综述与专论

Molecular Mechanisms of Cell-cell Recognition

WANG Jia-Huai

(*Dana-Farber Cancer Institute, Department of Pediatrics, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA.* 02115, USA)

Abstract Cell-cell recognition is the key for multicellular organisms to survive. This recognition critically depends on protein-protein interactions from opposing cell surfaces. Recent structural investigations reveal unique features of these cell surface receptors and how they interact. These interactions are specific, but usually relatively weak, with more hydrophilic forces involved in binding. The receptors appear to have specialized ways to present their key interacting elements for ligand-binding from the cell surface. Cell-cell contacts are multivalent. A large group of cell surface molecules are engaged in interactions. Characteristic weak interactions make possible for each individual molecule pair within the group to constantly associate-dissociate-reassociate, such that the cell-cell recognition becomes a dynamic process. The immunological synapse is a good example for immune receptors to be orchestrated in performing immunological function in a collective fashion.

Key words protein-protein recognition, cell adhesion, three-dimensional structure, immunological synapse, cellular immunity

1 Introduction

In multicellular organisms, cells are in a social context, both structurally and functionally. They adhere together to form various tissue types, and, eventually, a living organism. Cells coordinate the behavior of other cells during development and are involved in complicated and dynamic contacts in the immune response to environmental challenges. As another example, neuronal cells migrate along a defined pathway during axon extension to reach their target and establish a stable synapse. In a third and more dysregulated scenario, malignant cells invade healthy tissues through the bloodstream or lymphatic vessels to generate metastasis. All of these processes, commonly termed cell adhesion in most situations, require direct interactions between cells or between cells and the extra-cellular matrix. Ultimately, at the molecular level, these interactions can be attributed to, in majority cases, interactions between proteins from opposing cell surfaces or between proteins from cell surfaces and the extra-cellular matrix.

Genomic sequence data from the *C. elegans* to the human have revealed a surprisingly large number of cell adhesion molecules (CAMs). Interestingly these molecules fall into only four major categories of protein families: the immunoglobulin superfamily (IgSF), the cadherin family, the selectin family and the integrin family[1]. Extensive structure works have been performed in each of these cell surface receptor families over last the decade. More recently, a major endeavor has been made to define the structures of protein complexes. Exciting accomplishments in this area have unraveled many extremely important cell biology problems, in particular in the field of cellular immunity (see reviews[2,3]). Even more importantly, perhaps, principles of protein-protein interactions from opposing cell surfaces have begun to emerge from these structures, revealing the molecular mechanisms of cell-cell recognition. In this review, I will first briefly describe the structural features of each of the four protein families. Next, I will summarize current knowledge about protein-protein interactions. The central theme of this review will be a discussion of the characteristics of protein-protein interactions from opposing cell surfaces in the context of cell-cell recognition.

2 Structural features of the four cell adhesion families

Figure 1 shows ribbon drawings, depicting one example each for the four families: CD4 in the IgSF[4], C-cadherin in the cadherin family[5], P-selectin in the selectin family[6] and $\alpha_v\beta_3$ in the integrin family[7].

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Dr. Jia-huai Wang was a professor at Institute of Biophysics of CAS in mid 1980's. At that time he also served in the 11-member 863 committee on biotechnology. He is now an associate professor at Dana-Farber Cancer Institute and the Department of Pediatrics and Department of Biological Chemistry and Molecular Pharmacology at Harvard Medical School in the United States. As a structural biologist, he has been working on structures of many interesting subjects, including hormone, tRNA, plant toxins, DNA/protein interactions. Over the last one decade he has turned his interests to structures of cell surface receptors that are critical in immune system. Some of these receptors are also known as virus receptors.
Tel: 617-632-3983; Fax: 617-632-4393; E-mail: jwang@red.dfci.harvard.edu

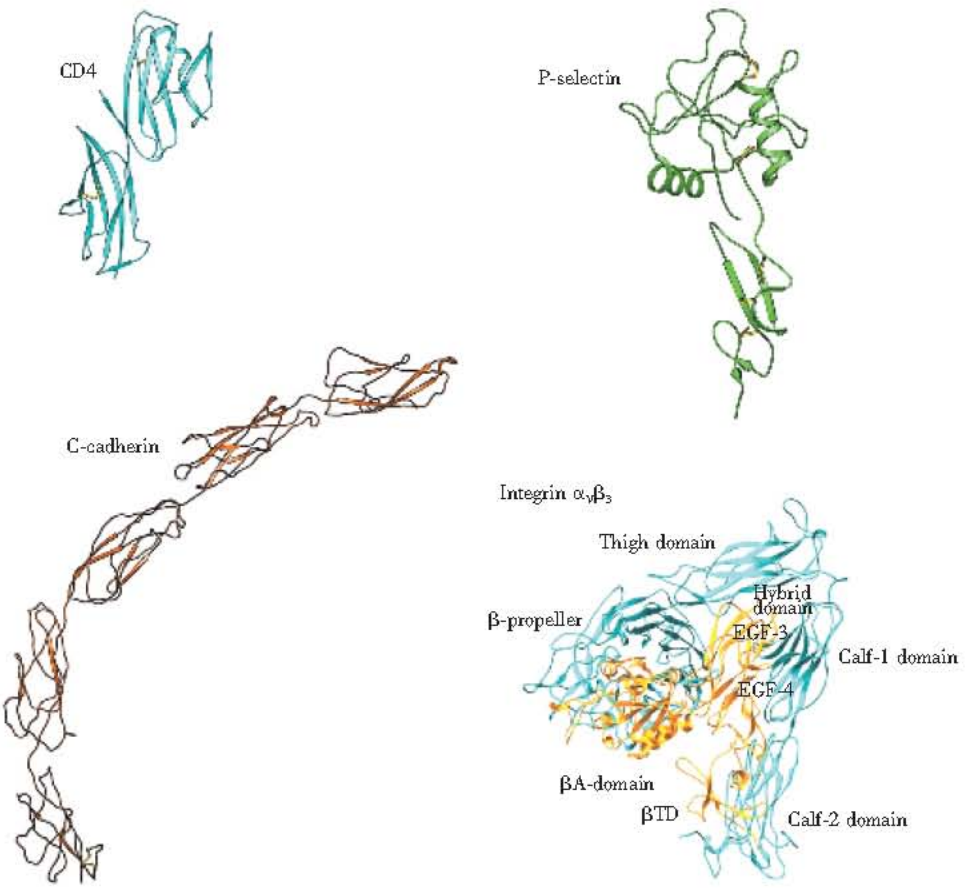

Fig. 1　Ribbon drawings of representatives of four major cell adhesion molecule families

Shown here are the CD4 N-terminal two domain fragment (PDB code: 3CD4)[4], the entire extra-cellular fragment of C-cadherin (PDB code: 1L3W)[5], P-selectin (PDB code: 1G1S)[6] and integrin $\alpha_V\beta_3$ (PDB code: 1JV2)[8]. Note that the hetero-dimeric integrin molecule (the α chain in cyan color and β chain in gold color) is in a bent and inactive conformation. Upon activation, the molecule assumes an extended conformation. The figure was prepared using the program SETOR[9].

2.1　IgSF

This is an extremely large and diverse superfamily. In the human genome, at least 1 192 proteins (5.6% of all assigned sequences) belong to the IgSF (http://supfam.org/SUPERFAMILY/cgi-bin/gen_list.cgi?bluff=0&password=rumplestiltskin). As cell surface receptors, their extracellular portions usually consist of several IgSF domains in tandem or interspersed with epidermal growth factor (EGF) and/or fibronectin type Ⅲ (Fn3) domains. IgSF proteins often have a relatively short cytoplasmic tail. Human CD4, for instance, is made up of four IgSF domains on the cell surface, a 24-residue transmembrane segment and a 35-residue cytoplasmic tail[10]. The IgSF is so named because Ig-like domains all have a similar β sandwich structure which is first found in the immunoglobulin molecule. In the majority cases, the final N-terminal IgSF domain is engaged in cell adhesion. IgSF proteins either participate in homophilic interactions as in NCAM (neural cell adhesion molecule), or in heterophilic interactions with other IgSF members or with integrins, as will be described in later sections. Many IgSF members play key roles in cellular immunity and in the nervous system. These include antigen-specific receptor like T cell receptor (TCR), the co-receptors CD4 and CD8, major histocompatibility antigens (MHCs), and costimulatory factors. Along with them are also many antigen non-specific immune receptors, such as CD2, CD58, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), etc (reviewed in [2, 3]). Well known in the nervous system are NCAM, L1, and axonin-1, etc[11].

2.2　Cadherins

Cadherins are major CAMs responsible for calcium-dependent cell adhesion[12]. They play a crucial role in development and tissue formation. The so-called classic cadherins are conserved in containing five distinct cadherin domains on the cell surface and homologous cytoplasmic tail. The cadherin domain is

similar to the IgSF domain in having a β sandwich structure. They differ, however, from IgSF protiens in several hallmark residues in their sequences. For example, cadherin domains usually do not have a disulfide bond linking two β sheets, a highly conserved feature of IgSF domains. Cadherins mediate almost exclusively homophilic cell adhesion with their N-terminal domains. So far only E-cadherin is known to be involved in heterophilic adhesion with integrin $\alpha_E\beta_7$[13] and possibly $\alpha_2\beta_1$[14].

2.3 Selectins

Selectins are cell surface proteins (lectins) that bind carbohydrates. They are only expressed on endothelium and blood cells mediating transient, calcium-dependent cell adhesion. The conserved lectin domain at the N-terminus binds to a specific oligosaccharide on the opposing cell. This binding is regarded as the first step in the recruitment of leukocytes from the bloodstream into tissue sites of infection or injury.

2.4 Integrins

Integrins are perhaps the most versatile of the adhesion molecules (reviewed in [15]). They are large heterodimeric proteins. The extracellular fragments of their α and β chains are composed of around 1 000 and 700 amino acid residues, respectively. Most integrins are cell surface receptors for extracellular matrix (ECM) proteins, such as fibronectins, laminins and collagens. There are some integrins, particularly those expressed on leukocytes (hence the name of leukocyte integrins), that bind to IgSF members on the endothelium, mediating cell-cell adhesion. Integrins and their ligands play key roles in development, immune responses, and in many human diseases including cancers and autoimmune diseases. Integrins are especially intriguing in that they signal across the plasma membrane in both directions ("inside-out" and "outside-in") through dramatic conformational changes in both the extracellular fragment and the cytoplasmic domain[16].

3 Unique features of protein-protein interactions that mediate cell-cell recognition

3.1 Protein-protein interactions in general

Many biological processes are carried out or regulated by direct protein-protein associations. There have been extensive reviews of the principles of protein-protein interactions[17,18]. A general view can be summarized as follows: Usually the binding is of relatively high affinity, ranging from 10^{-7} mol/L to 10^{-13} mol/L. On average, (16 ± 4) nm^2 of the proteins' surface area get buried upon complex formation. The interface tends to be flat and there is shape complementary between the binding partners. The average interface has the same hydrophobicity as that found on the external surface of a protein, which means that the buried interface is not necessarily dominated by hydrophobic residues. This is because each of the interacting partners has to be stable in solution when they are not part of a complex, and a large exposed hydrophobic surface would destabilize a protein. There is usually a "hot spot", often a tryptophan, tyrosine or arginine, at the center of the binding interface that provides most of the binding energy, whereas at the periphery more hydrophilic residues are seen[19,20]. A standard-size interface contains (9 ± 5) hydrogen bonds, ensuring binding specificity[18].

These principals were generated from protein complex structures in which either both binding partners are soluble proteins as in the case of enzyme/inhibitor, antigen/antibody, interacting signal transduction proteins or in cases where at least one component is a soluble protein, such as cytokine/receptor proteins. This paradigm must be modified when the proteins at issue are cell surface receptors.

3.2 Specific but weak interactions of cell surface receptors

Structure studies of protein-protein interactions of cell surface receptors have recently been made possible through the application of advanced molecular and structural biology techniques to difficult problems in cell biology. The hurdle of manufacturing and crystallizing heavily glycosylated proteins has been overcome in many cases, and a signigicant number of atomic resolution crystal structures of interacting protein complexes from opposing cell surfaces has recently become available. One striking example of this kind is the structure of the complex formed by the adhesive domains of human CD2 and CD58, both IgSF members (Figure 2)[21]. The extracellular fragments of transmembrane proteins CD2 and CD58 have two Ig-like domains each, of which the N-terminal domain is responsible for adhesion. The CD2/CD58 interaction was the first heterophilic cell adhesion to be directly identified[22]. In cellular immunity, unlike antigen/antibody recognition, protein antigens are first processed inside the cell into short peptides. These peptides are then presented by specialized molecules called MHC on the surface of the infected cell, and then a T cell receptor on the T lymphocyte surface will recognize a cognate antigenic peptide bound to the MHC (the complex is called pMHC) on an antigen-presenting cell (APC)[23]. This is a very complicated cell-cell recognition, involving multiple molecular interactions, among which interacting pairs of CD2s from a T cell and CD58s from an APC play an extremely important role.

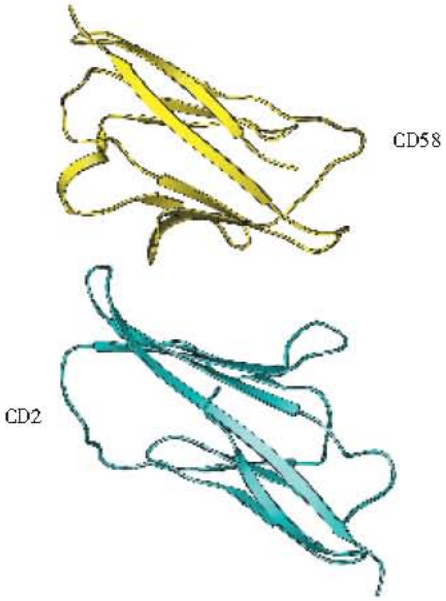

Fig. 2　Ribbon drawing of adhesive complex structure of human CD2 and CD58

Shown here only the adhesive domains of CD2 and CD58. This is a complex between two IgSF family members (PDB code: 1QA9). The CD2 is from the T cell surface and CD58 from the target cell surface. The figure was prepared using the program SETOR[9].

The most remarkable feature of the CD2/CD58 interface is its hydrophilic character. In fact, the interface is dominated by charged residues[21]. There are ten salt bridges and five hydrogen bonds, and only very few hydrophobic contacts between CD2 and CD58. The rich interdigitating hydrogen-bonding (charged plus neutral) network ensures that, among a huge pool of cell surface receptors, CD2 from a T cell and CD58 from an APC can recognize each other with high specificity. Figure 3 is the surface representation of the binding faces of CD2 and CD58. Notably the binding surface of CD2 is rich in basic residues (in blue color), whereas acidic residues (in red color) are predominant in that of CD58. An average protein contains about 11% of acidic and basic residues each, whereas CD2 adhesive domain has 21% positive residues and its CD58 counterpart has 20% negative residues. One can imagine that electrostatic attraction between the two molecules should facilitate their binding. Only about 11.60 nm^2 total surface area is buried upon complex formation, at the very low extreme of the binding statistics cited above for soluble proteins. Furthermore, even within the relatively small contact area, the CD2/CD58 interface does not have good shape complementarity. An *Sc* value has been widely utilized to evaluate the shape complementarity of protein-protein interfaces[24]. *Sc* values measure the degree of geometric match between two juxtaposed surfaces. Interfaces with $Sc = 1$ fit perfectly, whereas interfaces with $Sc = 0$ effectively define topologically uncorrelated surfaces. The *Sc* value ranges from 0.70 to 0.76 for protease/inhibitor interfaces and 0.64 ~ 0.68 for antigen/antibody interfaces[25]. The CD2/CD58 interface has *Sc* value around 0.58, again at the low end of the spectrum for soluble protein interactions. It was noticed[21] that although the CD2 molecule has a quite flat binding surface, CD58's binding surface is unusually uneven. Therefore cavities can be found embedded in the interface. In other words, CD2/CD58 complex has a charge complementary rather than shape complementary interface. These discoveries are surprising, because they contrast sharply with the "normal" protein-protein associations previously described for soluble proteins.

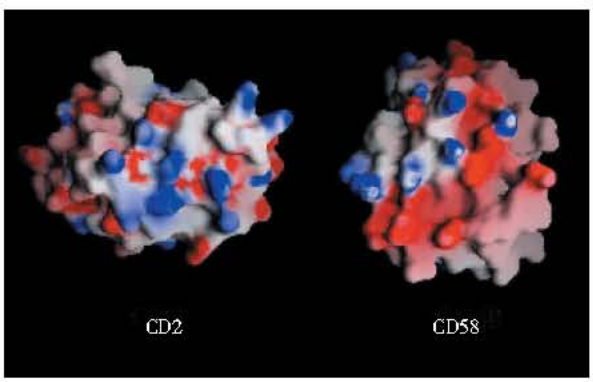

Fig. 3　Electrostatic potential surface representation of binding faces of CD2 and CD58

This figure was drawn as if the CD2/CD58 complex were open up like a book with the binding faces of both molecules facing the reader. In this drawing, molecular surfaces are colored from dark blue (most positive) to deep red (most negative) according to the local electrostatic potential on a relative scale. Hydrophobic residues are in white color. Note the striking contrast between CD2 (more positive surface) and CD58 (more negative surface). The figure was prepared using the program GRASP[26].

The above observations generated from the CD2/CD58 complex have been shown to be more or less common features of cell surface receptor complexes, particularly as demonstrated in recent structure works of the immune system[3,27,28]. In the physicochemical context, hydrophilic forces are much weaker than hydrophobic forces. The affinity measurement of cell adhesion molecule pairs has demonstrated that they are usually in the order of 10^{-3} ~ 10^{-6} mol/L[3]. By comparison, affinities for antigen/antibody are around 10^{-8} ~ 10^{-10} mol/L[25], whereas affinities for hormone/receptor like human insulin/receptor can be as high as 10^{-12} mol/L[29]. It is interesting to point out that, although the insulin receptor is also a cell surface molecule, insulin is a soluble molecule, circulating in the blood stream at very low concentrations. The binding affinity of the insulin/receptor complex has to

be very high so that a single insulin molecule, once firmly bound to the cell surface, can trigger downstream signaling inside the cell. This is a very different cell biology process from immune recognition as will be discussed below.

3.3 The "not-so-hot" hot spots

Albeit weak in character, there still exists an energetic "hot spot" in the interface of cell surface protein adhesion complexes. This has been elaborated through site-directed mutagenesis of the CD2/CD58 interface, in experiments stimulated by the structure work[30]. A systematic replacement of interface residues into an alanine showed that mutations of the most hydrophilic residues involved in hydrogen bonding networks did not have a substantial effect on CD2/CD58 binding. Figure 4 depicts a local region of the CD2/CD58 interface in detail. A mutation in R48A, however, affects affinity significantly (50-fold affinity reduction) because Arg48 is engaged in multiple hydrogen bonds as seen in Figure 4. Interestingly, whereas mutation of Y86F only has a slight effect (three-fold reduction), mutation of Y86A essentially abolished the binding. It was noticed in the CD2/CD58 complex structure that there are only three hydrophobic contacts[21]. The major one is where the aliphatic portion of the Lys34 sidechain of CD58 is sandwiched by aromatic rings from the Phe46 of CD58 and Tyr86 of CD2 (Figure 4). Although the replacement of Y86F causes the loss of one hydrogen bond and hence affects the affinity, the replacement of Y86A destroys key hydrophobic contacts. Apparently, in the energetic context, this is the "hot spot", similar to what has been observed in the well known example of human growth hormone interacting with its receptor[19]. In other words, the general rule still holds: hydrophobic interactions contribute the major binding energy. In the CD2/CD58 complex, there are very few hydrophobic contacts, which explains the low binding affinity.

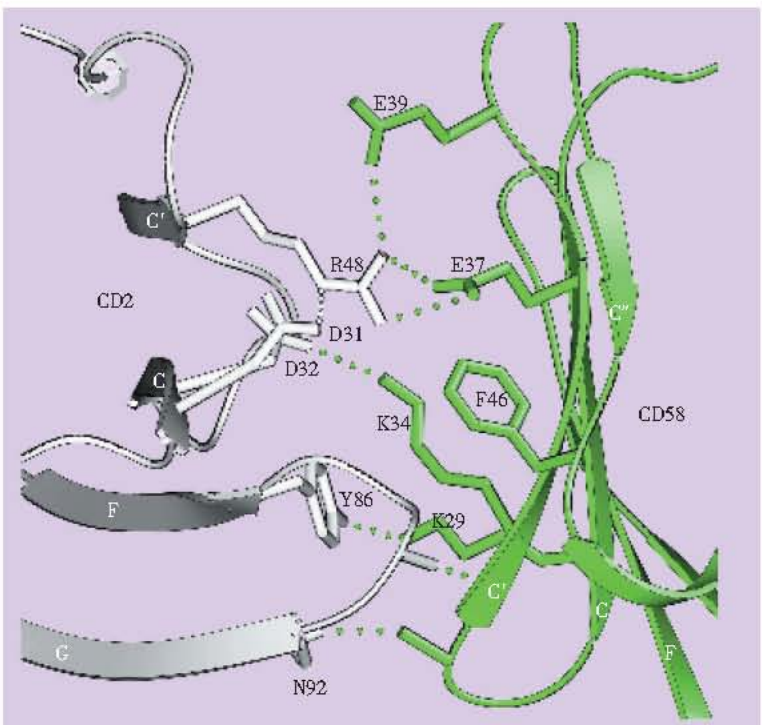

Fig. 4 The hot spots on the CD2/CD58 interface

The dotted lines represent hydrogen bonds. Note that the aliphatic portion of the Lys29 of CD58 is sandwiched by two aromatic rings from the Phe46 of CD58 and the Tyr86 of CD2. Hydrophobic interactions contribute the major energy for binding. This figure was adapted from the reference[30].

3.4 The binding site presentation

Cell adhesion receptors like the IgSF and the cadherin superfamily are composed of several domains in tandem like beads in a string on the cell surface. This kind of modular architecture is a result of evolution. The ICAM-1 molecule, for instance, consists of five Ig-like domains. Each of these structurally defined domains accurately corresponds to an exon[31]. Since there are no linker residues between domains, an exquisite inter-domain junction allows limited flexibility[32]. An elongated molecule can then extend out from the cell surface to reach a binding

partner from the opposing cell. In many cases, the central binding sites are located at the molecule's very N-terminal domain.

Structural studies have provided clues to how these binding sites might be presented. Here are two interesting examples: ICAM-2 has two Ig-like domains on the cell surface. As a glycosylated protein, each domain has three glycans attached. Remarkably, the three glycans on the membrane-proximal domain distribute evenly around the domain at about the same height. It is conceivable that these glycans will have their long and branched carbohydrate chains extending back to reach the cell membrane. The tripod-like glycans may therefore help hold the membrane-proximal domain standing vertically on the cell membrane to present the ligand-binding site on the N-terminal domain[33], as shown in Figure 5. A similar role glycans may play in positioning a cell surface molecule on the membrane can be found for many other molecules. CD2[34] and CD4[35], for instance, have one glycan each at the membrane-proximal domain, both pointing towards the membrane.

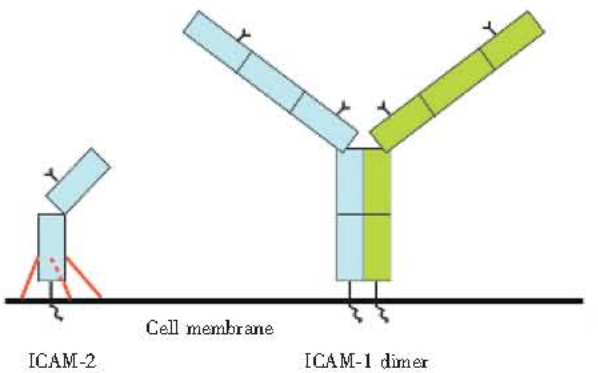

Fig. 5 Cartoon drawings showing how ICAM-2 and ICAM-1 may project their ligand-binding sites from the cell surface

In the case of ICAM-2, three glycans (represented by red sticks) on the membrane-proximal domain act as a tripod to help hold the molecule standing vertically on the cell surface. Moreover, the relatively rigid inter-domain relationship fixes the bending angle between domains 1 and 2 in a way that positions the ligand-binding site (represented by black side chains) pointing towards the opposing cell for interaction[33]. In the case of ICAM-1, the membrane-proximal domains 4 and 5 of two molecules form an intimate dimer (the two molecules in dimer are in cyan and green color, respectively). This offers the dimeric molecule a stem-like architecture as it stands on the cell surface. There is also a characteristic bend between domains 3 and 4 such that both ligand-binding sites (represented by black side chains) on domains 1 and 3 can be presented towards the opposing cell[31].

ICAM-1, on the other hand, uses a different strategy. The five-domain ICAM-1 is known to exist on the cell surface as a dimer[36,37]. There are strong structural evidences indicating that domain 4 of the two independent molecules come together so closely that their two β sheets essentially "merge" into a single super-sheet. The membrane-proximal domains 5 of the two molecules are brought together such that the two molecules' domain 4-5 form a stem-like configuration, which facilitates the standing of the ICAM-1 dimer on the cell surface. A characteristic bend between domains 3 and 4 allows the ligand-binding sites on the N-terminal domain and the domain 3 exposed towards the opposing cell for ligand-binding[31] (see Figure 5).

4 Multivalent and dynamic nature of cell-cell recognition

The unique features of protein-protein interactions from opposing cells described above are characteristic of cell-cell recognition. Recognition between living cells is usually a multivalent and dynamic process. In the nervous system, a synapse is known as a stable adhesive junction between two cells across which signal is relayed from one cell to the other. The cell-cell contacts in the specialized zone are mediated by a large group of cell surface molecules. In the immune system a similar adhesive junction between a T cell and an APC have been described as an immunological synapse[38]. T cells circulate in the blood stream or lymphoid fluid for immune surveillance. When a T cell encounters an APC, it stops migrating and develops contact points. Many molecule pairs are engaged in the contacts to help TCR/pMHC recognition. Other than CD2/CD58, these include adhesion pairs between ICAM-1 and leukocyte integrin LFA-1 (leukocyte function associate antigen-1), co-stimulatory molecule pairs between CD28 (or its homologous inhibitory molecule CTLA-4) and CD80 (B7), etc. In addition, TCR/pMHC recognition requires that a co-receptor CD4 or CD8 interact with the same MHC for signaling. A huge amount of molecules are clustering in the contacting zone between T cells and APCs. Apparently, an immunological synapse is not only a multivalent molecular interacting region between T cells and APCs, but this contact itself is a dynamic process[39]. At any moment some weakly interacting pairs may dissociate, while new pairs may form. Accordingly, most adhesion pairs have fast on and fast off binding kinetics (reviewed in [3]). Since a particular TCR only recognizes its cognate antigenic pMHC, a T cell has to scan the APC surface until the antigen presented on the infected cell surface is detected, and it takes about ten pMHC for a T cell to launch an immune response. It is the dynamic nature of cell-cell association that makes it possible for a particular T cell to execute this scan on an APC's surface for specific recognition. Were interacting pairs to have a very strong affinity, the T cell would immediately stick firmly onto one point of the APC surface, and probably would have never found a

specific antigen that is located somewhere else. Therefore, it is evolutionarily advantageous for adhesion molecules in the immunological synapse to have a weak binding affinity, as is in fact the case.

Figure 6 is a composite drawing of some known structures of interacting pairs within the core of an immunological synapse. In the middle is the ternary model of pMHC recognized by the TCR and co-receptor CD4[40], on the left side is the adhesion pair CD2/CD58[21], and on the right side is the inhibitory pair CTLA-4/B7-1[41,42]. On the periphery (not shown in the figure) are ICAM-1/LFA-1 pairs. The much longer ICAM-1/LFA-1 pairs form a band-like structure[31] to encircle the relatively shorter pairs in the central zone of a synapse as seen in a mature imuunological synapse[39].

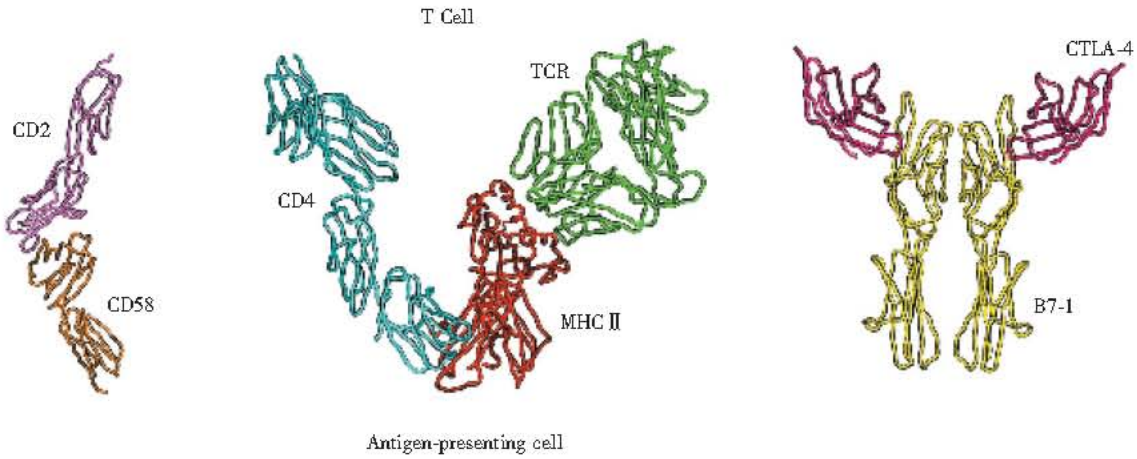

Fig. 6 Molecular complexes in the core region of an immunological synapse between a T cell and an APC

Shown in center is a ternary complex with a class Ⅱ MHC molecule from an APC simultaneously interacting with a TCR and its co-receptor CD4. This model was constructed based on four crystal structures: the TCR/pMHC Ⅱ complexes (PDB code: 1D9K[43] and 1FYT[44]), the CD4/pMHC Ⅱ complex[40], and the CD4 ecto-fragment structure[35]. On the left panel is a model of the complex between the entire extracellular fragments of CD2 and CD58. The model was constructed based on three crystal structures: the CD2/CD58 adhesive domain complex (PDB code: 1QA9)[21], the CD2 two domain structure (PDB code: 1HNG)[34] and a chimeric CD58-CD2 structure (PDB code: 1CCZ). On the right panel is the structure of a complex between dimeric B7-1 and the inhibitory molecule CTLA-4 (PDB code: 1I8L)[42]. The figure was prepared using the program SETOR[9].

In cell biology a special term "avidity" has been used to describe multivalent interactions. Avidity is a function not only of the binding affinity of individual interacting pairs, but also of the copy number of interacting components. In conclusion, cell-cell recognition is a dynamic, avidity-driven process. Each individual interacting pair usually has a specific but weak interaction. A large amount interacting pairs work together dynamically to fulfill important functions.

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