CELL ADHESION

A birth certificate for CD2

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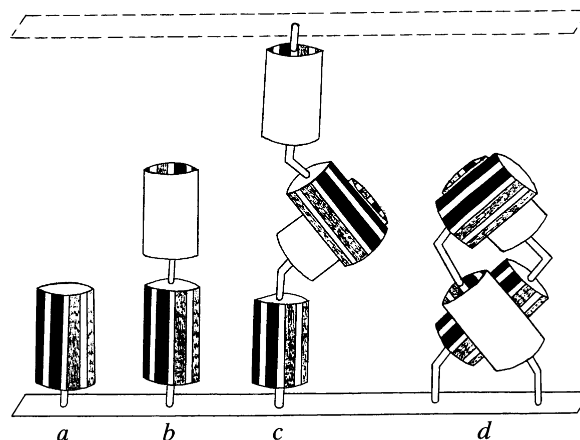
As the usual tag of 'superfamily' implies, the immunoglobulin-related molecules are a large and extended kindred. They are by far the most populous and functionally diverse family of molecules present on the cell surface. On page 762 of this issue[1], Driscoll and colleagues present a three-dimensional structure for the business end of CD2, an important T-lymphocyte adhesion receptor, and at the same time relieve any doubts about the legitimacy of this molecule as a member of the immunoglobulin (Ig) superfamily.

The CD2 adhesion molecule is one of a number of adhesion molecules on T lymphocytes that participate in interactions with other cells, and hence contribute to antigen-specific responses of both cytotoxic and helper T lymphocytes[2]. It functions by binding to LFA-3, a widely expressed molecule that is also a member of the Ig superfamily. Cell-adhesion and monoclonal antibody blocking experiments, and saturable binding of purified soluble CD2 or LFA-3 to the complementary molecule on the cell surface, have provided convincing evidence that CD2 binds to LFA-3 and measurements of the affinity of this interaction. Water-soluble monomeric molecules give a K_d for binding and a K_i for inhibition of adhesion in the range of 1 μM (refs 3–6). CD2 contains two Ig-like domains; mutagenesis and proteolysis studies have shown that the N-terminal, Ig-like domain binds to LFA-3 (refs 6,7).

The structure described on page 762 is of this N-terminal, ligand-binding domain in rat CD2. The domain was expressed in *Escherichia coli* and its structure in solution determined by ^{1}H and ^{15}N multidimensional heteronuclear NMR spectroscopy. Driscoll *et al.* present high-resolution data on the atoms of the polypeptide chain backbone, and no doubt side-chain data will be forthcoming soon. The polypeptide chain fold is that of an Ig V-region, clearly providing CD2 with an impeccable birth certificate as a true descendant in molecular evolution from the primordial Ig-like domain. Although studies on the binding of CD2 to LFA-3 have to date been conducted on the human and sheep molecules, and rat LFA-3 is yet to be identified, there is every reason to expect that the polypeptide fold will be the same in all species. The polypeptide conformation of CD2 is almost identical to previously solved immunoglobulin V-domains and the CD4 N-terminal V-like domain[8,9]; the only significant difference is a comparative truncation in β-strands B, D and E, and a compensating lengthening and kinking of the loop between β-strands B and C. Although the cysteines that form the intradomain disulphide loop in most Ig domains are not present in domain 1 of CD2, the homologous residues are the correct distance apart in the structure so that they could form a disulphide bond if they were cysteines.



An evolutionary pathway for dimerization in the immunoglobulin family[13]. *a*, A primordial, cell-surface Ig domain. The face of the Ig domain with the four-stranded β-sheet is shown with four stripes; the face with the other β-sheet (three-stranded in C-domains or five-stranded in V-domains because of the addition of strands C′ and C″) is plain. *b*, Tandem domain duplication with divergence to give rise to V (upper) and C (lower) domains, as seen in CD2 and LFA-3 (refs 10,15), the first two domains of CD4 (refs 1,8,9), and antibody H and L chains. Rotation by 180° of the V-domain relative to the C-domain is shown as in antibody H and L chains[13,14], and in CD4 (refs 8,9). *c*, Symmetric interaction between the V-domains of two adhesion molecules on opposite cells, as hypothesized for CD2 and LFA-3 and their ancestors. The V-domains are shown interacting exactly as in dimerization of V_L and V_H. *d*, Dimerization of V_H-C_H1 with V_L-C_L as found in Fab fragments of antibody[13,14] and hypothesized for the α- and β-subunits of T-cell receptors. The N-terminal (upper) V-domains interact through the five-stranded β-sheets whereas the C-domains interact through the opposite three-stranded β-sheet.

Prediction of membership of the Ig superfamily is no easy matter because primary sequences have diverged to the extent that a statistically significant relationship often cannot be proven by sequence comparison; hence the importance of three-dimensional structure, which is much more highly conserved. The pre-

diction that CD2 has an Ig-like structure, based on superfamily sequence patterns, is now clearly vindicated[10]; the prediction of secondary structure from primary sequence without taking into account similarities to known structures is fraught with difficulty[11], and it is not surprising that this approach failed to yield the correct structure for CD2 (ref. 6).

The new work brings us closer to a molecular definition of the binding site on CD2 for LFA-3. Alignment of the rat and human sequences shows that mutations that disrupt binding to LFA-3 (ref. 7) lie in the region of β-strands C′ and F. This leads to the prediction that the face of the β-sheet containing the five β-strands C″, C′, C, F and G binds to LFA-3. The structure of CD2 now provides a basis for designing further mutations, particularly in β-strands C″, C and G, that should define the extent of contact between CD2 and LFA-3.

Future structural imaging of CD2 bound to LFA-3, either in a crystal or in solution, would be of great interest. If the general rules for protein–protein association hold[12], the area of contact (calculated on the K_d of 10^{-6} M) will be about 620–680 $Å^2$, only slightly lower than the 700–800 $Å^2$ involved in the binding of antibodies to protein antigens (K_d 10^{-9}–10^{-10} M); yet the nature of the contact appears to be quite different.

Whereas in antibodies the contact site between the variable Ig domains and antigen is formed by the three hypervariable loops that connect strands in the β-sheets and are located at one end of the Ig domain, the ligand-binding residues in CD2 can now be inferred to be on the face of a β-sheet. This type of interaction between CD2 and LFA-3 would be more akin to interactions that underlie dimerization of Ig domains in immunoglobulins (see figure). The V-domains of the light and heavy chains of immunoglobulin, for example, interact through the five-stranded β-sheet that forms one face of the domain; known C-domains by contrast turn the other cheek, interacting through the four-stranded β-sheet forming the other face of the domain[13,14]. Both CD2 and LFA-3 have a single V-like domain N-terminal to a single C-like domain, and it is intriguing that dimerization of V-domains in immunoglobulin uses the same face as in interaction of the V-like domain of CD2 with LFA-3.

The sequence similarity between CD2 and LFA-3 (ref. 15), together with the close linkage of their genes[16], suggests that they arose by tandem gene duplication and this gives rise to a prediction about the symmetry of their interaction. The common ancestor might have participated in like–like (homophilic) interactions with the same molecule on another cell, which evolved to a like–unlike (heterophilic) interaction between CD2 and LFA-3 after the gene duplication event (*c* in the figure). The most optimal interaction between identical proteins is symmetric. This symmetry is seen in oligomeric proteins and is preserved within these proteins after gene duplication and divergence of the monomers, as in the pseudo-dyad axes that relate V_H to V_L and $C_{H}1$ to C_L in the antibody Fab fragment (*d* in the figure). The prediction therefore is that a primordial interaction between the common ancestors of CD2 and LFA-3 would be preserved in evolution as an interaction between corresponding faces of CD2 and LFA-3 with pseudo twofold rotational symmetry. Whether this prediction is true or false will emerge only when the structure of CD2 bound to LFA-3 is solved. □

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