

A Subtle Role for CD2 in T Cell Antigen Recognition

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After the demonstration almost 20 years ago that antibodies to CD2 are potent inhibitors of T cell function, this cell surface glycoprotein became one of the most intensively studied of all T cell antigens (1–3). The subsequent identification of LFA-3 (CD58) as a ligand for human CD2 led to the demonstration in cell culture experiments that the CD2–CD58 interaction dramatically enhanced T cell antigen recognition (1–3). Given this background, it was puzzling that no abnormality in T cell function was observed in the initial experiments on CD2-deficient mice (4, 5). A subsequent study did detect some alteration in T cell development and peripheral T cell responses, but the precise nature of the abnormality was unclear (6). Now work by Bachmann and colleagues, described in this issue (7), has clarified matters by demonstrating that T cells from CD2-deficient mice have a quantitative defect in antigen recognition: they require a higher concentration of antigen to produce the same response as wild-type T cells.

CD2 has an extracellular portion with two Ig-like domains, a single membrane-spanning region, and a positively charged, proline-rich cytoplasmic domain, which is highly conserved between species (2, 8). It is expressed on T cells and NK cells in all mammalian species studied to date, but is also found on B cells in the mouse and splenic macrophages in the rat and sheep (9). A more significant species difference is that in the mouse and rat, which do not express CD58, the major CD2 ligand is CD48 (8). Conversely, human CD2 binds very weakly to CD48. CD2 and its ligands, CD48 and CD58, are members of a family of structurally related cell surface molecules termed the CD2 family, which includes signaling lymphocytic activation molecule (SLAM), CD84, Ly-9, and 2B4 (8, 10). The observation that these molecules bind members of the same family suggests that the CD2 family evolved from a common ancestral gene that encoded a homophilic adhesion molecule. Further support for this notion has come from the recent demonstration that 2B4 binds CD48 (11), and that SLAM self-associates (12). It has also been reported that human CD2 binds the structurally unrelated molecule CD59, but this is controversial (8).

The strategy used by Bachmann et al. to study CD2 function was to cross CD2-deficient mice with transgenic mice expressing a TCR specific for the lymphocytic choriomeningitis virus (LCMV)-derived peptide, p33. In the absence of CD2, peripheral T cells required 3–10-fold more p33 peptide to produce the same response in vitro. A similar quantitative defect was seen when APCs lacking intracellular adhesion molecule 1 (ICAM-1, a ligand for the T cell

adhesion molecule LFA-1) were used to present antigen. These differences were more marked when an altered, presumably low affinity, peptide was used as stimulus. This suggests that CD2 may lower the affinity as well as the surface density of peptide–MHC required to activate T cells, a question requiring further study using TCR/peptide–MHC combinations with known binding properties.

A previous study had shown that CD2-deficient mice are no more susceptible to LCMV infection than wild-type mice (5). Bachmann et al. devised a more sensitive assay to probe for subtle defects. They transferred equal numbers of CD2-deficient and CD2-sufficient TCR-transgenic T cells into a mouse and compared the expansion of these two subsets in response to viral challenge. No difference was detected after infection with live virus competent to replicate (either LCMV or a recombinant vaccinia virus expressing the antigenic protein LCMV-GP [vacc-GP]). However, T cells lacking CD2 expanded poorly when the mice were challenged with either vacc-GP virus inactivated by UV light or the viral protein LCMV-GP (administered mixed together with cellular debris). They reasoned that CD2 only conferred an advantage to T cells in the latter experiments because much lower levels of viral antigen would have been presented than is the case after infection with active virus.

Taken together, these experiments suggest that CD2 quantitatively enhances T cell antigen recognition, thereby enabling lower concentrations of peptide–MHC to induce a T cell response. Although the defects observed in CD2-deficient mice are quite subtle, there are significant differences in the CD2–ligand system between humans and mice, suggesting that studies in mice may underestimate the contribution of CD2 to T cell function in humans. First, human CD2 binds its major ligand, CD58, with a 5–10-fold greater solution affinity than mouse (or rat) CD2 for CD48 (13). The difference in the more physiologically relevant “two-dimensional” affinity is even greater (40–50-fold [14]). This was measured as the surface density of membrane-anchored CD48 or CD58 in planar lipid bilayers required for half-maximal binding to CD2 on T cells (14). A second important difference is that, whereas CD58 is very widely expressed in both hematopoietic and nonhematopoietic cells, CD48 expression is largely confined to hematopoietic cells and endothelium (9). Finally, in vitro studies using blocking antibodies (15) or CD2-deficient murine T cell clones (16) suggest that mouse T cells are less dependent than human T cells on CD2–ligand interactions.

How might the CD2–ligand interaction enhance T cell

antigen recognition? One possibility is that the interaction enhances TCR engagement of peptide–MHC. In support of this, Bachmann et al. (7) observed that CD2 enhanced TCR downmodulation, which is thought to correlate with TCR/peptide–MHC engagement (17), to the same extent as it enhanced T cell proliferation and cytokine production. Structural studies have provided important clues to the mechanism by which CD2 enhances TCR/peptide–MHC engagement. The solution of the crystal structure of the extracellular portion of CD2 revealed that CD2 self-associates in the crystal lattice in a head-to-head orientation, making contact via its ligand-binding site (8). Given the structural similarity and evolutionary relationships within the CD2 family, it was proposed that the CD2 would interact with CD48 and CD58 in the same way (8). Complementary mutagenesis provided direct evidence that rat CD2 bound CD48 in a head-to-head orientation (8), and manual docking of human CD2 onto CD58 strongly supported a head-to-head orientation for this complex as well (13). The recent solution of the crystal structure of the CD2–CD58 complex has provided definitive proof that human CD2 binds CD58 in the same orientation (18). Since such a CD2–ligand complex would span the same distance as the TCR/peptide–MHC complex (~14 nm), it was suggested that CD2–ligand interactions might function to position the membranes of the T cell and APC at a separation distance optimal for TCR engagement of peptide–MHC, forming what have been termed “close contact zones” (8). In support of this is the demonstration that the dimensions of the CD2–CD48 complex have a critical influence on T cell antigen recognition (19): whereas the wild-type CD2–CD48 interaction enhances T cell antigen recognition, an elongated CD2–CD48 (>21 nm) interaction is strongly inhibitory.

Low-resolution structural data suggest that the LFA-1/ICAM-1 interaction would span a much greater distance (30–50 nm) than the CD2–ligand interaction (9), implying that the LFA-1/ICAM-1 interaction must enhance TCR/peptide–MHC engagement by a distinct mechanism. This is supported by the finding that the effects of CD2 and ICAM-1 are approximately additive (7). How then does the LFA-1/ICAM-1 interaction enhance TCR engagement of peptide–MHC? One possibility is that it helps form initial T cell–APC contacts. Dustin and colleagues observed that, after the formation of LFA-1/ICAM-1 adhesion contacts between T cells and planar lipid bilayers presenting peptide–MHC, more intimate close contacts formed surrounding the LFA-1/ICAM-1 contacts, despite the absence of any other adhesion molecules in the bilayers (20). They propose that T cells use LFA-1/ICAM-1 adhesion contacts as a fulcrum for “cytoskeletal protrusive mechanisms” that force the membranes into close proximity, thereby helping to form close contact zones.

A glaring omission from the model of CD2 function proposed above is any role for the cytoplasmic domain. This is very highly conserved between species, indicating that it must contribute in an important way to CD2 function. Indeed, truncation of the cytoplasmic domain decreased (although it did not abolish) the enhancing effect of CD2

on T cell antigen recognition (21, 22). Two distinct roles have been proposed for the cytoplasmic domain, transduction of a signal and the regulation of adhesion.

The notion that the CD2 cytoplasmic domain has a signaling role originates from observations many years ago that certain combinations of antibodies to human or rat CD2 can trigger T cell activation, and that these effects require an intact cytoplasmic domain (2, 23). Numerous subsequent studies have used antibody-induced T cell activation to delineate putative signaling pathways. However, given that there is no direct evidence that a physiological interaction between CD2 on T cells with a ligand on target cells or APCs transduces a signal to T cells, it remains possible that antibody-induced signaling is an artefact with little physiological relevance. The effects of anti-CD2 antibodies may simply be a consequence of the cross-linking of CD2-associated molecules such as the TCR–CD3 complex. The observation that CD2 enhances, but is not essential for, T cell function makes it unlikely that CD2 transduces a unique and functionally important signal, independent of the TCR–CD3 complex. It seems more likely that CD2 modulates TCR triggering by recruiting signaling molecules into the vicinity of the TCR–CD3 complex. Several such molecules have been implicated through their ability to bind directly to proline-rich motifs in the CD2 cytoplasmic domain, including the tyrosine kinase *lck* (24), the adaptor molecule CD2BP1 (25) (which binds to a tyrosine phosphatase), and CD2BP2 (26). The precise contributions of these molecules to CD2 function remain to be determined.

A second proposed role for the CD2 cytoplasmic domain is modulation of the adhesion function of CD2. The first evidence for such a role was the observation that activation of T cells enhanced their adhesion to surfaces coated with purified CD58, and that the cytoplasmic domain of CD2 was required for the effect (27). It was subsequently shown that the cytoplasmic domain of CD2 influences its distribution within adhesion contacts between T cells and planar lipid bilayers (28). Molecules segregate into distinct clusters (supramolecular activation clusters [SMACs]) at the interface between T cells and target cells or APCs, forming an organized interface termed the immunological synapse (20, 29; for a review, see reference 30). There is a central cluster (cSMAC) containing the TCR, CD28, and several signaling molecules, and a peripheral cluster (pSMAC) or outer adhesion ring containing LFA-1 and ICAM-1. Dustin and colleagues observed that CD2 segregates into a distinct “inner” adhesion ring surrounding the cSMAC but within the pSMAC (20). Given the similarity in size between CD2–ligand and TCR/peptide–MHC complexes, this finding was unexpected, and it raises several questions. What mechanisms drive the segregation of CD2 into this inner adhesion ring? It seems likely that cytoskeletally driven transport processes are responsible, and, given its role in influencing the distribution of CD2 in contact sites (28), the recently identified CD2-associated protein (CD2AP) is a plausible link between CD2 and the cytoskeleton. The availability of CD2AP-deficient mice (31) should enable this hypothesis to be tested in the near future. Interestingly,

these mice have a more severe defect in T cell activation than CD2-deficient mice, suggesting that CD2AP has a broader role than CD2 in T cell function.

What is the function of the inner adhesion ring? Dustin and colleagues (20) have proposed that it may function as a molecular size "filter," preventing molecules larger than ~15 nm from being transported into the cSMAC. In this context, it is interesting that CD2AP, which is widely expressed, is reported to associate with nephrin (31) and p130^{Cas} (32), proteins that are associated with specialized cell-cell junctions in other tissues. Furthermore, ectopic expression of CD2AP results in cytoskeletal changes (32). These data are consistent with the notion that CD2AP contributes to the formation of specialized cell-cell junctions such as the inner adhesion ring by coupling cell surface molecules to the cytoskeleton.

The "filtration model" for CD2 function does not explain why elongation of CD2-CD48 inhibits T cell antigen recognition to levels below that seen in the complete absence of CD2-CD48 (19). However, these data can be reconciled if it is proposed that CD2 has two distinct functions during T cell antigen recognition. Current evidence suggests that immunological synapse formation involves the active redistribution of cell surface molecules, and that this redistribution follows and is dependent on initial TCR triggering (20, 30). The first function of CD2-ligand may be to enhance this initial TCR triggering by positioning the membranes the optimal distance apart (8). After TCR triggering, CD2 is actively redistributed to the inner adhesion ring (20) where it may have a second function as a molecular filter, thereby helping to maintain the structure

of the immunological synapse (20). It is likely that the initial, membrane-approximation function can be fulfilled by any interaction, such as the CD28-ligand interaction, that has the same dimensions as the CD2-ligand complex.

The fact that CD2 has been conserved throughout mammalian evolution suggests that the quantitative effect that CD2 has on T cell antigen recognition must provide a significant survival advantage. What is this survival advantage? Perhaps the ability to recognize lower concentrations of peptide-MHC enables responses to be mounted earlier after infection and/or facilitates the elimination of residual infectious agent. Another possibility is that the CD2-ligand interaction alters the repertoire of T cells that are selected in the thymus. It has been pointed out that, because T cell clones carrying TCRs with a low affinity for peptide-MHC will be more common than those with a high affinity, an ability to respond to lower affinity peptide-MHC complexes would increase the effective size of the T cell repertoire, thereby increasing the likelihood that any particular T cell will respond to any particular antigen (8). Testing this hypothesis will require a technique for measuring small changes in the size of the T cell repertoire.

In conclusion, the CD2-ligand interaction enables T cells to respond to lower concentrations of antigens. One mechanism for this enhancement is the bringing together of T cell and APC or target cell plasma membranes to a separation distance that is optimal for TCR engagement of peptide-MHC. It seems likely that other lymphocyte molecules will also have quantitative effects on immune function. The lesson from CD2 is that sensitive experiments will be needed to detect these subtle effects.

I regret that, in order to conform to limits on the number of permitted references, it was necessary to cite appropriate reviews instead of original papers wherever possible. I thank Neil Barclay, Anna Cambiaggi, and Talitha Bakker for helpful comments on the manuscript.

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