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CD2/LFA-3

The CD2 and LFA-3 molecules are members of the immunoglobulin superfamily that interact directly to mediate adhesion of T lymphocytes expressing CD2 to diverse cells that express LFA-3.[1,2] The interaction of CD2 and LFA-3 is one of the best characterized heterophilic adhesion mechanisms.

Synonyms

CD2: T11, E rosette receptor; LFA-3: CD58.

Protein properties

CD2 is a 50 kDa M_r glycoprotein and LFA-3 is a 55–75 kDa M_r glycoprotein that migrates on SDS–PAGE as a broad smear. CD2 and LFA-3 form a heterophilic adhesion mechanism which has an important role in T lymphocyte interactions in the context of antigen recognition and T cell development.[3,4] The structural and functional relationship of these molecules makes it appropriate to discuss them both in the same chapter. CD2 was one of the first pan T cell markers in humans.[5] In fact, CD2 was used as a marker for human T cells prior to the advent of monoclonal antibodies in that the major clinical test for T cells is the 1970s and early 1980s was sheep erythrocyte rosetting.[6] This interaction is based on the interaction of human CD2 with the sheep homologueue of LFA-3, T11TS. CD2 is expressed on T cells and some B cells in rodents.[7] CD2 interacts with LFA-3 (CD58) in humans[8] and CD48 in rodents.[8,9] LFA-3 and CD48 are widely expressed glycoproteins.[8,10] Human CD2 does not interact with CD48 in a manner that supports physiological interactions.[11] CD2 plays an important role in T cell repertoire development and in mature T cell function.[10,12]

Activation of T cell through CD2

CD2 engagement with antibodies can trigger T cell activation in a T cell antigen receptor dependent manner.[13,14] In contrast to antibody cross-linking, the interaction of CD2 with its natural ligands does not directly activate T cells.[15] This discrepancy may be due to the fundamental difference between the high affinity and multivalent interaction of pairs of antibodies compared to the low affinity and transient interaction of CD2 with LFA-3 and other natural ligands.

Low affinity interaction of CD2 and LFA-3 in solution leads to a high 2D affinity in contact areas

The affinity of CD2 for LFA-3 has been determined by surface plasmon resonance.[11] The interaction of CD2 and LFA-3 has a low affinity in solution with a k_d of 15 μM at 37°C. The off-rate is estimated to be $< 5^{-1}$. The interaction or rat CD2 and its major ligand rat CD48 has an even lower affinity of 60–80 μM and a similar off-rate of $< 5^{-1}$.[16] Despite this low affinity in solution, both the human and rat adhesion mechanisms are remarkably efficient at forming bonds in cell–cell and cell–artificial bilayer contact areas. Recently, the 2D k_d for interaction of CD2 and CD58 has been determined. The 2D k_d for interaction of human CD2 and LFA-3 is on the order of 2 molecules/μm^2.[17,18] This 2D k_d indicates that physiological densities of CD2 and LFA-3, which are of the order of 100–200 molecules/μm^2, will drive efficient equilibrium

binding in a self-assembled contact area. The best explanation for the high 2D affinity of the CD2/LFA-3 interaction is that the cooperative activity of many bonds aligns the interacting cell membranes with ~1 nm precision, in effect concentrating the CD2 and LFA-3 binding sites in a very small volume.[17] The fast off-rate of the CD2/LFA-3 interaction is evident in rapid bond turnover in contact areas.[18] Thus, adhesion molecules produce a situation that is not typically encountered in cell–cell signalling through soluble molecules, an extremely large number (< 10 000 easily) of transient bonds. These unique kinetics may explain why cell–cell adhesion through CD2/LFA-3 interactions does not trigger T cell activation, the individual bonds may not exist long enough to allow assembly of signalling complexes. This is an important point since activation directly through CD2 alone could be disastrous in terms of T cell selection or autoimmunity.

The nature of the CD2/LFA-3 interaction

The crystal structure of rat CD2 showed not only the expected immunoglobulin like folding arrangement of the two domains, but revealed a bonus, a dimeric unit cell in which two CD2 molecules interact through the previously defined CD48 binding interface.[19] Alan Williams had proposed earlier that the CD2/LFA-3 interaction may have evolved from a homophilic interaction of ancestral CD2 molecules.[20] The Jones *et al.* crystal structure appeared to capture this ancestral interaction at 1.8 nm resolution, including prediction of several specific charged interactions that could be modelled on to a CD2/CD48 interaction (Fig. 1). These predictions have been tested by site directed mutagenesis and the importance of the predictions is elegantly upheld.[2] The interface between CD2 and CD48 contains many charged residues that form salt bridges. Interestingly, when mutated, these charged interactions are shown to make no contribution to the affinity of interaction, although they contribute to the specificity.[59] The structure of rat CD2 was also solved by NMR. Using NMR, it was possible to focus on the environment of the CD2 residues thought to be involved in binding CD48 in the absence and presence of the ligand.[21] The changes in NMR spectra for these residues directly demonstrated that they were involved in a binding interaction with CD48. The crystal structure of CD2 also predicts that the length of the bond is 15 nm. Combining this information with the 2D k_d studies, the conclusion can be reached that the CD2/LFA-3 interaction will hold interacting membranes at a distance of 15±1 nm.

A model for CD2 cooperation with the T cell antigen receptor

The T cell antigen receptor is a unique type of signalling machine in that it is tasked with distinguishing a subtle structural difference between MHC molecules with self-peptides and the same MHC molecules with foreign peptides. The affinity of the TCR interaction with activating foreign MHC–peptide complexes is low, with k_d of 50 μM,

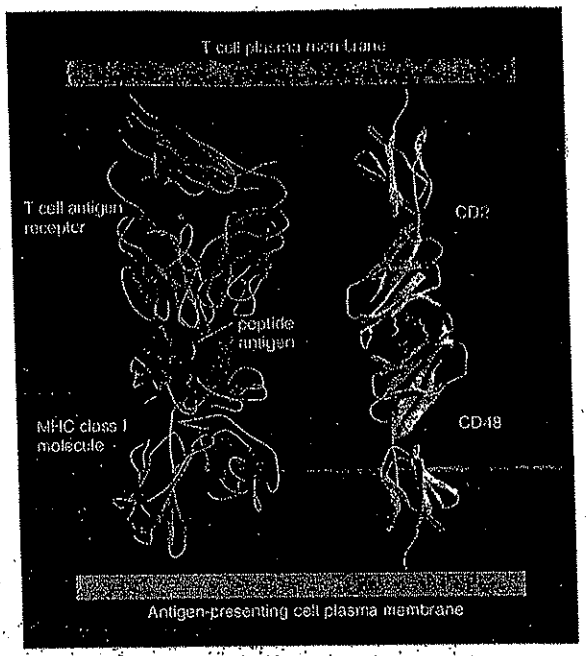

Figure 1. Model for interaction of rat CD2 and rat CD48. The T-cell receptor structure is included to demonstrate the similarity in the gap spanned for CD2/ligand and T-cell receptor/ligand interactions. (Figure provided by P. A. van der Merwe, Oxford University.)

and as few as 100 MHC peptide complexes on an antigen presenting cell (0.1 molecules/μm^2) can activate a T cell.[22,23] The TCR is also a relatively short molecule which when interacting with MHC peptide complexes, spans a gap of 15 nm (Fig. 1). The observation that CD2/LFA-3 and TCR/MHC interactions share the same bond length suggests that the numerically superior CD2/CD58 interaction may set up an alignment of the T cell and antigen presenting cell membranes in which both the CD2/LFA-3 and TCR/MHC interaction will have an optimal 2D affinity. Another T cell adhesion molecule that shares a ~15 nm bond length is the CD28/CD80 interaction.[24] The hypothesis is that T cells compensate for low antigen density on antigen presenting cells by using professional adhesion molecules to bring the membranes into alignment and increase the concentration of the TCR and MHC–peptides complexes. CD2/LFA-3, CD28/CD80, and other adhesion mechanisms may share this function. This may explain the subtle phenotypes of individual CD2 and CD28 knockout mice.[25,26] In fact, preliminary data on the phenotype of the CD2/CD28 double null mutant mouse support the idea that CD2 and CD28 have partially overlapping functions (J. Green and A. Shaw, personal communication).

CD2 mediated signalling

In addition to this extracellular function of aligning membranes, it is clear that CD2 can participate in signal

transduction.[27–29] CD2 has a large cytoplasmic tail of 116 amino acids. The tail contains three proline rich regions that can interact with proteins containing SH3 domains. It is know that src family tyrosine kinases interact with these sequences.[30] It is also likely that other classes of SH3 domain-containing molecules may interact with these sequences. Functionally, the cytoplasmic tail is required for optimal activation of T cell by antigen presenting cells bearing the appropriate MHC–peptide complexes and LFA-3.[31–33] These interactions appear to be important *in vivo* also since a human CD2 transgene in mice, which does not interact with mouse ligands, has a dominant negative phenotype for positive selection of thymocytes expressing a well characterized T cell receptor as a transgene.[12] The proximity of CD2 and the TCR in the same subdomains of the contact area, determined by the shared bond length or by direct lateral interactions, may contribute to this synergistic signalling since CD2 could then recruit kinases and cytoskeletal components to the site of TCR engagement.

Regulation of CD2 mediated adhesion

As long as LFA-3 is laterally mobile, CD2 is a constitutively active adhesion mechanism.[34] When LFA-3 is immobilized artificially on a substrate, CD2 is much less effective at mediating adhesion.[32,34] Under conditions of LFA-3 immobility, T cell activation results in a dramatic increase in T-cell adhesion. The mechanism of this increase in not known but may involve modulation of CD2 lateral mobility on the cell surface.[35] In cell–cell interactions, this antigen receptor mediated adhesion strengthening is subtle. This may reflect the lateral mobility of LFA-3 on the surface of antigen presenting cells. The CD2 adhesion mechanism is indirectly regulated by the surface charge of the interacting cells. CD2 and LFA-3 are buried in the glycocalyx of the T cell and antigen presenting cells and the repulsive activity of the negatively charged sialic acid may greatly reduce the rate of CD2/CD58 encounters until a contact area is established and the membranes are brought into close range.[1] Thymocytes and activated T cells have lower surface charge than resting T cells and these changes in surface charge may modulate the activity of the CD2/LFA-3 adhesion mechanism.

Purification

CD2 can be purified from human T cells using TS2/18 affinity chromatography.[36] Rat CD2 can be purified using OX34.[16] Human LFA-3 can be purified using TS2/9.[37] Rat CD48 can be purified using OX45.[16] All of these purification strategies involve elution at pH 3, which is well tolerated by Ig domains and results in full retention of activity. It is generally accepted that these proteins have fixed natural conformations and do not exist in low and high affinity forms.

Activities

Full length CD2 activity can be measured by direct binding to LFA-3 expressing cells following iodination and adsorption of detergent with excess BSA.[38] LFA-3 can be assayed by forming protein micelles by rapid detergent removal, iodinating the micelles, and testing for binding to CD2 positive cells.[39] These assays are multivalent. The most effective presentation for adhesion is to reconstitute LFA-3 (GPI form) or CD48 into liposomes followed by formation of glass supported planar bilayers.[40] The density of adhesion molecules is evaluated by binding of iodinated mAb. As little as 20 molecules/μm^2 of LFA-3 or 100 molecules/μm^2 rat CD48 will mediate significant adhesion of the appropriate T cells.

Antibodies

The anti-human CD2 mAb TS2/18 blocks binding to LFA-3[41] and is available from ATCC (cell line) or Endogen (Woburn, MA). The anti-human CD2 mAb 6F10.3 does not block binding to LFA-3[42] and is available form Immunotech (Westbrook, ME). Anti-rat (OX34) and anti-mouse (RM2–5) mAb are available from Biosource (Camarillo, CA) and Pharmingen (San Diego, CA), respectively. Anti-human LFA-3 mAb TS2/9 blocks binding to CD2[41] and is available from ATCC or Endogen (Woburn, MA). Anti-rat CD48 mAb OX45 is available from Serotech, USA, Westbrook, ME).

Genes

cDNA sequences have been reported for human CD2[43–45] (M16445, M16336, M14362), rat CD2 [51] (XO5111), mouse CD2[47–49] (Y00023, X06143, M18934), horse CD2[50] (X69884), human LFA-3[51,52] (X06296, Y00636), rat CD48[53] (X13016), and mouse CD48[54] (X17501, X53526). The genomic organization has been determined for the human and mouse CD2 genes[55,56] (J03622, J03623, X07871–74). PDB accession codes: rat CD2: 1HNG; human CD2: 1GYA and 1HNF.

Structures

LFA-3 is homologueous to CD2 in having two Ig-like domains.[51] LFA-3 is heavily glycosylated with six N-linked glycans. LFA-3 can be GPI-anchored or may have a short transmembrane domain.[57] Both isoforms arise from the same gene by alternative splicing.[52] There is no evidence that this splicing is regulated and all cells examined have had equal ratios of both forms. CD48 exists only in a glycolipid-anchored form.[53] The GPI-anchored forms are predicted to have a relatively high lateral mobility and to associate with glycolipid enriched membrane domains.[58]

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