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Supergene families meet in the immune system

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The organization of the immune system and its mobilization in response to foreign antigens are dependent on a network of transient cell–cell interactions. Two receptor–ligand pairs involved in these adhesive interactions have been defined to date, while many remain to be characterized (Table 1). The interaction between the CD2 and lymphocyte function associated antigen-3 (LFA-3) glycoproteins permits T lymphocytes to interact with a number of other cells[1]. More recently, it has been shown that LFA-1 mediates intercellular adhesion, at least in part, by binding to intercellular adhesion molecule-1 (ICAM-1)[2–4]. There are several aspects of ICAM-1 biology that are of interest, and the recent elucidation of the primary structure[5,6] of ICAM-1 establishes an unexpected intersection in the study of two supergene families[5,7]

ICAM-1 was identified independently as a putative LFA-1 ligand expressed on B-lymphoblastoid cells[2] and as a B-cell activation marker[8]. ICAM-1 is a single chain glycoprotein of 76–114 kDa expressed on different cells[8,9]. The core polypeptide has a molecular mass of 55 kDa (Ref. 9).

Analysis of ICAM-1 expression reveals two major themes. (1) While ICAM-1 is weakly expressed on the surface of resting peripheral blood leukocytes[9,10], its expression is increased by activation of T and B lymphocytes and monocytes[8–10]. (2) ICAM-1 expression on nonhematopoietic cells is variable (strongest on endothelium), but is up-regulated by certain cytokines *in vitro* (Table 2) and at sites of inflammation *in vivo* (Refs 9, 14 and G.L. Wantzin, E. Ralfkiaer, C. Avnstorp *et al.*, submitted). ICAM-1 expression on transformed cells appears to correlate roughly with the state of normal differentiation to which the phenotype of the transformed cell is most similar (unpublished)[5,9]. Cells freshly isolated from some Epstein–Barr virus (EBV)-positive Burkitt's lymphoma tumors do not express ICAM-1 or the CD2 ligand, LFA-3 (Refs 15, 16): these cells are relatively resistant to EBV-specific cytolytic T lymphocytes (CTL)[16]. ICAM-1 and LFA-3 are expressed on some of the EBV-positive lines after *in-vitro* culture and expression is correlated with acquisition of susceptibility to EBV-specific CTL[16]. Therefore, down-regulation of ICAM-1 and LFA-3 may be one mechanism by which tumor cells evade the immune response.

The role of ICAM-1 in LFA-1-dependent adhesion was defined using phorbol-ester-induced, LFA-1-dependent homotypic aggregation of B-lymphoblastoid cells as a simple assay. One monoclonal antibody (mAb) was identified on the basis of its ability to inhibit the aggregation of these cells[2], while other mAbs defined initially by different criteria were later identified as potential ICAM-1 mAbs using the aggregation assay[17,18]. Furthermore, immunoaffinity-purified ICAM-1 in planar lipid membranes[3] or cross-linked to solid substrates[4] mediates LFA-1-dependent adhesion, showing that ICAM-1 is an adhesion ligand. Expression of an ICAM-1 cDNA clone has recently confirmed that ICAM-1 is a ligand for

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Table 1. Characteristics of leukocyte adhesion proteins

Receptor	Size (kDa)	Distribution	Ligand	Size (kDa)	Distribution
CD2	50	T lymphocytes, thymocytes	LFA-3	45–75	Broad
LFA-1	α,190; β,95	All leukocytes	ICAM-1 others?	80–114	Broad (see Table 2)
Mac-1 (Mo1, CR3)	α,165; β,95	Monocytes, macrophages, granulocytes, LGL	C3bi, others?		NA
p150, 95	α,150; β,95	Monocytes, macrophages, granulocytes, CTL lines	C3bi, others?		NA

The β subunit is an identical polypeptide in LFA-1, Mac-1 and p150,95. LGL: large granular lymphocytes; CTL: cytolytic T lymphocyte; NA: not applicable.

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Table 2. Regulation of ICAM-1 expression on cultured cells

Cell type	Basal expression[a]	Up-regulated by[b]	Ref.
Fibroblasts	++	IL-1α and β, TNF-α, IFN-γ, LPS	9, unpublished
Umbilical vein endothelial cells	+	IL-1α and β, TNF-α and - β, IFN-γ, LPS	11, 12
Epidermal keratinocytes	+/−	IFN-γ, TNF-α (not by IL-1α or β, or LPS)	13
Thymic epithelial cells	+	IFN-γ, TNF-α (not by IL-1α or β, or LPS)	unpublished
Astrocytes	+/−	IFN-γ, TNF-α	E. Frohman, unpublished

[a]Each '+' represents approximately 50 000 sites per cell, although basal expression varies with culture conditions.
[b]Abbreviations used – IL-1α, β: interleukin 1α, β; TNF-α, β: tumor necrosis factor-α, β; IFN-γ: gamma-interferon; LPS: lipopolysaccharide.

LFA-1-dependent adhesion[5]. Reciprocally, we have found that purified LFA-1 in planar membranes mediates adhesion of cells expressing ICAM-1 (M.L. Dustin and T.A. Springer, unpublished).

LFA-1 and ICAM-1 feature as a receptor–ligand pair in cell adhesion in a number of interactions besides aggregation. ICAM-1 mAbs, as well as LFA-1 mAbs, inhibit non-antigen-specific adhesion of lymphocytes to fibroblasts and gamma-interferon (IFN-γ)-treated epidermal keratinocytes[13]. Non-antigen-specific conjugate formation between CTL and some hematopoietic tumors is inhibited by ICAM-1 mAb[19]. ICAM-1 also contributes to the adhesion of granulocytes to lipopolysaccharide (LPS)-stimulated endothelial cells (C.W. Smith, R.Rothlein, B. Hughes *et al.*, submitted).

However, there is substantial functional evidence that other LFA-1 ligands exist. For example, the homotypic aggregation of some T-cell tumors[4], the interaction of CTL with many hematopoietic targets[19], and the adhesion of T lymphocytes to endothelial cells[20] are all partially or totally inhibited by LFA-1 mAb, but are not affected by ICAM-1 mAb. Despite the possibility of other LFA-1 ligands, ICAM-1 is clearly important for *in-vitro* immune responses since ICAM-1 mAbs inhibit the proliferative response of peripheral blood mononuclear cells to soluble antigens, possibly by inhibiting antigen presentation by monocytes[10]. In contrast, CTL conjugate formation with cultured monocytes is blocked by LFA-1 mAb, but not by ICAM-1 mAb[19]. This suggests that high levels of ICAM-1 expression on cells such as cultured monocytes, which appear to express other LFA-1 ligand(s), may be more critical for interactions with resting lymphocytes than for interactions with activated lymphocytes such as CTL.

LFA-1 is a member of the integrin supergene family of heterodimeric receptors and together with the Mac-1 and p150,95 heterodimers, which share a common β subunit with LFA-1, make up the leukocyte integrin subfamily (Table 1)[21]. While LFA-1 is known to be involved in cell–cell interactions, Mac-1 and p150,95 are involved in binding opsonized particles and various solid substrates in addition to being implicated in intercellular adhesion[1]. Members of the other two integrin subfamilies, also defined by shared β subunits, are extracellular matrix receptors, many of which recognize the core sequence Arg-Gly-Asp (RGD) in their ligands[22]. Mac-1-dependent adhesion to an immobilized RGD-containing peptide derived from the complement protein fragment C3bi has been observed[23];however, it remains to be confirmed that binding involves RGD and can be inhibited by peptides. Binding of LFA-1 to ICAM-1 is not blocked by the fibronectin hexapeptide Gly-Arg-Gly-Asp-Ser-Pro (GRGDSP)[3], but this does not exclude a role for an RGD sequence with critical ICAM-1-specific flanking sequences. It was anticipated that the cloning of ICAM-1 would answer two major questions: (1) is ICAM-1 related to other known adhesion molecules (as LFA-1 was)?; (2) does ICAM-1 contain RGD sequences which could serve as recognition sites for LFA-1? To these questions, the primary structure of ICAM-1 (Refs 5,6) provided two surprising answers: (1) ICAM-1 is a member of the immunoglobulin supergene family, but (2) ICAM-1 contains no RGD sequences.

The interaction between LFA-1 and ICAM-1 is the first known intersection of the integrin and immunoglobulin superfamilies. The combination of integrin and immunoglobulin supergene family members in intercellular adhesion, but not in cell–matrix adhesion systems identified to date, is consistent with the involvement of many immunoglobulin family members in cell–cell recognition[24]. ICAM-1 is most closely related to two adhesion proteins of the adult nervous system, neural cell adhesion molecule (NCAM)[5,7] and myelin-associated glycoprotein (MAG)[25,28]. NCAM, MAG and ICAM-1 each have five immunoglobulin-like domains (Fig. 1). ICAM-1 is 24% homologous with MAG and is 20% homologous with NCAM over the five immunoglobulin-like domains.

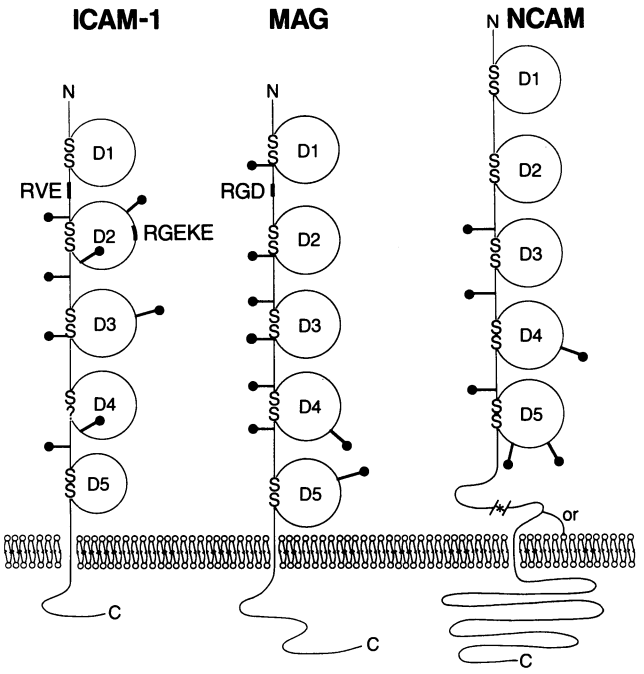

Fig. 1. *Predicted domain structure of ICAM-1, MAG and NCAM. Representations of immunoglobulin-like domains after Williams[24]. The fifth domain of ICAM-1 lacks a β-strand found in other constant (C1/C2) type domains[24]. NCAM exists in alternate transmembrane polypeptide and glycosylphosphatidylinositol-anchored forms. *NCAM also contains a 200 amino acid non-immunoglobulin-like sequence at the carboxy-terminal end of its extracellular domain.*

Localization of the genes for ICAM-1 (Ref. 26) and MAG[27] to chromosome 19 may reflect a closer evolutionary relationship of ICAM-1 with MAG than with NCAM, the gene for which is on chromosome 11 (Ref. 7). MAG is involved in adhesion in the nervous system by what appears to be a Ca^{2+}-independent heterophilic molecular interaction with an unknown molecule[28]. ICAM-1 also has about 20% homology with several retroviral envelope proteins. This includes gp110 of the human immunodeficiency virus (D.E. Staunton and T.A. Springer, unpublished), which has been previously shown to have strong immunoglobulin homology over half-domains[29] and is involved in the interaction between the virus and its cell surface receptor (CD4).

While ICAM-1 contains no RGD sequences, it does contain the sequences Arg-Val-Glu (RVE) and Arg-Gly-Glu-Lys-Glu (RGEKE) between the first and second domains and within the second domain, respectively (Fig. 1). The RVE sequence can readily be aligned with an RGD sequence in MAG. However, a peptide containing the first sequence did not inhibit adhesion (R. Rothlein, unpublished). It is possible that a relatively large recognition region may be involved in interactions between LFA-1 and ICAM-1 since mAbs to two distinct epitopes on ICAM-1 can block adhesion[4].

Does the interaction between the LFA-1 and ICAM-1 molecules set a precedent for interactions between integrin and immunoglobulin superfamilies in intercellular adhesion? Identification of other LFA-1 ligands and the ligands of Mac-1 and p150,95 will reveal whether this is a prevalent feature of integrin-mediated intercellular adhesion or another example of the versatility displayed by the other integrins, which bind to a wide array of extracellular matrix proteins sharing little sequence similarity apart from the RGD sequence itself[22].

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