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Review

# The multiple roles of the CD8 coreceptor in T cell biology: opportunities for the selective modulation of self-reactive cytotoxic T cells

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## ABSTRACT

**Short peptide fragments generated by intracellular protein cleavage are presented on the surface of most nucleated cells bound to highly polymorphic MHCI molecules. These pMHCI complexes constitute an interface that allows the immune system to identify and eradicate anomalous cells, such as those that harbor infectious agents, through the activation of CTLs. Molecular recognition of pMHCI complexes is mediated primarily by clonally distributed TCRs expressed on the surface of CTLs. The coreceptor CD8 contributes to this antigen-recognition process by binding to a largely invariant region of the MHCI molecule and by promoting intracellular signaling, the effects of which serve to enhance TCR stimuli triggered by cognate ligands. Recent investigations have shed light on the role of CD8 in the activation of MHCI-restricted, antigen-experienced T cells and in the processes of T cell selection and lineage commitment in the thymus. Here, we review these data and discuss their implications for the development of potential therapeutic strategies that selectively target pathogenic CTL responses erroneously directed against self-derived antigens.** ***J. Leukoc. Biol.*** **90: 1089–1099; 2011.**

## Introduction

The CD4 and CD8 molecules were identified initially as phenotypic markers on T lymphocytes restricted by MHC class II and class I proteins, respectively [1]. Subsequent data showing that CD4 and CD8 were functional components of the T cell antigen recognition machinery—most notably, the key findings that CD4 and CD8 physically engage the same ligand as the TCR and facilitate downstream proximal signaling events triggered by TCR ligation through interaction with the $p56^{lck}$ tyrosine kinase—led to the concept of the T cell "coreceptor" [2]. The present review addresses particular aspects of pMHCI recognition and will focus accordingly on the CD8 coreceptor. CD8 is a transmembrane, disulfide-linked glycoprotein that exists on the cell surface in $\alpha\alpha$ homodimeric or $\alpha\beta$ heterodimeric form [3–5]. Each chain consists of a short cytoplasmic region, a single transmembrane domain, a long glycosylated stalk region, and a globular variable Ig-like domain. The $\alpha\beta$ isoform of CD8 is expressed exclusively by conventional MHCI-restricted $\alpha\beta$ T cells. In contrast, the CD8$\alpha\alpha$ homodimer has a more promiscuous expression pattern in humans and rodents; distinct lymphoid cells, such as intraepithelial lymphocytes, $\gamma\delta$ T cells, and NK cells, and also certain myeloid cell types, all express the $\alpha\alpha$ isoform of CD8 [6, 7]. Although CD8$\alpha\alpha$ is able to engage MHCI molecules and associates with $p56^{lck}$, its role is less well-understood. Indeed, the evidence gathered to date points to a regulatory role for CD8$\alpha\alpha$ through the engagement of nonclassical MHC molecules and/or via inhibition of the coreceptor activity of the $\alpha\beta$ isoform in $CD8^+$ CTLs [6, 8]. It is therefore believed that only CD8$\alpha\beta$ is able to act as a bona fide coreceptor in the activation of developing and mature T cells that express a pMHCI-specific TCR.

## MECHANISTIC ASPECTS OF CD8 CORECEPTOR FUNCTION IN THE PROCESS OF ANTIGEN RECOGNITION

The involvement of CD8 in the recognition of target cells by CTLs was appreciated prior to the identification of the TCR. Early reports showed that antibodies recognizing the $\alpha$ or $\beta$ subunit of CD8 were able to block the killing of target cells by CTLs in vitro [3, 9], thereby hinting at the involvement of CD8 in the molecular processes of antigen recognition. Subsequent characterization of the TCR subunits [10] and their coding genes [11, 12] led to the understanding that CTL activation was mainly mediated by interaction of the TCR with the polymorphic residues of MHCI molecules combined with peptide fragments derived from intracytosolic proteins [13–15]. Nevertheless, the discovery

Abbreviations: $\alpha$-CPM=$\alpha$-connecting peptide motif, CDR=complementarity-determining region, DP=double-positive, MS=multiple sclerosis, pMHCI=peptide MHC class I, SP=single-positive, SPR=surface plasmon resonance, T1D=type 1 diabetes, TIL=tumor-infiltrating lymphocyte

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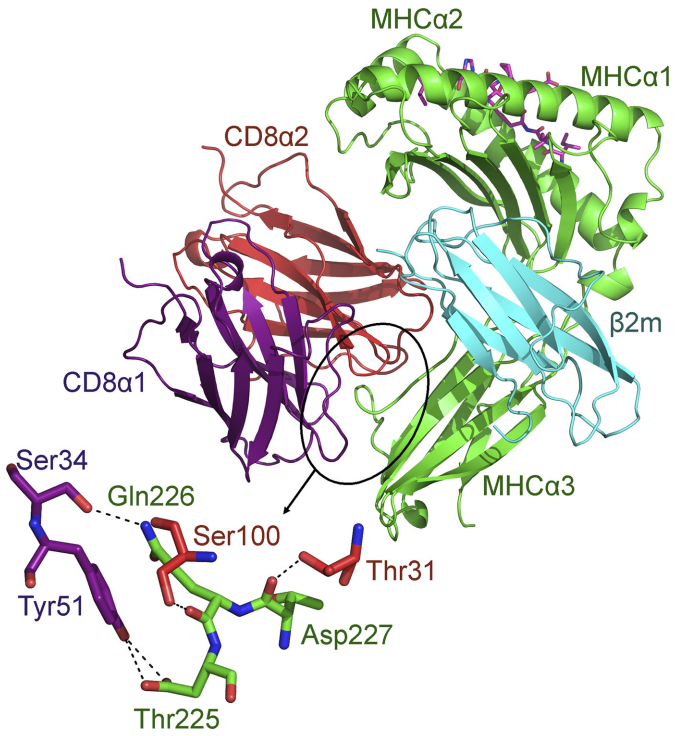


**Figure 1. Overview of the molecular interactions between CD8 and MHCI molecules.** Ribbon representation of the cocrystal complex between CD8αα and pMHCI. CD8αα is shown in red (α2) and purple (α1), binding mainly to the α3 domain of MHCI (green). The CDR-like loops of the CD8 molecule and residues 223–227 of the MHCI α3 domain comprise the main binding interface. The enlargement of the main binding interface between CD8 and MHCI shows the two CDR-like loops of the CD8 molecule forming a "clamp"-like topology around the MHCI loop encompassing residues 223–227 of the α3 domain. The most important contacts are made between the CD8 α1-chain Ser34/Tyr51 and the MHCI α-chain Thr225/Gln226 and between the CD8 α2-chain Thr31/Ser100 and the MHCI α-chain Gln226/Asp227. Determination of the crystal structure of murine CD8αβ in complex with H2-D$^d$ [21] has confirmed the general coreceptor binding topology inferred from the pMHCI/CD8αα complexes and has shown that the CD8β-chain occupies the T cell proximal position in the heterodimer (in place of the CD8α2-chain in this picture).

that CD8 coprecipitates with p56$^{lck}$ tyrosine kinase [16], the enzyme that catalyzes the phosphorylation of CD3ζ ITAM residues (reviewed in ref. [17]), pointed to an important role for CD8 as a conveyor of proximal T cell activation signals. This notion was reinforced by the demonstration that CD8 binds MHCI molecules [18] via interactions with largely nonpolymorphic amino acid residues situated in the α3, and to a lesser extent, the α2 domain of the heavy-chain and β2-microbulin [19, 20] (**Fig. 1**). The MHCI/CD8 interaction is characterized by a particularly weak affinity and rapid kinetics [22]. Despite these seemingly unfavorable binding characteristics, however, the engagement of MHCI molecules by CD8 at the cell surface enhances the association rate of pMHCI complexes with TCRs and increases the half-life of cognate TCR/pMHCI interactions [23–25]. These data suggest that CD8 binding helps to increase the degree of TCR occupancy at the T cell surface and stabilize the interaction between the TCR and pMHCI, two parameters that critically determine the potency of TCR agonist ligands [26]. Thus, the promotion of intracellular signaling events and TCR/pMHCI interactions by the CD8 coreceptor likely combine to enhance cognate antigen recognition. As a result of the importance of p56$^{lck}$ in the initiation of proximal phosphorylation events, however, the main contribution of CD8 is believed to result from the promotion of signaling and second messenger pathways downstream of TCR triggering [27, 28].

The existence of a physical association between the TCR and CD8 on the T cell surface was first suggested by studies using comodulation [29], coprecipitation [30, 31], and affinity chromatography [32]. Subsequently, the nature of this interaction and its functional importance were revealed by the finding that palmitoylation of the CD8β-chain enables the coreceptor to interact directly with CD3δ and recruit TCR/CD3 complexes to membrane microdomains that promote signaling through the exclusion of inhibitory phosphatase proteins [33–35]. Furthermore, the conserved α-CPM, located on the membrane proximal domain of the TCR α-chain, facilitates the recruitment of CD8 in close proximity to the TCR/CD3 complex [36, 37] (**Fig. 2**). In view of the apparent importance of CD8 in the recruitment of p56$^{lck}$ tyrosine kinase to the TCR signaling complex, the most straightforward interpretation of the biochemical data documenting the *cis* association between TCR and CD8 and the *trans* interaction between TCR and pMHCI was that the TCR and its coreceptor coordinately engage the same cognate pMHCI ligand [2]. In this TCR/CD8 heterodimeric configuration, binding of the coreceptor to MHCI drives the recruitment of CD8-associated p56$^{lck}$ to the vicinity of an engaged TCR/CD3 signaling complex, resulting in phosphorylation of the CD3ζ ITAMs (Fig. 2A). However, this scenario was challenged by observations that CTLs from mice lacking both coreceptors can be activated by cognate viral or alloantigens [38], which suggests that free p56$^{lck}$ can initiate signaling, and by structural data describing a binding angle between CD8 and pMHCI, seemingly incompatible with the formation of a tripartite TCR/pMHCI/CD8 molecular complex involving direct contacts between the TCR and CD8 [26].

In addition to spatial considerations, the timing of coreceptor activity during antigen recognition is crucial for a full mechanistic understanding of this process. A large proportion of CD8 and TCR molecules is constitutively associated on primary CTLs in the absence of TCR engagement by agonist ligands, which suggests that the TCR and CD8 pre-exist as bispecific receptors that can engage pMHCI agonist ligands in a coordinate manner [30, 31, 35, 39]. However, studies of the dynamic interactions between CD8 and TCR/CD3, based on the use of fluorescence resonance energy transfer, have shown that TCR binding to pMHCI occurs first, thereby satisfying the antigen-specific component of the interaction, and that the recruitment of CD8 occurs subsequently [40, 41]. These data, obtained in T cell hybridomas, point to a chronological sequence according to which the pivotal antigen-specific proofreading event provided by the TCR occurs prior to the association of CD8 with the TCR/CD3 complex. Such a mechanism would ensure that CD8 coreceptor functions, resulting in the amplification of downstream signaling cascades, are only elaborated for ligands that engage the TCR with favorable affinities and kinetics. In this scenario, the activity of the TCR

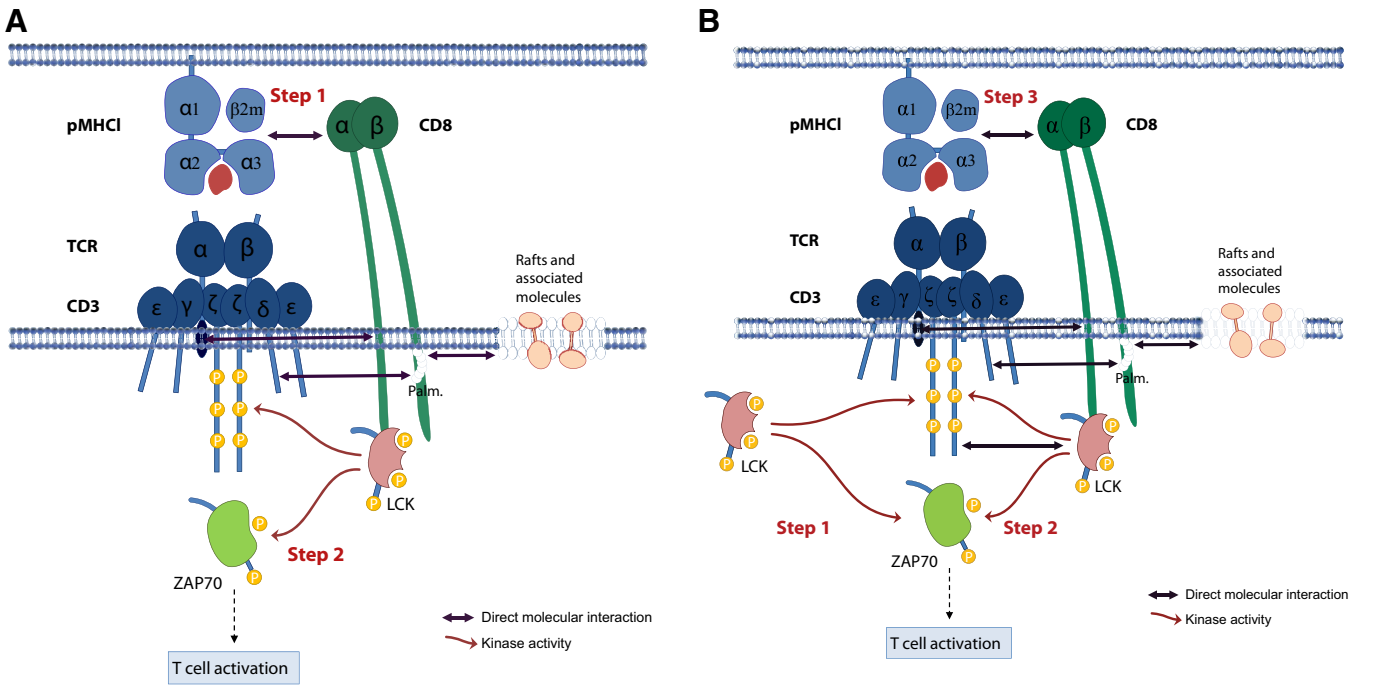


**Figure 2. Schematic representation of CD8 coreceptor functions in early T cell activation events**. The extracellular portion of CD8$\alpha\beta$ interacts directly with the MHCI $\alpha$-chain and $\beta$2-microglobulin, facilitating TCR/pMHCI interactions. Direct molecular *cis*-interactions between CD8 and the TCR complex also take place on the T cell surface. These involve direct contacts between the CD3$\delta$- and CD8$\beta$-chain and require the native $\alpha$-CPM sequence of the TCR $\alpha$-chain (represented by a dark-blue oval shape). Palmitoylation (Palm.) of CD8$\beta$ facilitates these contacts and also permits partitioning of the TCR/CD8 complex in membrane lipid rafts, which provide an enzymatic environment that favors phosphorylation events via the sequestration of phosphatases. (A) The classical view of T cell activation is that CD8 is recruited to the TCR complex before phosphorylation takes place and that $p56^{lck}$ bound to the CD8$\alpha$ cytoplasmic tail catalyzes the initial CD3$\zeta$ ITAM phosphorylation events, which then allow for the recruitment of additional $p56^{lck}$ molecules and signal amplification. (B) Alternatively, recent experimental data favor a model whereby free $p56^{lck}$ is responsible for the initial phosphorylation events. Phosphorylated CD3$\zeta$ ITAMs then allow the recruitment of $p56^{lck}$ bound to CD8$\alpha$ in close proximity to CD3. In this scenario, the interaction between the TCR and CD8 occurs after the initial phosphorylation events and is driven intracellularly by interactions between CD3- and CD8$\alpha$-bound $p56^{lck}$.

could also reinforce the definition of a sharp TCR/pMHCI affinity/dwell-time threshold that serves to discriminate agonist and nonagonist pMHCI complexes, with coreceptor recruitment being enabled only in the context of agonist-ligand interactions with the TCR. Notably, a recent study addressing the effect of CD8 on the kinetics of pMHCI engagement by primary mouse $CD8^+$ T cells in two dimensions reported that the coreceptor cooperatively enhanced cell-cell adhesion mediated by the TCR [42]. This CD8-mediated effect required prior TCR engagement by agonist ligands and the tyrosine kinase activity of $p56^{lck}$. Thus, in addition to the necessity for a prerequisite TCR/pMHCI agonist-ligand interaction, these results suggest that the exertion of CD8 coreceptor functions requires the initiation of signaling by free $p56^{lck}$ and identifies coreceptor-bound $p56^{lck}$ as the mediator/adaptor molecule that recruits CD8 to the TCR/CD3 complex, a scenario proposed previously by Thome et al. [43] (Fig. 2B). Importantly, this notion also reconciles the mechanistic scenario of coreceptor activity with structural studies, as recruitment of CD8 to the TCR complex via $p56^{lck}$, rather than by direct interactions with MHCI molecules, seems compatible with the overall binding configuration of the multimolecular complex [44]. In addition, Jiang et al. [42] proposed a cooperative binding effect for CD8 following its recruitment via $p56^{lck}$, which may establish interactions with agonist as well as nonagonist pMHCI molecules on the APC surface. According to this model, the induced recruitment of CD8 serves to stabilize molecular contact between the CTL and the APC, thereby unveiling another potential role for the coreceptor. This phenomenon may also help to explain previous observations, indicating that the ability of CD8 to interact with nonstimulatory pMHCI complexes lowers T cell activation thresholds and enables CTL to respond to low copy numbers of specific pMHCI molecules [40, 45].

## EXPERIMENTAL SYSTEMS FOR THE ASSESSMENT OF CD8 FUNCTION DURING CTL ACTIVATION

Preventing the association between CD8 and MHCI molecules in cellular assays, often using anti-CD8 blocking antibodies, has been the most common experimental approach used to address the role of CD8 in CTL activation. Although it had been appreciated for a long time that a single anti-CD8 antibody paratope could have weak or strong inhibitory effects on the activation of different CTL clones [46, 47], the reasons underlying these discrepancies remained elusive until recently. It has since become clear that anti-CD8 antibodies can have

**TABLE 1. The Heterogeneity of Antihuman CD8 Antibodies**

| Ab clone | α or β | Tetramer binding | MIP-1β | MIP-1α | RANTES | IFN-γ | TNF-α | IL-2 | Cytotoxicity |
|---|---|---|---|---|---|---|---|---|---|
| OKT8 | α | Enhance | Yes | Yes | Yes | No | No | No | Yes |
| SK1 | α | Inhibit | No | No | No | No | No | No | No |
| MCD8 | α | Neutral | No | No | No | No | No | No | No |
| 32/M4 | α | Neutral | No | No | No | No | No | No | No |
| C8/144B | α | Neutral | No | No | No | No | No | No | No |
| DK25 | α | Inhibit | No | No | No | No | No | No | No |
| 2ST8.5H7 | β | Inhibit | No | No | No | No | No | No | No |

Summary of the effects exerted by antihuman CD8 antibodies on pMHCI tetramer binding and $CD8^+$ T cell activation in the absence of TCR engagement. Table reproduced from ref. [54] (Copyright 2011, The American Association of Immunologists).

very distinct or even opposing effects on TCR/pMHCI interactions and the recognition of agonist epitopes by CTLs. Furthermore, these effects can be largely unrelated to disruption of the pMHCI/CD8 interaction. Indeed, some anti-CD8 antibodies can completely or partially inhibit TCR binding by identical pMHCI ligands, and others can enhance pMHCI engagement and antigen recognition or even stimulate CTLs in their own right [48–54] (summarized in **Tables 1** and **2**). These heterogeneous effects, which are inherent to the binding mode and impacted by steric hindrance resulting from the use of macromolecules to block the engagement of MHCI molecules by CD8, impose important caveats on the interpretation of data generated with such approaches. Indeed, given that the TCR complex and CD8 become very closely associated on the surface of CTLs during antigen-induced TCR triggering, it is not surprising that the inhibitory effects of large molecules bound to CD8 can be independent from the pMHCI/CD8 interaction itself. More elaborate systems that circumvent these experimental problems have been provided by the design of point-mutated MHCI molecules that fail to interact with the coreceptor [28, 55]. These molecules, expressed on the surface of APCs or in the form of soluble recombinant proteins, have allowed in-depth investigations into the importance of the pMHCI/CD8 interaction in the process of CTL activation. Hybridomas expressing monoclonal TCRs delivered by viral vectors, with or without the cotransduction of CD8 α- and β-chains, have also provided suitable systems for the study of coreceptor function [56, 57].

**TABLE 2. The Heterogeneity of Antimouse CD8 Antibodies**

| Ab clone | α or β | Tetramer binding | MIP-1β | IFN-γ | IL-2 |
|---|---|---|---|---|---|
| CT-CD8a | α | Inhibit | Yes | No | No |
| 53.6.7 | α | Enhance | Weak | No | No |
| CT-CD8b | β | Enhance | Yes | No | No |
| KT112 | β | Enhance | Weak | NT | NT |

Summary of the effects exerted by antimouse CD8 antibodies on pMHCI tetramer binding and $CD8^+$ T cell activation in the absence of TCR engagement. NT, Not tested. Table reproduced from ref. [54] (Copyright 2011, The American Association of Immunologists).

## UNRAVELING THE ROLE OF CD8: ENHANCEMENT OF CTL ACTIVATION BY LOW-AFFINITY ANTIGENIC LIGANDS

The experimental approaches described above have been used to characterize many monoclonal CTLs or hybridomas that recognize epitopes in a CD8-dependent or CD8-independent manner [48, 56, 58–61]. From these studies, it appeared that the degree of dependency on CD8 for efficient recognition roughly correlated with the potency of the considered ligand, as defined by the concentration of peptide required to elicit half-maximum activation in dose-response titrations (**Fig. 3**). As biophysical investigations using SPR had determined that the affinity and/or the dwell time of TCR/pMHCI interactions correlated well with the potency of agonist ligands [26], it was inferred that CD8 dependency was probably linked with these parameters. Two studies tested this possibility directly by systematically assessing the CD8 dependency of several agonist ligands with a wide range of defined $K_D$ values and half-lives for their respective cognate TCR interactions. In both cases, the experimental results established a tight correlation between the requirement for CD8 activity and TCR/pMHCI af-

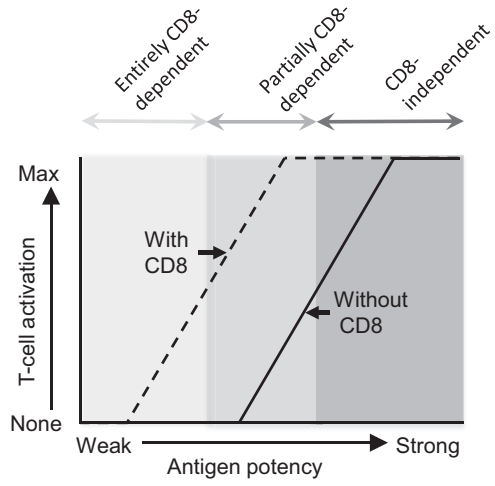


**Figure 3. Model illustrating the degree of CD8 coreceptor dependency as a function of antigen sensitivity and TCR/pMHCI affinity in the process of CTL activation.**

finity. In the murine 2C TCR system, Holler et al. [62] used the WT TCR and high-affinity mutants bearing amino acid substitutions in the CDR loops to show that T cell hybridomas were not fully activated in the absence of CD8 surface expression by agonist ligands with $K_D$ >100 nM [63]. At $K_D$ values >3 $\mu$M, antigen recognition became strictly dependent on the presence of the coreceptor, as indicated by the failure of CD8-negative hybridomas to produce IL-2 in response to such agonists. As most syngeneic TCR/pMHCI interactions measured by SPR have $K_D$s above this value [26], the authors concluded that the majority of CTLs that express TCRs specific for antigens relevant to a natural setting were likely to rely heavily on the coreceptor to recognize cognate epitopes efficiently. Using a human CTL clone specific for a putative tumor epitope, together with several peptide variants in the context of WT HLA A*0201 or point-mutated HLA A*0201 molecules that fail to interact with CD8, we found a qualitatively similar but less stringent correlation between coreceptor binding and CTL activation. The degree of dependency on CD8 for CTL activation correlated well with the $EC_{50}$ of 13 syngeneic epitopes comprising the index peptide and 12 variants bearing single amino acid substitutions [64]. The affinity of agonist pMHCI complexes for the TCR paralleled their potency, yet only ligands with a $K_D$ ≥35–40 $\mu$M displayed some degree of dependency on CD8 engagement in peptide titration assays [24]. Furthermore, in our system, entirely CD8-dependent agonists displayed very low affinities characterized by $K_D$ values >100–200 $\mu$M. Several explanations could account for the observed differences in CD8 dependency as a function of TCR/pMHCI affinity reported in these two studies. First, such discrepant results might simply reflect fundamental differences between the human and murine systems. Second, the coreceptor was expressed on the cell surface in our study, whereas Holler et al. used CD8-positive or CD8-negative hybridoma cells. Therefore, the differences observed in our system could only be accounted for by the disruption of pMHCI/CD8 interactions. In contrast, the greater degree of CD8 dependency observed by Holler et al. [63] might reflect an unappreciated role for CD8, which is independent of MHCI engagement, such as might relate to membrane organization of the T cell antigen recognition machinery. Third, the answer could lie in the functional readout used to assess activation. Hybridomas only express IL-2, and within the arsenal of effector functions deployed by CTLs, the expression of this cytokine requires strong TCR stimuli and is highly reliant on CD8 coreceptor functions [65]. Whatever the reasons for these differences, it would undoubtedly be of interest to determine accurately and in different systems the range of TCR/pMHCI affinities for which human T cells at different stages of their development are reliant on the coreceptor role of CD8 to respond adequately to cognate ligands. Such knowledge would reveal the nature of ligands for which the coreceptor is important in T cell biology and illuminate the potential pathological contexts in which modulation of CTL activity via CD8 might be desirable. It is also noteworthy that CD8 can determine whether low-affinity cognate pMHCI molecules behave as null ligands, coagonists, or antagonists, at least in the 2C TCR system [66]. These recent findings add an extra dimension to the role of CD8 during CTL stimulation.

## THE CORECEPTOR FUNCTIONS OF CD8 ARE ESSENTIAL FOR T CELL DEVELOPMENT IN THE THYMUS

In early life, and to a lesser extent, during adulthood, lymphoid progenitors that originate from bone marrow hematopoietic stem cells migrate to the thymus, where they differentiate and develop into mature T cells, ready to populate peripheral lymphoid organs [67]. Differential expression of the CD4 and CD8 coreceptors is a reliable way to identify thymocytes at different stages of their development. At the $CD4^+CD8^+$ DP stage, thymocytes express clonotypic TCRs and undergo positive selection, a process during which TCRs with minimal affinities for self-pMHC convey survival signals that permit continued thymocyte development [68]. At the DP and SP stages, cells that express TCRs with high affinities for self-pMHC are deleted by the process of negative selection [69]. Thymic education thus ensures the stringent selection of peripheral T cell clonotypes that express TCRs with weak/moderate affinities for self-pMHC molecules, thereby minimizing the risks of autoreactivity [70] but enabling strong interactions with foreign epitopes. Alternatively, thymocytes "die by neglect" if the expressed TCR fails to interact with pMHC molecules. An elegant study by Van Laethem and colleagues [71] revealed recently that the CD4 and CD8 coreceptors impose MHC reactivity on the TCRs borne by mature T cells. By studying the peripheral T cell repertoire of mice with MHCI and MHCII deficiency, these authors observed the development of mature T cells with reactivity against non-MHC ligands in the concomitant absence of CD4 and CD8. Conversely, the presence of both coreceptors on the MHC null background resulted in the thymic deletion of these cells. These data are consistent with the sequestration of intracellular $p56^{lck}$ by CD4 and CD8, which would ensure that coengagement of MHCI or MHCII by the TCR and CD8 or CD4, respectively, is required to trigger the signals that elicit positive or negative selection in the thymus. Thus, according to this mechanism, TCRs without MHC specificity are unable to support thymocyte survival and differentiation.

Mice lacking CD8$\alpha$ do not have mature T cells with cytotoxic properties [72] and, as a consequence, are more susceptible to viral infection [73]. This phenotype, as well as other in vivo and in vitro observations, points to a crucial role for CD8 in the process of CTL precursor development in the thymus up to the $CD3^{lo/int}$ DP stage [74, 75]. Positively selecting pMHCI complexes display comparatively low binding affinities for cognate TCRs [76, 77], and a likely consequence of this is that intact CD8 coreceptor activity is essential for the delivery of survival signals that promote the positive selection of DP thymocytes bearing MHCI-restricted TCRs [78]. The study of thymic selection in transgenic mice bearing $\alpha$-CPM-defective TCRs provided further experimental support for this hypothesis. Deletion of thymocytes occurs normally in these mice, but positive selection is prevented as a result of impaired coreceptor recruitment and signaling [79, 80]. Altogether, these data

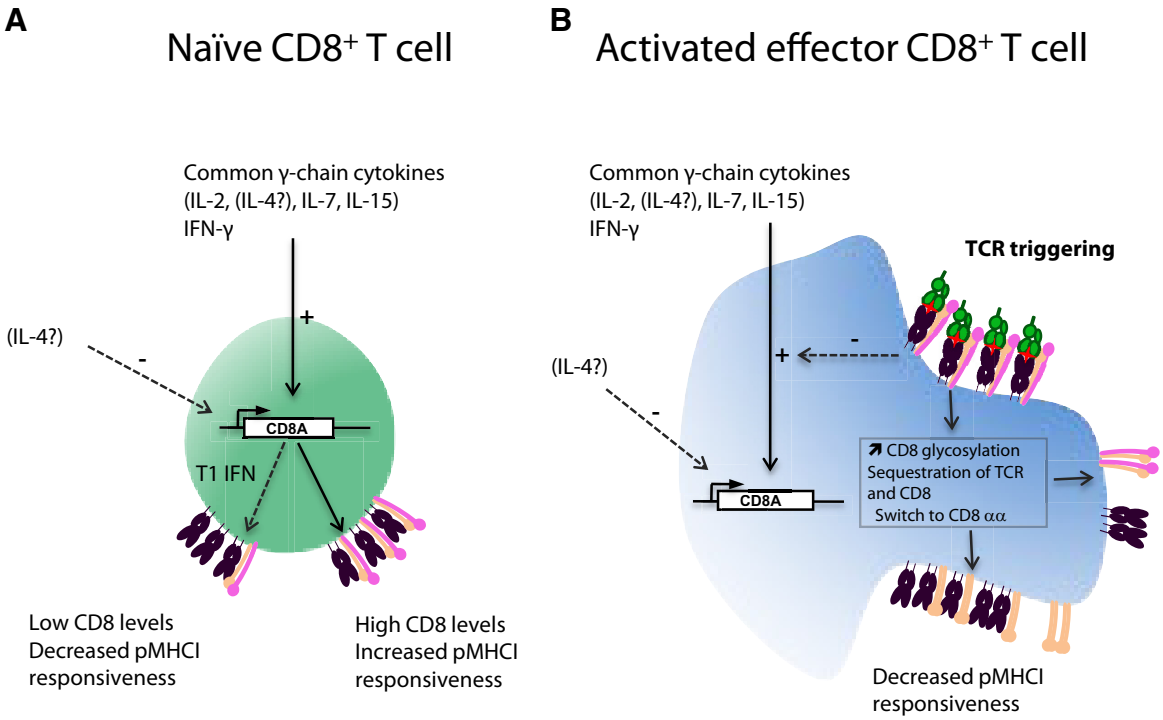


**Figure 4. Cues regulating CD8 expression levels and coreceptor functions in relation to the cellular activation status of peripheral CD8$^+$ T cells.** (A) In naïve CD8$^+$ T cells, modulation of CD8 functions is mostly known to occur via the regulation of CD8A transcription, which tunes coreceptor expression levels on the cell surface and thereby alters pMHCI recognition. Physiologically, CD8A transcriptional regulation in naïve T cells is thought to modulate survival signals elicited by self-pMHCI and regulate homeostatic expansion. (B) In addition to cytokine signals, the functions of CD8 are regulated at the transcriptional and post-translational levels following T cell activation by antigen. For instance, TCR stimuli result in the inhibition of IL-7R signals that up-regulate CD8A expression, thereby resulting in lower coreceptor expression levels on the cell surface. Altogether, these mechanisms down-regulate the functions of CD8, probably to prevent excessive and deleterious CTL activation by self-pMHCI ligands.

support a model, termed the "coreceptor zipper" by the authors, whereby the coreceptor is crucial for the elicitation of survival signals by increasing the apparent affinity of the interaction between an individual TCR and a positively selecting ligand, as well as by directly promoting consecutive, proximal intracellular signaling events [77, 79].

In addition, several reports have suggested an important role for CD8 in the process of DP thymocyte progression toward the more mature SP CD4$^+$ or CD8$^+$ stage. The kinetic signaling model of T cell lineage decision, which accommodates most experimental results that address lineage commitment, postulates that the key parameter in the DP thymocyte commitment to Th or CTL differentiation, is the duration of TCR-mediated signaling elicited by positively selecting ligands (reviewed in ref. [81]). Sustained signaling is associated with CD4$^+$ Th cell-lineage commitment, whereas short-lived signals promote differentiation into CD8$^+$ CTLs [82, 83]. As the initiation of TCR signaling in DP thymocytes is concomitant with the down-regulation of CD8 gene expression, independently of MHC restriction and later lineage commitment [84, 85], it is in a CD4$^+$CD8$^{low}$ state of differentiation that signal duration determines lineage fate. Cessation of TCR signals elicited by pMHCI interactions subsequent to CD8 down-regulation specifies commitment to the CTL lineage. Conversely, CD8-independent signals, elicited by the engagement of pMHCII, remain unaffected by CD8 down-regulation and thus, promote differentiation into the CD4$^+$ Th cell lineage. Therefore, one of the fundamental postulates underlying the kinetic signaling model of thymic selection, as well as other nonstochastic models not discussed here, relies on the premise that signals elicited by low-affinity, self-pMHCI ligands supporting the positive selection of DP thymocytes and their commitment to the CD8$^+$ CTL lineage are heavily dependent on CD8 coreceptor functions in the thymus.

## PERIPHERAL REGULATION OF CD8 CORECEPTOR FUNCTIONS AS A POTENTIAL MECHANISM TO MODULATE ANTIGEN SENSITIVITY IN VIVO

Several lines of evidence have recently given credence to the hypothesis that efficient regulation of murine CTL activity in the periphery can be achieved through the modification of CD8 functions (**Fig. 4**). Indeed, TCR-dependent activation of naïve CD8$^+$ T cells in the periphery appears to be a major checkpoint for such modulatory pathways. The mechanisms that underlie the modulation of CD8 functions include: (i) expression-switching to the CD8$\alpha\alpha$ homodimer, which is

known to be a poor coreceptor compared with the $\alpha\beta$ isoform as a result of an inability to recruit TCR complexes to membrane microdomains and suboptimal engagement of pMHCI molecules [6, 86]; (ii) post-translational modifications of CD8$\alpha\beta$ glycosylation [87, 88]; and (iii) modulation of coreceptor expression levels on the cell surface [60, 89, 90]. In addition to activation-dependent cues, the activity of several cytokines can tune CD8 expression levels on the surface of CTLs through the regulation of CD8A gene transcription. Park and colleagues [91] reported that the common $\gamma$-chain cytokines IL-2, IL-4, IL-7, and IL-15 enhance CD8A transcription and coreceptor expression on the cell surface. It is known that in steady-state, weak nonimmunogenic TCR signals elicited by self-pMHCI complexes are required to mediate the survival of naïve $CD8^+$ T cells (reviewed in ref. [92]). Accordingly, the authors' interpretation of their data was that under these conditions, signaling via homeostatic cytokines such as IL-7 and IL-15 would promote $CD8^+$ T cell survival by favoring their responsiveness to weak, CD8-dependent, nonstimulatory self-pMHCI complexes. Somewhat in contrast with these findings, another group linked the down-regulation of CD8 expression and subsequent inhibition of antigen recognition by CTLs to the direct effects of IL-4 [93, 94], an activity that they later reported was antagonized by autocrine IFN-$\gamma$ [95]. These authors also suggested that, in addition to the altered functional phenotype induced by IL-4, down-regulation of CD8 expression on CTLs could contribute to their nonresponsiveness to antigen and that this mechanism could be relevant to the immunosuppressive action of IL-4-secreting tumors [95]. In addition, Xiao and colleagues [96] found that type I IFN also down-regulated the expression of CD8 via nontranscriptional mechanisms. The significance of this latter observation remains unclear.

Perhaps the most compelling evidence that modulation of CTL activity can be achieved through CD8 in vivo comes from the study of human $CD8^+$ TILs. Demotte and colleagues [39] proposed that the physical dissociation between CD8 and the TCR mediated by galectin-3 could be responsible for a form of nonresponsiveness to antigen in the cancer setting. This hypothesis stemmed from the characterization of freshly isolated $CD8^+$ TILs that were unable to kill target tumor cells or peptide-pulsed APCs. The authors found that CD8 and TCR were separated in distinct membrane locations on the TIL surface and that CD8/TCR interactions were restored by treatment of these cells with galectin-3 disaccharide ligands. This treatment also reversed the coreceptor/TCR dissociation observed on cells cultured with tumor ascite supernatants and correlated with reversion of the anergic state [39, 97]. Although they did not suggest a causal effect, these authors also reported that galectin-3 ligands induce superior in vivo antitumor adaptive-immune responses in mice [97].

In summary, there is substantial evidence for the existence of multiple transcriptional and post-translational mechanisms that act transiently to down-regulate or increase coreceptor functions. These pathways can be activated as a direct consequence of TCR triggering and $CD8^+$ T cell activation in a cell-autonomous or autocrine manner, or locally through the paracrine action of cytokines and soluble factors, such as occurs in the tumor microenvironment. It is conceivable that TCR-mediated regulatory pathways act to decrease CTL responsiveness to antigen in the context of an ongoing immune response in vivo, thereby ensuring the nonresponsiveness of effector CTLs to self-determinants and contributing to the maintenance of peripheral tolerance. Conversely, in steady-state conditions, homeostatic cytokines might act to promote nonactivating self-pMHCI-dependent survival cues through the transcriptional enhancement of CD8 expression, thereby indirectly promoting naïve T cell persistence and expansion in the periphery.

## THE POTENTIAL PHYSIOLOGICAL IMPORTANCE OF CD8 IN CTL BIOLOGY

Although the importance of CD8 for CTL activation in response to low-affinity ligands in vitro is well-established, the actual role of the coreceptor during an immune response to microbial, tumoral, or self-derived antigens remains to be formally established in vivo. Indeed, despite the wealth of experimental data that document the enhancement of mature CTL activation and the detailed biochemical characterization of the mechanisms that pertain to this activity, the only firmly proven physiological role for the coreceptor concerns its importance in the events that govern T cell selection in the thymus. However, based on current evidence, it seems likely that CD8 helps to drive the priming and expansion of CTL clonotypes with low functional avidities for cognate antigen. Such a role could enhance the clonotypic diversity of CTL responses to microbial determinants and, more obviously, might contribute to the onset of a response directed against self-determinants in an autoimmune context. Support for the former scenario was provided by the finding that ex vivo activation of subdominant CTL clonotypes specific for epitopes derived from EBV and human CMV relied more heavily on CD8 engagement compared with numerically dominant clonotypes with the same antigen specificity, thereby indicating that CD8 likely augments clonotypic diversity within the antigen-specific CTL pool and prevents avidity-based selection proceeding to unity during chronic viral infections [98]. Perhaps an even more important putative role for CD8 could relate to maintenance of the naïve $CD8^+$ T cell pool in the steady-state. As discussed previously, several reports have demonstrated the importance of suboptimal TCR engagement by self-ligands, resulting in low-level signaling without concomitant activation, for the survival of naïve $CD8^+$ T cells in the periphery [92]. In contrast, memory $CD8^+$ T cell persistence only requires the presence of homeostatic cytokines and does not rely on suboptimal TCR stimuli [92]. It is therefore likely that coreceptor functions are required for the survival of naïve, but not memory, $CD8^+$ T cells. Rigorous testing of these predictions would require an approach that allows the deletion of CD8 or disruption of the MHCI/CD8 interaction at different stages of T cell differentiation in the periphery. Conditional ablation of CD8$\alpha$ in transgenic mice with floxed CD8A alleles is one such option. The best-suited existing system for conditional expression of the Cre recombinase is probably the distal Lck promoter, which is switched on during the late stages of thymic development; this model has been used by Killeen and colleagues [99] to assess the role of CD4 in mature T cells. However, detailed investigations of this promoter system with the

ROSA26 reporter mouse strain revealed an incomplete penetrance in T cells, as well as variations in the timing of Cre expression with respect to thymocyte development [100]. Consequently, a novel conditional or inducible system, strictly limited to mature T cells of the cytotoxic lineage, is required to investigate the functions of CD8 in the periphery.

## PHARMACOLOGICAL INHIBITION OF CORECEPTOR FUNCTION TO DAMPEN AUTOIMMUNE CD8$^+$ T CELL-MEDIATED PATHOLOGY

Inappropriate T cell responses are likely involved in the etiology of many autoimmune and chronic inflammatory pathologies. Modulation of T cell functions therefore represents an attractive therapeutic strategy to mitigate pathogenic immune responses. However, the design of efficient and safe therapies that target T cell activity faces major challenges. On the one hand, targeting the entire T cell compartment threatens harmful immunosuppression. On the other hand, specific T cell targeting approaches require detailed knowledge of the putative self-antigens that drive autoimmune pathogenesis, which is presently an unrealistic goal considering the tremendous diversity of potential T cell antigens and the vast allelic variability within the human MHC locus.

It was thought previously that CD8 involvement was critical for the majority of CTL responses and that targeting the pMHCI/CD8 interaction using soluble CD8 or variants thereof [101, 102], antibodies, or various small molecules [103, 104] represented a generic way of disrupting CD8$^+$ T cell activity. However, as discussed above, recent data have refined our understanding of the role of the coreceptor in the process of CTL activation by showing that the degree of CD8 dependence is inversely related to the strength of the TCR/pMHCI interaction. Accordingly, CD8 blockade should enable the selective targeting of weak TCR/pMHCI interactions without affecting high-affinity cognate TCRs, such as those expressed by CTLs specific for microbial epitopes. In support of this idea, accumulating data suggest that CTLs specific for autoimmune and tumoral self-determinants bear TCRs with lower affinities for their cognate ligands compared with microbe-specific and alloreactive CTLs [26, 105–110] (unpublished results). Thus, CD8 may be a valid therapeutic target in the setting of autoimmune diseases with etiologies that are linked to the activity of CTLs. CD8$^+$ T cells are known to infiltrate damaged tissues in high numbers in many autoimmune conditions, but their roles in disease initiation and progression are uncertain. However, recent investigations have produced several lines of evidence that implicate CD8$^+$ T cells in the pathogenesis of autoimmunity. First, genetic association and genome-wide analysis studies have linked several HLA I alleles with disease susceptibility or protection, independently of HLA II alleles [111, 112]. In addition, the identification of HLA I epitopes presented by target tissues and recognized by patients' CD8$^+$ T cells, combined with the development of transgenic animal models of T1D [113–115] and MS [116], which are entirely dependent on CD8$^+$ T cells, provides compelling evidence for a role of CD8$^+$ T cells in effecting tissue damage in the context of certain autoimmune pathologies. In addition to T1D and MS, such conditions may include vitiligo [117], neurodegenerative diseases, such as certain paraneoplastic syndromes [118], Hashimoto's thyroiditis, autoimmune myocarditis, and autoimmune hepatitis [119].

## CONCLUDING REMARKS

Recent developments have refined our understanding of CD8$^+$ T cell activation and the role of the coreceptor in the process of antigen recognition. Detailed biochemical and cellular investigations have established that CD8 coreceptor activity is essential for the recognition of weak, low-affinity ligands but dispensable for potent, high-affinity ligands. This knowledge holds translational promise, especially in the setting of autoimmunity. Compounds that are currently approved for the treatment of autoimmune and inflammatory diseases or those in the later stages of development usually rely on the induction of profound and/or long-lasting immunosuppression. This is often achieved by global depletion of entire cell types or lineages in the periphery, by the prevention of peripheral leukocyte trafficking and egress from lymphoid organs, or by targeting the systemic activity of cytokines involved in disease pathogenesis [120–122]. Although these treatments often show good efficacy, their adverse effects can outweigh their benefits. Potentially severe opportunistic infections or reactivation of latent viral infections with clinically disastrous sequelae are unacceptable side-effects in the treatment of autoimmune diseases with less aggressive profiles. More targeted and less disruptive treatments are therefore required for certain pathologies. Based on accumulated knowledge of CD8 biology, we propose that specific targeting of coreceptor functions might preferentially inhibit pathogenic CTL responses directed against self-determinants and leave cellular immunity to microbial pathogens largely intact. This hypothesis must still be validated experimentally in terms of efficacy and innocuity, particularly with respect to T cell development and homeostasis. Nevertheless, the possibility of selective therapeutic intervention without a priori knowledge of precise antigenic targets is an exciting and realistic goal.

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