CD4 and CD8: modulators of T-cell receptor recognition of antigen and of immune responses?

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The response of T cells to antigen involves the participation of a number of distinct receptor--ligand engagements. The major players in the recognition of complexes of major histocompatibility complex molecules and peptide antigens are the T-cell receptors and the co-receptors CD4 and CD8. Progress in understanding the physical structures of these molecules, and how complexes between them are formed, is helping our understanding of how they participate in regulating the signals transduced to T cells.

Introduction

The cell-surface glycoproteins CD4 and CD8 were initially described as specific markers of peripheral T cells with distinct effector functions; CD4 was found to be expressed by MHC class II-restricted helper T cells and CD8 by MHC class I-restricted cytotoxic T cells [1]. Subsequently, CD4 and CD8 were themselves shown to be receptors for MHC molecules and mutational analysis established that the binding sites for CD4 [2,3] and for CD8 [4,5] mapped to structurally similar regions of the constant domains of MHC class II and class I molecules, respectively. The observation that the binding sites for CD4 and CD8 on MHC molecules were separate from the peptide-binding domain of MHC molecules and therefore, from the site of interaction with the T-cell receptor (TCR), suggested a single MHC molecule could be bound simultaneously by both TCR and CD4 or CD8, increasing the overall avidity of the interaction. In addition, it was discovered that the cytoplasmic domains of CD4 and CD8 were associated with a T-cell-specific intracellular protein tyrosine kinase, p56lkx (Lck) [6,7]. Lck is a Src family kinase whose activity is critical for initiation of the intracellular tyrosine kinase cascade in response to TCR triggering [8]. Thus, the simultaneous binding of CD4 or CD8 to the same MHC complex as the TCR could juxtapose Lck and the TCR, leading to increased tyrosine phosphorylation and further recruitment and activation of downstream signalling effector molecules [9]. Indeed it was shown that co-ligating CD4 or CD8 to the TCR provided a more potent stimulus than simply ligating TCR alone [10-12] giving rise to the hypothesis that CD4 and CD8 acted as co-receptors in concert with the antigen-specific TCR for recognition of peptide--MHC complexes [13].

A current preoccupation of biologists studying T cells is how recognition of MHC–peptide ligands by receptors on T cells can induce a number of distinct outcomes during differentiation and activation of mature T cells [14*,15*]. It is clear that involvement of the co-receptor can have a significant influence on the outcome of peptide–MHC engagement. What is less clear, is precisely how these effects are mediated. Recent controversies have focused on whether the primary role of the co-receptors is to augment the relatively weak affinity of the TCR for MHC molecules, or whether it is mainly to recruit sufficient Lck to the signalling complex in order to trigger the response. With the advance of technologies that allow direct measurement of affinities and stabilities of these intermolecular interactions, we are making progress in assessing the contributions of the individual components to the formation of stable complexes necessary for signal transduction. Furthermore, progress in solving crystal structures of the individual components of the TCR signalling machinery, together with structural determination of complexes between some of the individual components, is leading to a greater understanding of how these structures interact. However, while these biophysical measurements help us to understand the basics of these interactions, they have yet to explain how these receptor–ligand engagements lead to diverse outcomes. Here I review some of the more recent findings that bear on these questions.

TCR–co-receptor structures and the recognition of antigen–MHC complexes

Despite the fact that CD4 and CD8 interact with structurally homologous sites on their MHC ligands, they have very little in common. Although both use basic immunoglobulin domains for their ligand-binding structures, these are arranged quite differently in the two molecules. CD4 is a single polypeptide, folded into four external immunoglobulin-related domains, that has a unique strand topology between domains 1 and 2 (D1 and D2) and between domains 3 and 4 (D3 and D4), as elucidated in the crystal structure of a human D1D2 fragment [16,17] and of rat D3D4 fragment [18]. The recently solved structure of the entire external portion of CD4 [19*] has added to our understanding of this

Abbreviations

APL altered peptide ligand
CDR complementarity determining region
MHC major histocompatibility complex
TCR T-cell receptor
Tg-TCR transgene-encoded T-cell receptor
molecule by indicating significant flexibility between D2 and D3 and a tendency of the molecule to dimerise at high protein concentrations, through interactions between opposing D4 domains. The local concentration of CD4 would be expected to increase in the area of contact between a T cell and antigen-presenting cell; therefore, the ability to dimerise may be particularly important if the TCR complex needs to form lattices for successful signal transduction. Several reports indicate that optimal signal transduction occurs when TCR and CD4 form stable associations with each other [13] and there are indications that TCRs themselves can oligomerise upon binding MHC-peptide complexes [20**]. Thus dimerisation of two CD4, or two TCR, molecules, as well as interactions between CD4 and TCR, may be important in signal transduction. Chimaeric CD4 molecules, in which human D3 and D4 domains were substituted for their mouse counterparts, were shown to act in a dominant negative fashion, interfering with wild-type CD4 function [21*]. These data were interpreted as a failure of these chimaeric CD4s to form a close association with the TCR, as demonstrated by a decrease in fluorescent resonance energy transfer between the hybrid CD4 and TCR. However, it is also possible that human and mouse D4 domains fail to dimerise efficiently, which may in part explain why these hybrid molecules were so efficient at disrupting productive signal transduction elicited by a wild-type CD4 molecule.

CD8, by contrast, is a disulphide-bonded heterodimer of two polypeptides, α and β, encoded by distinct genes that are physically linked and are predicted to show conserved overall structural topology although they share only ~20% residue identity [22]. Both polypeptides have an immunoglobulin-like amino-terminal domain linked to the transmembrane domain by an extended polypeptide region that contains a number of O-linked sugars. Currently, the available crystal structure information is for CD8αα homodimers, which indicates that the amino-terminal immunoglobulin-like domains fold very similarly to an Fv-like homodimer [23]. More recently, the structure of a complex between CD8 and HLA-A2 has been solved; this structure confirms mutagenesis studies that the major binding interface between these two molecules occurs between a highly flexible loop in the α3 domain of MHC class I (residues 223–229) that is clamped between the CDR1- and CDR3-like loops of both the CD8 subunits [24**]. Surprisingly, the contribution of the two CD8 subunits to the interaction is not equivalent, with ~70% of the solvent-accessible area of the CD8α1 domain interacting with parts of the HLA-A2 α2 domain and β2-microglobulin, in addition to the α3 domain interactions. This structure is unlikely to form interactions with more than one MHC molecule as had been speculated; however, a similar latticing to that postulated for CD4 could occur, as the existence of CD8 multimers has been described [25], formed between free cysteine residues in the hinge region.

Although CD8 can form αα homodimers, the major species expressed on the surface of mature MHC class I-restricted T cells is a heterodimer of α and β. CD8-null mice develop only ~20% of the normal numbers of peripheral CD8+ cells, indicating that the CD8β polypeptide supplies a unique function [26,27]. Although the cytoplasmic domain of the β polypeptide does not interact with Lck, there have been suggestions that the CD8β polypeptide can modify CD8-associated Lck activity [28]. There are clear indications that, although the heterodimer and homodimer have similar affinity for MHC [29**], the heterodimer has a more significant effect in influencing the binding of TCR to MHC [29**,30*]. Furthermore, in a series of confocal studies we have shown that anti-CD8α antibodies are significantly more efficient than anti-CD8α antibodies at inducing co-capping of the TCR (Kwan Lim et al. unpublished data; see Note added in proof), suggesting that antibodies to the CD8β polypeptide may preferentially promote a conformation of CD8 that stabilises an association with the TCR, independently of their binding to MHC.

Further examples of dynamic interactions between TCRs, co-receptors and their MHC–peptide ligands have been provided in a series of studies which showed that minimal occupancy of the TCR increased the capacity of CD8 to bind MHC molecules [31]. This suggests that ligation of the TCR can promote binding of CD8, resulting in an overall avidity and dissociation kinetics which exceeds the sum of the individual affinities. Indeed there appear to be preferred conformational states which are stabilised by both TCR and CD8 being bound to the same MHC molecule [29**,32]. On the T-cell surface these interactions may be further promoted by cytoskeletal rearrangements that redistribute the molecules to areas of interaction with the MHC, as agents that disrupt cytoskeletal function have been shown to disrupt CD8 binding to MHC class I [33].

Role of the co-receptors during differentiation

The co-receptors have a significant role during T-cell differentiation, as mice that lack expression of CD4 (CD4(null) [34] or CD8(8Dnull) [35] have severely impaired differentiation of MHC class II- and class I-restricted T cells, respectively. For both CD4 [36] and CD8 [37], if the endogenous molecules are replaced by mutants that lack cytoplasmic domains, differentiation of these subpopulations is somewhat restored, although, characteristically, high levels of expression of such tailless molecules are required. These results suggest that the Lck-binding function of the co-receptors is not an absolute requirement for the function of these molecules during differentiation, although the T-cell repertoire that develops in these mice may well be altered. It has now been confirmed that T-cell differentiation can occur in the absence of CD4 and CD8 expression, implying that the co-receptors do not contribute a unique differentiation signal. Thus, a small subpopulation of CD4−CD8− double (DN) negative cells,
with T-helper phenotype was shown to differentiate in CD4null mice [34], although no equivalent population with cytotoxic phenotype could be demonstrated in CD8null mice [35]. However, in CD8null mice expressing class I-restricted transgenic TCRs (Tg-TCR), the differentiation of TCR+ double-negative cells with cytolytic function could be induced by culture of fetal thymus lobes with peptides that were weakly stimulatory for CD8+ Tg-TCR-expressing cells [38,39]. These data argue that although the co-receptors may not provide a unique signal to direct differentiation to the individual lineages, during normal differentiation they provide a significant contribution to the selection of a broad TCR repertoire on endogenous thymic MHC–peptide ligands.

**Contribution of the co-receptors to anergy versus activation and to differentiation of effector T cell subsets**

In addition to their ability to alter the dynamics of TCR–ligand binding, recent evidence has shown that the co-receptors can have a significant influence on the outcome of antigen engagement. It had been shown previously that expression of co-receptors could influence the fine specificity of a response. For example, co-receptor-negative T cells that were responsive to particular antigens could broaden their recognition and acquire the ability to respond to related antigens once transfected with the appropriate co-receptor [40,41]. It has now been shown that a co-receptor involvement can have a significant influence on the nature of the ensuing response.

While some peptide antigens are able to stimulate full activation of T cells, there are variant peptides, generally referred to as altered peptide ligands (APLs), that stimulate partial responses. These peptides may be weak agonists or may be fully antagonistic and drive the cells into an anergic state [14†]. It had been shown that for class I-restricted cells, CD8 blockade can convert a poor antigen to a good antigen [42], and that expression of the CD8β chain can influence whether a particular APL is seen as an agonist or partial agonist [43]. Similarly, reduction in the level of CD4 can change a partial or weak agonist into an antagonist [44,45]. It has now been shown that the co-receptor has a more direct influence on the nature of the signal that is transduced upon encounter with antigen. Madrenas et al. [46**] found that if they stimulated a T-cell clone in the presence of anti-CD4 antibody or with mutant MHC class II molecules that fail to interact with CD4, they could change a characteristic pattern of tyrosine phosphorylation seen after stimulation with agonist peptides to that resembling the pattern found upon encounter with antagonist peptides. These data suggest that involvement of the co-receptor in recruiting Lck to the TCR complex can have a significant influence on the activation of specific intracellular signalling pathways. A practical consequence of this has been known for some time: non-depleting anti-CD4 antibodies function as efficient immune modulators in vivo capable of generating long-lasting transplantation tolerance [47–49]. Furthermore, a similar effect has been described recently for non-stimulatory anti-CD3 antibodies [50**]. Such reagents are able to engage the TCR without co-localising the co-receptor and appear to induce a state of anergy in T cells similar to that induced by antagonistic peptides.

In addition to influencing whether a cell may be anergised or activated in response to peptide, the involvement of CD4 can influence the nature of the T-cell response elicited by antigen. A recent study by Fowell et al. [51**] showed that in CD4null mice, only Th1 responses were stimulated by pathogens such as *Nippostrongylus brasiliensis* that generally preferentially stimulate a Th2 response. This change was not simply due to an altered TCR repertoire in these animals; in the same study, a significant difference on the ability of CD4+ and CD4- cells expressing the same Tg-TCR to differentiate into Th2 cells was shown. In the absence of CD4 interaction with MHC class II, the T cells appear to alter the balance of cytokines they produce; in particular, they fail to produce interleukin 4 (IL-4), causing differentiation to be pushed exclusively towards the Th1 lineage. It thus seems that there are a number of subtle influences that the co-receptors can have on the nature of the response, both by influencing the sensitivity of antigen recognition and potentially the precise nature of the response.

**Co-receptor dependence in T-cell activation**

It has long been recognised that some T cells require active participation of the co-receptor to initiate a productive response to antigen, while others appear to be activated independently of the co-receptor. Co-receptor independence was initially defined as the resistance of some T-cell clones to inhibition by anti-CD4 and CD8 antibodies [13]. Such antibody blocking studies were complicated by the observation that, in addition to the antibody preventing the co-receptor binding the MHC molecule, engagement of the co-receptors by antibody could generate an inhibitory or negative signal [13]. The latter was thought to occur through the activation of Lck. Nevertheless, it was subsequently shown that if individual TCRs were expressed in the absence of the co-receptors, either in hybridomas or T-cell clones, in general co-receptor-independent TCRs would respond to antigen while co-receptor-dependent TCRs would not [41,52].

The property of co-receptor dependence was thought to reflect the affinity of the TCR for its ligand and it has certainly been shown to be an inherent property of the TCR. For example, transgenic mice made from an alloreactive CD8-dependent or -independent TCR retained this phenotype in their cytotoxic lymphocyte precursors [53]. However, there appear to be differences in the efficiency of signal transduction between CD8-dependent and -independent TCRs which are unrelated to their affinity for peptide–MHC complexes. Thus, we showed that a variety
of CD8-independent hybridomas had markedly increased sensitivity to stimulation with anti-CD3 antibodies than a comparable panel of CD8-dependent hybridomas [52], a property that is unlikely to be related to their affinity for antigen. Furthermore, Yelon et al. [54] found that CD4 cells expressing a Tg-TCR showed considerable CD4 dependency as immature thymocytes, which became less marked when they became peripheral T cells. When repeatedly stimulated to create a long-term line, the same Tg-TCR-expressing cells acquired a CD4-independent phenotype. We observed a similar progression from CD8 dependence to CD8 independence after repeated stimulation of a class I-restricted TCR line from a transgenic mouse mutant for the RAG-1 gene, which confirms that this progression to co-receptor independence involves only the Tg-TCR (G Kwan-Lim, R Zamoyska, unpublished data). Given that the TCR from these transgenic animals would be of the same affinity at all stages of maturation and stimulation, the change to co-receptor independence must reflect an alteration in the sensitivity of the T cell to stimulation.

Recently, Anel et al. [55] have compared the tyrosine phosphorylation patterns induced by stimulation with antigen or anti-CD3 antibody in cytotoxic lymphocyte-precursors and clones expressing either a CD8-dependent or -independent TCR. They concluded that the intensity and duration of the signal induced by TCR engagement was greater in CD8-independent clones, even when stimulation was with anti-CD3 antibody and therefore was unlikely to be simply a reflection of the affinity of the TCR for MHC. An intriguing observation noted by ourselves and by Anel et al., [52,55] was that CD8-dependent TCRs seem to be expressed in greater abundance than CD8-independent TCRs, on the surface of T cells, and it will be interesting to explore whether this is linked to their different behaviour with respect to the co-receptors. It is interesting to note that the crystal structure of the TCR utilised in Anel et al.'s study (KB5-C20), which is remarkably CD8 dependent, has been solved recently [56**] and differs from previously published crystal structures of the TCRs 2C [57] and A6 [58] in that it has a very long CDR3β loop. Furthermore, whereas both 2C and A6 present relatively flat surfaces for interaction with the MHC-peptide complex, KB5-C20 does not. Therefore, it is postulated that KB5-C20 will have to undergo significant conformational change when binding MHC-peptide ligands, which may explain its dependence on CD8 for this interaction. It will be interesting to see whether the structures of other co-receptor-dependent and -independent TCRs will be so diverse.

Conclusions
It is apparent that the nature of the response elicited by contact between a T cell and an antigen-presenting cell is influenced by the intermolecular interactions that occur between them. In contrast to other receptor signalling systems, which generally involve the interaction between soluble ligands and a single receptor species, stimulation of T cells requires the binding of a number of unassociated and unrelated molecules. Despite this, similar questions arise of how extracellular engagement of surface molecules is able to transmit a signal into the cell. Receptor signalling is envisaged to occur in two principal ways, either by receptor aggregation initiating trans-activation of signalling domains or by conformational changes resulting from receptor–ligand binding directly relaying activation signals inside the cell. The data which are accumulating on the interactions between TCRs and co-receptors and MHC molecules suggest that, for T cells, both types of interactions are important. It seems likely that for successful signal transduction, aggregation of distinct receptor species may be facilitated by conformational changes that stabilise these interactions. We are beginning to gain an understanding of the subtleties involved in receptor–ligand engagements on T cells which will be of benefit in designing strategies for regulating immune responses in the future.

Note added in proof
The citation which appears as (Kwan Lim et al., unpublished data) has now been accepted for publication [59].

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest

A recent review of the molecules involved in proximal tyrosine phosphorylation events which accompany TCR triggering.


A comprehensive review of altered peptide ligands, encompassing an overview of current hypotheses of how they exert their effects.


A refreshing look at T-cell activation which discusses how the physical attributes (size, glycosylation and charge) of various receptors on T cells may affect their interactions with each other and their ligands, and pays particular attention to the different classes of receptors which participate in T cell activation.


This paper describes how TCRs have the potential to oligomerise in a concentration-dependent manner when binding to antigenic MHC/peptide complexes, suggesting that T cell signalling may indeed require TCR multimerisation.


Chimaeric mouse CD4 molecules, in which the membrane proximal domains (D3 and D4) were substituted for a variety of other structures, were analysed for their ability to act as co-receptors. Most interesting were hybrids containing mouse D1 and D2 and human D3 and D4 domains, which not only failed to restore T-cell signalling but acted as a dominant negative interfering molecule for the wild-type CD4 function.


The solution of the crystal structure of the intact CD4 is described, indicating the presence of dimers at high protein concentration.


Surface plasmon resonance was used to measure the binding kinetics of CD8αβ homodimers and αβ heterodimers to class I MHC. The binding kinetics of the two forms were found to be similar, except that the heterodimer had a slightly faster off rate. The CD8 heterodimer was found to have a significant influence on the interaction between TCR and MHC-peptide molecules, by stabilising the complex formed between them.


A contemporaneous study to [30] reaching similar conclusions for a second class I restricted transgenic T cell receptor.

35. Blok R, Margules D, Pease L, Ribaudo R, Schneek J, McCluskey J: CD8 expression alters the fine specificity of an alloreactive TCR.


This is a functional and biochemical analysis of the consequence of limiting the association of CD4 with the T-cell receptor (TCR) upon ligand engagement. The effect of preventing the involvement of CD4 was to alter the response to an individual peptide from that described for agonist peptides to that described for antagonist peptides. This study provides biochemical evidence for the participation of the co-receptor in a qualitative manner in the TCR signal transduction cascade.


This study shows that engaging the TCR with non-stimulatory anti-CD3 antibodies, which fail to involve the co-receptors, induces energy in the targeted T-cell population. These results have important implications for therapeutic modulation of T-cell responses in vivo.


An interesting demonstration of the influence of the co-receptor CD4 on the nature of the response which is elicited by a pathogen. The data show that the availability of CD4 can dictate whether T cells can differentiate into the Th2 or Th1 lineages, as CD4+ cells were found to differentiate only into the Th1 subset.


Biocchemical evaluation of the differences in signalling between a CD8-independent and a CD8-dependent T-cell receptors (TCRs) expressed in cytotoxic T lymphocytes (CTL)-precursor cells and clones. The data indicate that the response of the former is stronger and more sustained than the latter, even when anti-CD3 antibodies are used as the stimulus, indicating these differences in signalling potential are acquired during development.


The crystal structure of a CD8-dependent TCR which differs from other published TCR structures in the nature of its CDR3β loop. This TCR is predicted to have to undergo a structural change in order to bind its MHC-peptide ligand, and it will be interesting to determine whether this requirement for conformational modification will relate to its CD8 dependency.

