Review

CD8: Adhesion Molecule, Co-Receptor and Immuno-Modulator

David K Cole[1] and George F Gao[1, 2]

CD8 is a cell surface glycoprotein found in cytotoxic T lymphocytes, which are important components in cellular immunity, esp. in the immune response to cancer and chronic infections. There are two forms of CD8, either as an αα homodimer or αβ heterodimer. It acts as an "assistant" or co-receptor in the function of cytotoxic T cells where specific immunity is mediated by interaction of specific T cell receptor (αβTCR) and its ligand peptide major histocompatibility complex (pMHC). CD8 also binds to pMHC but away from the interface of pMHC and TCR contact, thereof no influence on the specificity of this interaction. If the TCR and CD8 bind to the same pMHC at the same time, CD8 is defined as a co-receptor, functioning through its signalling *via* its cytoplasmic tyrosine phosphorylation pathway; if CD8 binds to pMHC independently of the TCR, it is defined as an adhesion molecule. At present, the co-receptor function theory is dominated in the field. Recent study has also shown that murine CD8αα binds to TL antigen, an MHC homologue, therefore acts as an immuno-modulator. In this review, we discuss these current understandings of the three aspects of the CD8 functions and their structural basis. *Cellular & Molecular Immunology*. 2004;1(2):81-88.

Key Words: CD8, T cell, adhesion, co-receptor, immuno-modulator

Introduction

As science progresses, and the body of information concerning the functions and mechanisms of biological pathways invariably increases through experiment and discussion, our knowledge of the nature of the proteins involved typically broadens to encapsulate the new facts. With this always being the case in science, we often find that the more we know, the more there is to learn. One such example of these is CD8, a cell surface glycoprotein expressed on all cytotoxic T lymphocytes (CTLs), or killer cells.

CD8 was first discovered as a cell surface marker in mice, useful only to distinguish between cytotoxic T cells and the two subsets of CD4 helper T (Th) cells (Th1 and Th2) (1, 2). At this time, these cell surface markers were termed lyt-1 (expressed on helper T cells) or lyt-2/3 (expressed on cytotoxic T cells) (3). Later experiments revealed human homologues of these markers (leu-1, 2a & 2b) (4) suggesting an important functional role due to their evolutionary conservation. This function, due to the ability of CD8 to bind to the same peptide/major histocompatibility complex (pMHC) as its corresponding T cell receptor (TCR), was first thought to be that of an adhesion molecule (5), increasing the avidity and/or stability of the TCR/pMHC interaction (3, 6). This idea was later revised due to the extremely low binding affinity of CD8/pMHC and the failure to detect any specific enhancement to TCR/pMHC binding (7). Presently, CD8 is described most often as a co-receptor to TCR function because it binds to the same pMHC as the TCR (8). As a co-receptor, it is thought to have an important role in the signalling *via* $p56^{lck}$ to induce TCR activation through the CD3ζ chain tyrosine phosphorylation pathway (9, 10). There is also evidence to suggest that CD8 is involved in the recruitment of lipid rafts (11), which interact with the TCR/CD3 complex, also possible important in TCR signalling and activation (12, 13). Although CD8 is primarily recognized as a co-receptor, and the evidence is very much in favour of this being its role, there has recently become available data, which expands the function of CD8 further to that of an immuno-modulator. Leishman et al. carried out experiments to show that intestinal intraepithelial T lymphocytes (iIELs) express CD8 that has the ability to bind to a non-classical MHC class I-like molecule, TL antigen (TL), independently of TCR/CD3 but still signal the TCR through $p56^{lck}$ type pathways (14). These data provide evidence that CD8 can modulate the immunological response of the TCR without being classed as a co-receptor or adhesion molecule by definition.

This review will expand upon the issues of CD8 as an adhesion molecule, a co-receptor (Figure 1) and an immuno-modulator (Figure 2). A more detailed account of the structure and function of CD8 (in comparison with CD4) as well as an account of its evolutionary and cross-species dynamics are described. Lastly, possible future research will be considered to uncover more about its function and the potential for manipulation of this molecule for use in therapy.

[1]Nuffield Department of Clinical Medicine, John Radcliffe Hospital, University of Oxford, Headington Oxford OX3 9DU, United Kingdom.

[2]Corresponding to: Dr. George F Gao, Nuffield Dept of Clinical Medicine, John Radcliffe Hospital, University of Oxford, Headington Oxford OX3 9DU, UK. Tel: 44-1865-228927, Fax: 44-1865-222901, E-mail: george.gao@ndm.ox.ac.uk or ggao66@yahoo.com.

Received for publication Jan 18, 2004. Accepted for publication Feb 26, 2004.

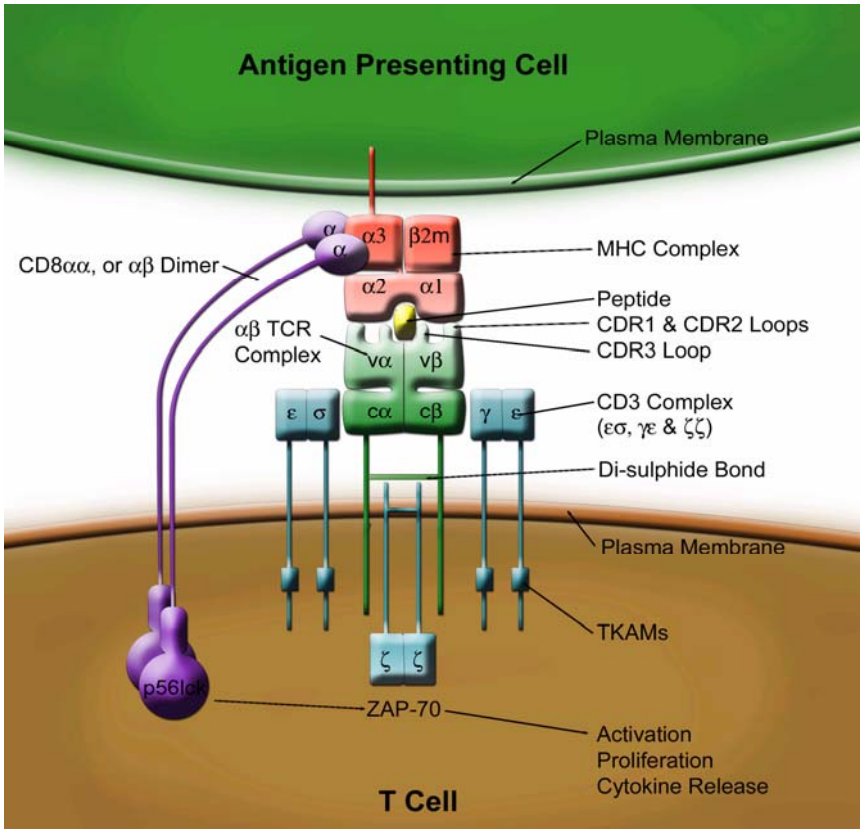

Figure 1. Schematic of the interaction between TCR, pMHC, CD8αα co-receptor and the CD3 complex (it is thought that this complex forms a V-shape in reality (reviewed in Gao et al.)). pMHC I (red), anchored through its transmembrane domain to its antigen presenting cell (green), presents short peptides (yellow) to a T cell receptor (TCR in green, αβTCR heterodimer) bound to a T cytotoxic cell (brown) *via* their transmembrane domains. The TCR recognizes the pMHC I *via* CDR1, 2 and 3 loops from its variable α and β regions (vα and vβ). CD8αα (purple) anchored to the T cell *via* its transmembrane domain, binds to the α3 domain of pMHC independently of the dominant TCR/pMHC interaction. This is thought to be of importance in activation signals transduced *via* CD8 associated $p56^{lck}$ tyrosine kinase to TCR/CD3ζζ mediated T cell activation. CD3 molecules contain tyrosine kinase activation motifs (TKAMs), CD3εσ and γε have one each, ζζ possesses three. These TKAMs are thought to be important in acting as part of $p56^{lck}$ to ZAP-70 kinase pathways and TCR signalling.

Comparative view of the structure and function of CD8αα, CD8αβ and CD4

CD8 forms dimers in its native state; these dimers can be of two sorts. The first is the αα homodimer form, encoded by the *leu-2a* gene in human (*lyt-2* in mice). The second is the αβ heterodimer encoded by *leu-2a* and *leu-2b* (*lyt-3* in mice) genes (3, 15). The structural and functional differences between these two forms are considered.

Structure and function of CD8αα

The α chain of CD8 is physically larger than the β chain at 34-37 kD. It is comprised of an extracellular 122 amino acids N-terminal Ig-like domain with a 48 amino acids stalk region. A transmembrane domain separates this from the cytoplasmic region, which is comprised of 28 amino acids (15) and contains two vicinal cysteine motifs responsible for src kinase $p56^{lck}$ interaction *via* a zinc chelate complex (16). Two α subunits, covalently linked by a disulphide bond, form the homodimer CD8 structure (17).

The expression of CD8αα is present on αβ T cells (although CD8αβ is predominant) and exclusively on NK cells and γδ T cells (18). The binding affinity (Kd) of CD8αα/pMHC analyzed by surface plasmon resonance (SPR) using Biacore™ is around 0.2 mM with kinetics of around 2 to 3 orders of magnitude faster than that of TCR/pMHC binding (7). The heterologous form of CD8 (CD8αβ) shares these characteristics. The fast kinetics is thought to be important in ensuring that the binding of pMHC is dominated by the TCR allowing immune recognition and response (19). The main function of the α chain has previously been determined as important for interaction with $p56^{lck}$ through its cytoplasmic domain, possibly resulting in a signalling cascade and T cell activation (20). The homologous form of CD8, however, has been shown to be a less effective T cell activator than its heterologous counterpart (21, 22). More recent data have alluded to a role as an immuno-modulator due to the ability of two α chains to bind in a cross-link fashion with non-classical MHC molecule (TL antigen) independently of TCR/CD3 complex (only shown in murine systems) (14). Evidence has also provided a role for the α chain in the recruitment of lipid rafts, *via* palmitoylation events, important in TCR signalling (23).

Structure and function of CD8αβ

The CD8β chain is 32 kD and its amino acid sequence shares homology with around 20% of that of the α chain (24). Both the stalk region of the β chain (10-13 amino acids shorter than the α chain (15)) and the cytoplasmic region are shorter than that of the α chain (22). There is no $p56^{lck}$ binding domain in the cytoplasmic region of the β chain, therefore it seems to have no involvement in signalling. Combined with the longer α chain, a recent proposed orientation by Kern et al. could confer greater thermodynamic stability to this form of CD8, explaining to some degree, its greater ability to induce αβ T cell activation (15). These conformational differences have been the main area of study in alluding to the differences observed in the two forms of CD8.

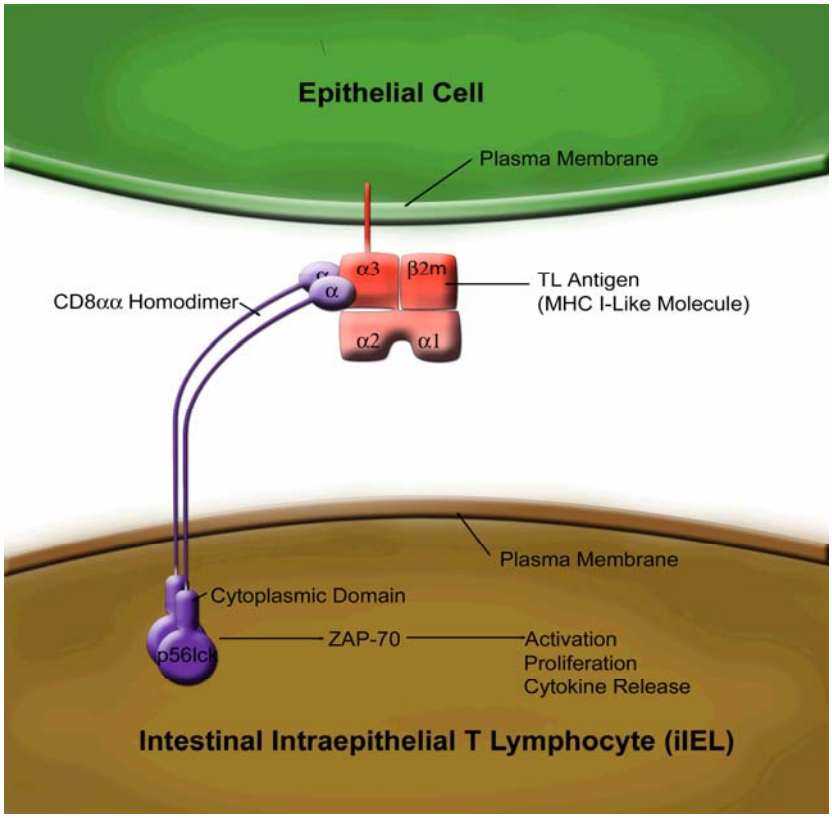

Figure 2. Schematic of CD8αα (purple) bound to TL antigen (MHC I-like molecule in red). CD8αα, bound to an intestinal intraepithelial T lymphocyte (iIEL in brown) *via* its transmembrane domain can bind to TL independently of TCR. TL, bound to an epithelial cell (green) *via* its transmembrane domain, interacts with CD8αα *via* its α3 domain. This interaction causes CD8αα associated $p56^{lck}$ activation and subsequent initiation of ZAP-70 pathways. This can mediate iIEL responses in the absence of TCR/pMHC binding. In this way CD8αα acts as an immuno-modulator.

Although the αβ form shares much of its binding affinity characteristics with CD8αα, data have shown T cell activation to be more effective when it is expressed (25, 26). A recent report, using a chimeric form of CD8α with the stalk region replaced by the CD8β region, has implicated the stalk region in mediating this difference (27). This is most likely due to specifically regulated *O*-linked glycosylation modifications that are important during binding to pMHC, which only occurs in the β chain (19, 27). This function is also of importance when considering negative and positive selection events during thymocyte maturation, due to the restriction in TCR activating peptide repertoire these glycosylations would invoke. Increased T cell production of interleukin-2, a cytokine important for CTL maturation and activation (28), is another characteristic associated with CD8β expression only (29). Expression of high affinity CD8αβ/pMHC is thought to be crucial during positive selection when binding to self-pMHC is required, and this interaction becomes much weaker during negative selection allowing the TCR to become dominant to ensure self-reactive T cells are destroyed (30, 31). As with the α chain, the β chain is palmitoylated, leading to lipid raft recruitment thought to be important in signalling pathways (11, 16, 31-33).

Structure and function of CD4

CD4, unlike CD8, is expressed exclusively on T helper, rather than T cytotoxic cells and interacts with MHC class II (pMHC II) rather than MHC class I (pMHC I) molecules (8). Also unlike CD8 species, it classically forms only a monomeric rather than a dimeric molecule on the cell surface (34), although new evidence has highlighted the possible role of dimeric and oligomeric forms of functional CD4 (35). The monomeric form of CD4 comprises of four Ig-like domains (D1-D4), a single transmembrane domain followed by a short cytoplasmic tail (36, 37). Crystal structures of CD4 have led to the identification of short linker regions between D2, D3 and D4 allowing for a degree of CD4 flexibility (34, 36, 38), unlike the rigidity retained by CD8 molecule. The N-terminal Ig-like V domain interacts with the α2 and β2 proximal domains of the pMHC II molecule (34). In this way, it binds in a dissimilar orientation to CD8, which forms a "clamp" like bond to pMHC. Although key differences exist in the binding modes of CD4 and CD8, both bind to pMHC independently of the TCR/pMHC and do not play a physical role in the dominant peptide interaction.

The main consensus for the function of CD4, is that of a co-receptor involved in TCR signalling, but not critical for TCR/pMHC binding (34, 39, 40). $p56^{lck}$ interaction and subsequently TCR activation *via* phosphorylation events involving the CD3ζ chain occurs *via* the N-terminus of the cytoplasmic tail of CD4 *via* a zinc clasp (41, 42). More in function than in structure, CD4 and CD8 are much alike.

Evolutionary facts and functional diversity of CD8 in different species

CD8 has been studied in a number of model organisms including mice, human and fish. By comparing the function and identifying gene homology, a broader picture of a conserved role (if any) for CD8 can be identified.

As previously discussed, CD8 was first discovered as a cell surface marker in mice, used to identify different populations of T cells. As described by Ledbetter et al., lyt-1 (CD4) is expressed only on helper T cells, whereas lyt-2 (CD8α) and lyt-3 (CD8β) are expressed exclusively on cytotoxic T cells (4, 15). Human homologues to these genes were later described by Ratnofsky et al., *leu-3* (*lyt-1*), *leu-2a* (*lyt-2*) and *leu-2b* (*lyt-3*) (3). It is commonly accepted that CD8 homologues exist in all mammals due to the evolutionary conservation of the genes and the similarities that exist in the workings of the immune systems of these organisms. Not only mammalian species share CD8 co-receptor homology, birds and fish also share many immunological genes, which confer similar functions. Chickens are one of the more widely studied avian species sharing specific sequences in genes that encode for both CD4 (CT4) and CD8 (CT8)-like proteins (43). Similar findings have been described in rainbow trout, using a degenerate primer based PCR protocol to identify CD8α-like sequences. This study indicates that CD8 genes have been conserved for over 400 million years and have evolved little in vertebrate systems (44).

The genes may well be conserved for CD8, but the immunology and physiology of different species such as human, mice, birds and fish are diverse. Is then the function of CD8 co-receptor also conserved, or does it have a multitude of roles as we consider different organisms?

One well-documented fact about human CD8 (hCD8) is its role as a co-receptor and its ability to bind to pMHC "away" from the direct contact interface of TCR/pMHC I acting as a T cell activator *via* $p56^{lck}$ mediated pathways (8, 39, 41). When compared to murine CD8 (mCD8), we find almost identical functionality. Some key differences, however, include the affinity of mCD8, which is in the region of four times stronger than that of hCD8 with more overall contact, although this does not seem to endow mCD8 with any greater functionality (32), although some evidence exists implicating mCD8 with a greater role for TCR/pMHC stability than hCD8 (45). Studies comparing the crystal structures of hCD8 and mCD8 to pMHC I, have confirmed that, although some structural differences in binding orientation do exist between the two species, overall functional homology is maintained, i.e. neither mCD8 nor hCD8 has any contact with the peptide of the TCR/pMHC complex (46). Both bind to the α3 domain of the pMHC molecule inducing a conformational twist either away (mCD8) or toward (hCD8) the T cell to allow binding to occur (there has been some discussion on whether this shift mediates a similar change to the TCR/pMHC interaction, e.g. Gao and Jakobsen, 2000). There are also some differences in the pattern of hydrogen bonding between the two species. In human, three hydrogen bonds are formed between the T cell proximal CD8αα subunit and the MHC α2 domain, in addition to the hydrogen-bonding between CD8αα and pMHC heavy chain α3 domain, light chain β_2m (17). In the murine model hydrogen bonds are exclusively formed between the heavy chain α3 domain and light chain β_2m of the pMHC and N-terminal amino acids in the CD8α chain (47). Recent data, using murine CD8αα on intestinal intra-epithelial T lymphocytes have suggested an immuno-modulator type role for CD8 (14, 48). Whether this is also true for hCD8 has not yet been explored, although this will be discussed in more depth later. The function of CD8 seems to be of a similar nature in avian models as well. Experimental data indicate that CD8 in avian models also interact with a tyrosine kinase molecule sharing homology to the mammalian $p56^{lck}$ (49) mimicking the hCD8 in TCR signalling and activation. As we move away from mammalian and avian models and consider fish, we do observe more of a difference in CD8, both structurally and functionally. Studies involving rainbow trout confirm that the cytoplasmic tail of the CD8α molecule in this species lacks the consensus sequence required for interaction with a $p56^{lck}$-like molecule (44). Whether it interacts with other signalling molecules is not yet known, although it does contain a highly charged proximal portion of the consensus motif, allowing this possibility to be the likely target for future work (44).

CD8 functions: adhesion molecule versus co-receptor

CD8 was first discovered as a cell surface marker important for the initial discovery that a heterologous population of lymphocytes existed as part of the immune system (50). Later, this information was expanded somewhat describing not only different populations according to cell surface marker, but also in terms of immunological function (2, 51, 52). This indicated that specific roles existed for these cell surface markers in immune functions. Experiments using antibodies against the markers provided evidence that T cell activation is reliant on their functions (51, 53, 54). Definitively, CD8 can be classed as a co-receptor (Figure 1). This statement is validated due to the nature of CD8 to bind to the same pMHC as its TCR, without being directly involved in peptide recognition (17).

Adhesion molecule

CD8 has been shown to attach to the α3 domain of pMHC I in an antibody/antigen type conformation with (in human complex) or without (in murine complex) hydrogen bonding between the α2 and β_2m domains (17, 46). For the CD8αα/pMHC I complex structure, it has been determined that binding is asymmetric with one chain contributing around 75% of the interaction. What percentage of the binding the β chain commands is still under scrutiny (17, 21). Mutagenesis data indicate that residues 223-229 of the MHC α3 chain are of particular importance in the CD8 interaction (55). These residues form a loop, which remains flexible pre-CD8 binding, but becomes locked between CDR1-like and CDR3-like loops within the extracellular Ig-like domain of both CD8α chains (17). Residues Gln 115, Asp 112 and Glu 128 have also been shown as crucial factors in the MHC α2 domain (by further mutagenesis work) for successful CD8 binding (47). It was first thought that this CD8 (and CD4)/pMHC complex was needed solely to add stability to the TCR/pMHC complex, endowing additional affinity of the T cells to pMHC on antigen presenting cells (5, 6, 56). This theory was later expanded upon giving rise to the idea of CD8 and TCR

signalling *via* tyrosine kinase $p56^{lck}$ pathways (6, 42, 56, 57). Presently, the idea of CD8 as an adhesion molecule is unlikely due to SPR analysis indicating a significantly lower Kd (binding affinity) when compared to other adhesion molecules such as CD2 and CD48 (58, 59). Also, no conformational changes to the TCR/pMHC complex occur with/without CD8 binding; therefore, CD8 does not induce an energetically favourable shift in binding properties. Therefore the most commonly accepted role for CD8 is that of a TCR signalling molecule as a co-receptor.

TCR signalling

The most important function of CD8 when considering αβTCR is its involvement in activation pathways. The CD8α tail region is associated with tyrosine kinase $p56^{lck}$ *via* two vicinal cysteines that interact through a zinc chelate complex (22, 57, 60). Upon binding with the α3, α2 and β_2m of pMHC, activation pathways are thought to initiate creating a signalling cascade originating from CD8α linked $p56^{lck}$ and leading to ZAP-70 activation on the TCR/CD3 complex (61). CD3 is a signal transducer with three subunits. CD3δε and γε heterodimers containing a single tyrosine kinase activation motif, and a disulphide linked ζζ homodimer with three tyrosine kinase activation motifs are responsible for transmitting the signal from the CD8α chain to the TCR (47, 62). Knockout studies including the CD3 subunits have confirmed the data (63-67).

CD8β chain is not directly involved in $p56^{lck}$ signalling as there is no cytoplasmic tail sequence with a tyrosine phosphorylation site, so it would not be unexpected to find the αβ heterodimer as a poorer TCR activator than the homodimer. This, however, is not the case. It has been experimentally proven the heterodimeric form of CD8 is a more potent activator than the homodimeric form (25). It has been suggested that the unique structure of CD8β stabilizes the interaction between CD8α and $p56^{lck}$ allowing more efficient transduction (29, 68). Perhaps more interesting is the role of the β chain in the creating of lipid rafts. The CD8β cytoplasmic tail is palmitoylated at a membrane-proximal cysteine partitioning CD8 into lipid rafts, or detergent-insoluble membranes (11). These rafts are made up of ordered microdomains, enriched with sphingolipids and cholesterol (11-13, 69, 70). Molecules such as phosphatases (CD45) are excluded allowing uninhibited TCR signalling to take place. Other important molecules such as lck and the linker for activation of T cells (LAT) have been shown to associate increasingly with CD8β during raft recruitment, molecules vital to T cell activation (71, 72). These events inevitably lead to lck phosphorylation, mobilization of intracellular calcium and ZAP-70/CD3 activation (16). Moreover, it has been postulated that CD8β can form a functional link with CD3δ, which in turn is part of the TCR/CD3 complex, creating an important bond between TCR and co-receptor (16). How this interaction is formed and how it functions are not yet known, but knockout mice lacking either CD8β or CD3δ have been shown to suffer from extremely poor TCR mediated activation (73). There is also evidence to suggest that CD8/pMHC binding is enhanced by the β chain due to the presence of unique glycans on the stalk region that modulate the distal binding surface of the CD8αβ globular head domain and therefore, its link to pMHC (74).

Immuno-modulator

Recent evidence has implicated a new role for CD8αα in murine intestinal intraepithelial T lymphocytes (iIELs) that reside in the epithelial tissue layer barrier, which shields internal tissues from the external environment (75). This role is that of an immuno-modulator, rather than a co-receptor or adhesion molecule (Figure 2). The distinction between these two functions is in the ability of CD8αα to bind to an MHC I-like thymus leukemia antigen molecule (TL) independently of the TCR (14).

The expression of the monomeric form of CD8 is unusual on αβTCR expressing T cells because of the increased efficiency observed in TCR mediated T cell activation in cells expressing the dimeric form (21). iIELs may mature in a more specific environment compared to other T cells, in this way they may only be exposed to peptides expressed by intestinal epithelial cells, which in turn could influence activation pathways in the periphery (76). Lieshmann et al. found that TL binds very strongly to CD8αα due to cross-linkage interaction involving both CD8 chains (76). The crystal structure of this complex has shown that one CD8αα homodimer binds to one TL molecule (77). The affinity of binding between CD8αβ and TL was not of the same affinity and deemed insufficient to operate independently of TCR/TL interaction (14). Binding data have indicated that the complementary binding loops of CD8α interact differently with the β_2m domain of TL and the conventional MHC is perhaps in part responsible for this difference (78). In this model, the TCR does not interact with TL, rather, the high affinity bond between CD8 and TL, and the subsequent $p56^{lck}$ signalling pathways are sufficient to induce or suppress immune response (48). Whether the ability of CD8αα to act as an immuno-modulator can be transferred to NK cells and γδ T cells, where it is also commonly expressed, is still a matter for scrutiny. Recent data concerning the functions of TL have indicated that both αβ and γδTCR recognize it independently of antigen (79). Also, due to the self-reactive nature of CTL that bind TL and the low affinity of this interaction, it has been postulated that CD8αα may be required for activation in this unique situation (48). Therefore, the role of CD8 as an immuno-modulator may only occur in this isolated environment and may not contribute towards a broader function for CD8αα expression.

Conclusion and future perspectives

Although evidence exists for a new role for CD8 as an immuno-modulator, this function seems to be isolated to a unique subpopulation of T cells in the murine system. Therefore, the present experimental data point strongly to the major role of co-receptor for CD8 important in TCR signalling and T cell activation. With this in mind, one of the major benefits of CD8 mediated research may come in the form of therapeutic drugs for regulation of immune

responses for conditions such as cancer, allergy and autoreactive diseases such as type I diabetes. It has already been shown that blocking CD8 with anti-CD8 antibody, soluble CD8, or synthetic peptides drastically reduces immune responsiveness (reviewed in Gao et al., 2002). Refinement in the control of CD8 could, therefore be useful in immune suppression for organ transplantation patients.

There are still important discoveries to come in the future for CD8 based research. The structure of CD8αβ has not yet been fully described, and a broader understanding of the function of CD8αα as an immuno-modulator and in other potential roles will, no doubt, be useful. Although many models have been proposed, we still lack the triple complex crystal structure of CD8/pMHC/TCR needed to understand the physical orientation of CD8 in relation to TCR and CD3. These discoveries and others will be key for our continuing increase in knowledge of antigen recognition and TCR signalling and will help to define what there is still to learn.

Acknowledgement

David K Cole is supported by an MRC studentship of the United Kingdom.

References

1. Cantor H, Boyse EA. Functional subclasses of T-lymphocytes bearing different Ly antigens. I. The generation of functionally distinct T-cell subclasses is a differentiative process independent of antigen. J Exp Med. 1975;141:1376-1389.
2. Cantor H, Boyse EA. Regulation of cellular and humoral immune responses by T-cell subclasses. Cold Spring Harb Symp Quant Biol. 1977;41:23-32.
3. Ratnofsky SE, Peterson A, Greenstein JL, Burakoff SJ. Expression and function of CD8 in a murine T cell hybridoma. J Exp Med. 1987;166:1747-1757.
4. Ledbetter JA, Evans RL, Lipinski M, Cunningham-Rundles C, Good RA, Herzenberg LA. Evolutionary conservation of surface molecules that distinguish T lymphocyte helper/inducer and cytotoxic/suppressor subpopulations in mouse and man. J Exp Med. 1981;153:310-323.
5. Norment AM, Salter RD, Parham P, Engelhard VH, Littman DR. Cell-cell adhesion mediated by CD8 and MHC class I molecules. Nature. 1988;336:79-81.
6. MacDonald HR, Thiernesse N, Cerottini JC. Inhibition of T cell-mediated cytolysis by monoclonal antibodies directed against Lyt-2: heterogeneity of inhibition at the clonal level. J Immunol. 1981;126:1671-1675.
7. Wyer JR, Willcox BE, Gao GF, et al. T cell receptor and coreceptor CD8αα bind peptide-MHC independently and with distinct kinetics. Immunity. 1999;10:219-225.
8. Janeway CA Jr. The T cell receptor as a multicomponent signalling machine: CD4/CD8 coreceptors and CD45 in T cell activation. Annu Rev Immunol. 1992;10:645-674.
9. Anel A, O'Rourke AM, Kleinfeld AM, Mescher MF. T cell receptor and CD8-dependent tyrosine phosphorylation events in cytotoxic T lymphocytes: activation of $p56^{lck}$ by CD8 binding to class I protein. Eur J Immunol. 1996;26:2310-2319.
10. Kersh EN, Shaw AS, Allen PM. Fidelity of T cell activation through multistep T cell receptor ζ phosphorylation. Science. 1998;281:572-575.
11. Arcaro A, Gregoire C, Boucheron N, et al. Essential role of CD8 palmitoylation in CD8 coreceptor function. J Immunol. 2000;165:2068-2076.
12. Doucey MA, Legler DF, Boucheron N, Cerottini JC, Bron C, Luescher IF. CTL activation is induced by cross-linking of TCR/MHC-peptide-CD8/$p56^{lck}$ adducts in rafts. Eur J Immunol. 2001;31:1561-1570.
13. Montixi C, Langlet C, Bernard AM, et al. Engagement of T cell receptor triggers its recruitment to low-density detergent-insoluble membrane domains. EMBO J. 1998;17:5334-5348.
14. Leishman AJ, Naidenko OV, Attinger A, et al. T cell responses modulated through interaction between CD8αα and the nonclassical MHC class I molecule, TL. Science. 2001;294:1936-1939.
15. Kern P, Hussey RE, Spoerl R, Reinherz EL, Chang HC. Expression, purification, and functional analysis of murine ectodomain fragments of CD8αα and CD8αα dimers. J Biol Chem. 1999;274:27237-27243.
16. Arcaro A, Gregoire C, Bakker TR, et al. CD8β endows CD8 with efficient coreceptor function by coupling T cell receptor/CD3 to raft-associated CD8/p56(lck) complexes. J Exp Med. 2001;194:1485-1495.
17. Gao GF, Tormo J, Gerth UC, et al. Crystal structure of the complex between human CD8α(α) and HLA-A2. Nature. 1997;387:630-634.
18. Moebius U, Kober G, Griscelli AL, Hercend T, Meuer SC. Expression of different CD8 isoforms on distinct human lymphocyte subpopulations. Eur J Immunol. 1991;21:1793-1800.
19. Gao GF, Rao Z, Bell JI. Molecular coordination of αβT-cell receptors and coreceptors CD8 and CD4 in their recognition of peptide-MHC ligands. Trends Immunol. 2002;23:408-413.
20. Thome M, Duplay P, Guttinger M, Acuto O. Syk and ZAP-70 mediate recruitment of p56lck/CD4 to the activated T cell receptor/CD3/ζ complex. J Exp Med. 1995;181:1997-2006.
21. Gao GF, Jakobsen BK. Molecular interactions of coreceptor CD8 and MHC class I: the molecular basis for functional coordination with the T-cell receptor. Immunol Today. 2000;21:630-636.
22. Zamoyska R. The CD8 coreceptor revisited: one chain good, two chains better. Immunity. 1994;1:243-246.
23. van Der Merwe PA, Davis SJ. Immunology. The immunological synapse--a multitasking system. Science. 2002;295:1479-1480.
24. Nakauchi H, Nolan GP, Hsu C, Huang HS, Kavathas P, Herzenberg LA. Molecular cloning of Lyt-2, a membrane glycoprotein marking a subset of mouse T lymphocytes: molecular homology to its human counterpart, Leu-2/T8, and to immunoglobulin variable regions. Proc Natl Acad Sci U S A. 1985;82:5126-5130.
25. Renard V, Romero P, Vivier E, Malissen B, Luescher IF. CD8β increases CD8 coreceptor function and participation in TCR-ligand binding. J Exp Med. 1996;184:2439-2444.
26. Witte T, Spoerl R, Chang HC. The CD8β ectodomain contributes to the augmented coreceptor function of CD8αβ heterodimers relative to CD8αα homodimers. Cell Immunol. 1999;191:90-96.
27. Wong JS, Wang X, Witte T, et al. Stalk region of β-chain enhances the coreceptor function of CD8. J Immunol. 2003;171:867-874.
28. Janeway CA, Travers P, Walport M, Shlomchik M. Immunobiology (5th Edition). New York, NY: Garland Publishing; 2002.
29. Wheeler CJ, von Hoegen P, Parnes JR. An immunological role for the CD8β-chain. Nature. 1992;357:247-249.
30. Iwabuchi K, Negishi I, Arase H, et al. Positive selection of a T-cell subpopulation in the thymus in which it develops. Proc Natl Acad Sci U S A. 1989;86:5089-5093.

31. Sha WC, Nelson CA, Newberry RD, Kranz DM, Russell JH, Loh DY. Positive and negative selection of an antigen receptor on T cells in transgenic mice. Nature. 1988;336: 73-76.
32. Hutchinson SL, Wooldridge L, Tafuro S, et al. The CD8 T cell coreceptor exhibits disproportionate biological activity at extremely low binding affinities. J Biol Chem. 2003;278: 24285-24293.
33. Sun J, Kavathas PB. Comparison of the roles of CD8αα and CD8αβ in interaction with MHC class I. J Immunol. 1997;159: 6077-6082.
34. Wang JH, Meijers R, Xiong Y, et al. Crystal structure of the human CD4 N-terminal two-domain fragment complexed to a class II MHC molecule. Proc Natl Acad Sci U S A. 2001;98: 10799-10804.
35. Moldovan MC, Yachou A, Levesque K, et al. CD4 dimers constitute the functional component required for T cell activation. J Immunol. 2002;169:6261-6268.
36. Maddon PJ, Littman DR, Godfrey M, Maddon DE, Chess L, Axel R. The isolation and nucleotide sequence of a cDNA encoding the T cell surface protein T4: a new member of the immunoglobulin gene family. Cell. 1985;42:93-104.
37. Wu H, Kwong PD, Hendrickson WA. Dimeric association and segmental variability in the structure of human CD4. Nature. 1997;387:527-530.
38. Ryu SE, Kwong PD, Truneh A, et al. Crystal structure of an HIV-binding recombinant fragment of human CD4. Nature. 1990;348:419-426.
39. Campanelli R, Palermo B, Garbelli S, et al. Human CD8 co-receptor is strictly involved in MHC-peptide tetramer-TCR binding and T cell activation. Int Immunol. 2002;14:39-44.
40. Hamad AR, O'Herrin SM, Lebowitz MS, et al. Potent T cell activation with dimeric peptide-major histocompatibility complex class II ligand: the role of CD4 coreceptor. J Exp Med. 1998;188:1633-1640.
41. Kim PW, Sun ZY, Blacklow SC, Wagner G, Eck MJ. A zinc clasp structure tethers Lck to T cell coreceptors CD4 and CD8. Science. 2003;301:1725-1728.
42. Veillette A, Bookman MA, Horak EM, Bolen JB. The CD4 and CD8 T cell surface antigens are associated with the internal membrane tyrosine-protein kinase $p56^{lck}$. Cell. 1988; 55:301-308.
43. Chan MM, Chen CL, Ager LL, Cooper MD. Identification of the avian homologues of mammalian CD4 and CD8 antigens. J Immunol. 1988;140:2133-2138.
44. Hansen JD, Strassburger P. Description of an ectothermic TCR coreceptor, CD8α, in rainbow trout. J Immunol. 2000; 164:3132-3139.
45. Purbhoo MA, Boulter JM, Price DA, et al. The human CD8 coreceptor effects cytotoxic T cell activation and antigen sensitivity primarily by mediating complete phosphorylation of the T cell receptor ζ chain. J Biol Chem. 2001;276:32786-32792.
46. Kern PS, Teng MK, Smolyar A, et al. Structural basis of CD8 coreceptor function revealed by crystallographic analysis of a murine CD8αα ectodomain fragment in complex with $H\text{-}2K^b$. Immunity. 1998;9:519-530.
47. Sun J, Leahy DJ, Kavathas PB. Interaction between CD8 and major histocompatibility complex (MHC) class I mediated by multiple contact surfaces that include the α2 and α3 domains of MHC class I. J Exp Med. 1995;182:1275-1280.
48. Tsujimura K, Obata Y, Kondo E, et al. Thymus leukemia antigen (TL)-specific cytotoxic T lymphocytes recognize the α1/α2 domain of TL free from antigenic peptides. Int Immunol. 2003;15:1319-1326.
49. Veillette A, Ratcliffe MJ. Avian CD4 and CD8 interact with a cellular tyrosine protein kinase homologous to mammalian $p56^{lck}$. Eur J Immunol. 1991;21:397-401.
50. Shiku H, Kisielow P, Bean MA, et al. Expression of T-cell differentiation antigens on effector cells in cell-mediated cytotoxicity *in vitro*. Evidence for functional heterogeneity related to the surface phenotype of T cells. J Exp Med. 1975; 141:227-241.
51. Cantor H, Boyse EA. Lymphocytes as models for the study of mammalian cellular differentiation. Immunol Rev. 1977;33: 105-124.
52. Jandinski J, Cantor H, Tadakuma T, Peavy DL, Pierce CW. Separation of helper T cells from suppressor T cells expressing different Ly components. I. Polyclonal activation: suppressor and helper activities are inherent properties of distinct T-cell subclasses. J Exp Med. 1976;143:1382-1390.
53. Dembic Z, Haas W, Zamoyska R, Parnes J, Steinmetz M, von Boehmer H. Transfection of the CD8 gene enhances T-cell recognition. Nature. 1987;326:510-511.
54. Swain SL. Significance of Lyt phenotypes: Lyt2 antibodies block activities of T cells that recognize class I major histocompatibility complex antigens regardless of their function. Proc Natl Acad Sci U S A. 1981;78:7101-7105.
55. Salter RD, Benjamin RJ, Wesley PK, et al. A binding site for the T-cell co-receptor CD8 on the α3 domain of HLA-A2. Nature. 1990;345:41-46.
56. Moretta A, Pantaleo G, Mingari MC, Moretta L, Cerottini JC. Clonal heterogeneity in the requirement for T3, T4, and T8 molecules in human cytolytic T lymphocyte function. J Exp Med. 1984;159:921-934.
57. Turner JM, Brodsky MH, Irving BA, Levin SD, Perlmutter RM, Littman DR. Interaction of the unique N-terminal region of tyrosine kinase $p56^{lck}$ with cytoplasmic domains of CD4 and CD8 is mediated by cysteine motifs. Cell. 1990;60:755-765.
58. Davis SJ, Ikemizu S, Wild MK, Van Der Merwe PA. CD2 and the nature of protein interactions mediating cell-cell recognition. Immunol Rev. 1998;163:217-236.
59. Davis SJ, Davies EA, Tucknott MG, Jones EY, Van Der Merwe PA. The role of charged residues mediating low affinity protein-protein recognition at the cell surface by CD2. Proc Natl Acad Sci U S A. 1998;95:5490-5494.
60. Van Oers NS, Garvin AM, Davis CB, et al. Disruption of CD8-dependent negative and positive selection of thymocytes is correlated with a decreased association between CD8 and the protein tyrosine kinase, $p56^{lck}$. Eur J Immunol. 1992;22: 735-743.
61. Delon J, Gregoire C, Malissen B, et al. CD8 expression allows T cell signaling by monomeric peptide-MHC complexes. Immunity. 1998;9:467-473.
62. Borroto A, Mallabiabarrena A, Albar JP, Martinez A, Alarcon B. Characterization of the region involved in CD3 pairwise interactions within the T cell receptor complex. J Biol Chem. 1998;273:12807-12816.
63. Dave VP, Cao Z, Browne C, et al. CD3δ deficiency arrests development of the αβ but not the γδT cell lineage. EMBO J. 1997;16:1360-1370.
64. DeJarnette JB, Sommers CL, Huang K, et al. Specific requirement for CD3ε in T cell development. Proc Natl Acad Sci U S A. 1998;95:14909-14914.
65. Shores EW, Huang K, Tran T, Lee E, Grinberg A, Love PE. Role of TCRζ chain in T cell development and selection. Science. 1994;266:1047-1050.
66. Wang B, Wang N, Salio M, et al. Essential and partially overlapping role of CD3γ and CD3δ for development of αβ and γδ T lymphocytes. J Exp Med. 1998;188:1375-1380.
67. Wang B, Wang N, Whitehurst CE, She J, Chen J, Terhorst C. T lymphocyte development in the absence of CD3ε or CD3γδεζ. J Immunol. 1999;162:88-94.
68. Doucey MA, Legler DF, Faroudi M, et al. The β1 and β3 integrins promote T cell receptor-mediated cytotoxic T

lymphocyte activation. J Biol Chem. 2003;278:26983-26991.
69. Xavier R, Brennan T, Li Q, McCormack C, Seed B. Membrane compartmentation is required for efficient T cell activation. Immunity. 1998;8:723-732.
70. Zhang W, Trible RP, Samelson LE. LAT palmitoylation: its essential role in membrane microdomain targeting and tyrosine phosphorylation during T cell activation. Immunity. 1998;9:239-246.
71. Bosselut R, Zhang W, Ashe JM, Kopacz JL, Samelson LE, Singer A. Association of the adaptor molecule LAT with CD4 and CD8 coreceptors identifies a new coreceptor function in T cell receptor signal transduction. J Exp Med. 1999;190:1517-1526.
72. Bosselut R, Kubo S, Guinter T, et al. Role of CD8β domains in CD8 coreceptor function: importance for MHC I binding, signaling, and positive selection of $CD8^+$ T cells in the thymus. Immunity. 2000;12:409-418.
73. Doucey MA, Goffin L, Naeher D, et al. CD3δ establishes a functional link between the T cell receptor and CD8. J Biol Chem. 2003;278:3257-3264.
74. Moody AM, Chui D, Reche PA, Priatel JJ, Marth JD, Reinherz EL. Developmentally regulated glycosylation of the CD8αβ coreceptor stalk modulates ligand binding. Cell. 2001;107:501-512.
75. Hayday A, Theodoridis E, Ramsburg E, Shires J. Intra-epithelial lymphocytes: exploring the Third Way in immunology. Nat Immunol. 2001;2:997-1003.
76. Bonneville M, Lang F. CD8: from coreceptor to comodulator. Nat Immunol. 2002;3:12-14.
77. Liu Y, Xiong Y, Naidenko OV, et al. The crystal structure of a TL/CD8αα complex at 2.1 A resolution: implications for modulation of T cell activation and memory. Immunity. 2003;18:205-215.
78. Devine L, Rogozinski L, Naidenko OV, Cheroutre H, Kavathas PB. The complementarity-determining region-like loops of CD8α interact differently with β_2-microglobulin of the class I molecules $H\text{-}2K^b$ and thymic leukemia antigen, while similarly with their α3 domains. J Immunol. 2002;168:3881-3886.
79. Weber DA, Attinger A, Kemball CC, et al. Peptide-independent folding and CD8αα binding by the nonclassical class I molecule, thymic leukemia antigen. J Immunol. 2002;169:5708-5714.