 8–16-wk-old mice of either sex were used. Mice were housed in a specific pathogen-free colony at the Vivarium of the Robert Wood Johnson Medical School. The animal protocols for the experiments described in this paper were approved by the Institutional Animal Care and Use Committee of the Robert Wood Johnson Medical School.

Fluorochrome-conjugated antibodies against H-2K^b/D^b (28-8-6), I-A^b (25-9-17), CD3 (145.2C11), TCR $\alpha\beta$ (H-57), CD8 α (53-6.7), CD4 (GK1.5), pan-NK marker CD49b (DX5), CD44 (IM7), β_7 integrin (M293), CD122 (TM- β 1), and Ly6C (AL-21); anti-IgG, anti-BrdU-FITC, biotin-conjugated anti-Qa-1^b (6A8.6F10.1A), and anti-Qa-2 (1-1-2); and purified anti-H2-M3 (H2-M3.130) antibodies were purchased from BD Biosciences. Anti-NKG2C FITC (20d5), anti-CD11b-FITC, and anti-IL-12-PE (C17.8) for intracellular staining was purchased from eBioscience.

FACS staining and analysis. Cells were harvested from the spleen, lymph nodes, thymus, or peripheral blood. Red blood cells were lysed by the ACK lysis buffer (Biosource International). Cells were suspended in staining buffer (PBS, 3% FCS, 0.01% Na-azide) at a concentration of 10^7 cells/ml. 100 μ l of suspension was incubated either with directly conjugated antibodies or biotinylated antibodies for 30 min on ice. Streptavidin-PE was used as the secondary reagent with an additional incubation of 30 min on ice. Cells were washed twice with the staining buffer and fixed with 1% paraformaldehyde. Fluorescence intensity was measured by flow cytometry on a flow cytometer (FACStar; BD Immunocytometry).

T cell adoptive transfer. CD4⁺ or CD8⁺ T cells from OT-II or OT-I TCR Tg mice and from K^b-/-D^b-/-CIITA^{-/-} mice, respectively, were purified using Dynal Beads (Dyna), according to the supplier's instructions. For T cell survival experiments, cells were stained with CFSE, and 2 × 10⁶ cells were injected into syngeneic RAG-2^{-/-} or K^b-/-D^b-/-CIITA^{-/-} triple knockout mice. After 7 d, cells from spleen and lymph nodes were harvested, and the presence of CFSE-labeled cells was estimated on a flow cytometer. RAG-2^{-/-}pfn^{-/-} mice were also adoptively transferred with CD8⁺ T cells isolated from K^b-/-D^b-/-CIITA^{-/-} mice and analyzed for the development of autoimmunity.

T cell activation and cytokine detection. Purified CD8 T cells from different strains of mice were activated with plate-bound anti-CD3 and anti-CD28 antibodies for 16 h. Cytokine and chemokine levels in culture supernatants were determined by multiplexed bead array immunoassay using Luminescence Technology (Bio-Plex; Bio-Rad Laboratories). Unpurified splenocytes were activated with anti-CD3 antibodies for 18 h to examine IL-12 production. IL-12 was assayed by intracellular staining after treatment with 10 μg/ml brefeldin A during the last 6 h of culture. Cells were stained for surface markers, fixed with 1% paraformaldehyde for 30 min, washed, and resuspended in a permeabilization buffer provided in the Cytotfix/Cytoperm kit (BD Biosciences), then stained with PE-conjugated rat antimurine IL-12. Stained cells were then analyzed by flow cytometry on a cytometer (FACScan; Becton Dickinson).

In vivo T cell proliferation assay by BrdU. Mice were treated with BrdU in drinking water at 0.8 mg/ml (Sigma-Aldrich) for 9 d for assessing in vivo cell proliferation. Spleen and thymus were harvested, and single-cell suspensions were made. Cells were stained for surface markers using appropriate antibodies and fluorochromes. After washing, cells were resuspended in 0.15 M NaCl and fixed by gradual addition of ice-cold 95% ethanol. Cells were washed and incubated with 1% paraformaldehyde for 30 min at room temperature. Cells were stained with anti-BrdU-FITC in the presence of DNase I. Samples were analyzed using a FACScan flow cytometer.

Gross examination of mice and histology. Photographs of spleens, lymph nodes, or open abdominal cavities from 12-wk-old mice were taken using a digital camera (DSC V-3; Sony). For histology, pieces of intestine and pancreas were washed thoroughly in PBS and fixed in periodate-lysine-paraformaldehyde. Tissues were embedded in paraffin, and 5- to 6-μm sections were cut. Sections were stained with hematoxylin and eosin (H&E) and examined microscopically.

Online supplemental material. Fig. S1 shows the importance of IFN-γ in anti-CD3-induced IL-12 production in splenocytes of K^b-/-D^b-/-CIITA^{-/-} mice. Table S1 demonstrates thymocyte phenotypes in different strains of mice. Table S2 reveals TCR Vβ usage in CD8⁺ T cells of different strains of mice. Online supplemental material is available at <http://www.jem.org/cgi/content/full/jem.20060936/DC1>.

The authors would like to thank Dr. Sidney Pestka and Arthur Roberts for critical review of this manuscript.

This work was supported in part by United States Public Health Service grants (AI43384 and AI50222), a National Space Biomedical Research Institute grant (IH00208), which is supported by the National Aeronautics and Space Administration through Cooperative Agreement NCC 9-58, and the University Professor Fund of the University of Medicine and Dentistry of New Jersey (to Y.F. Shi).

The authors have no conflicting financial interests.

Submitted: 2 May 2006

Accepted: 5 October 2006

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