Determining Compatibility of Adhesion Molecules

M. Finn Morgan and Anthony N. Warrens

Graft 2001; 4; 40

Determining Compatibility of Adhesion Molecules

M. Finn Morgan and Anthony N. Warrens

Adhesion molecules, a heterogeneous group of membrane proteins, play a major role in the adhesion of leukocytes to activated vascular epithelium and their subsequent migration (Fig. 1). Many of these molecules also play a part in hemopoiesis and maturation of leukocytes within organs such as the thymus. As progress is made towards the first pig-to-human xenografts, the interactions between adhesion molecules and their ligands across the species barrier need to be understood for at least two reasons. First, if any of these interactions fail to occur, or occur in an altered fashion, we must be aware of the functional consequences of this and address how to circumvent these problems. Secondly, there may be potential benefit in not having certain functionally effective interactions (if, for example, they promote the host's anti-porcine xenograft response). Before proceeding to modify an interaction, it is clearly crucial to understand the natural consequences of the interaction of a pig receptor with its human ligand and vice versa.

Before considering individual receptor-ligand pairs, it is important to emphasize that these interactions are not only important in the development of an immune response, but also in the maturation of cells of the immune system. Adhesion molecules are involved in hemopoeisis and lymphopoiesis, which may be relevant if xenogeneic bone marrow precursors are to be used to induce xenograft tolerance. When investigating the receptor-ligand interactions across a species barrier, the use of functional assays (such as T-cell activation and proliferation) provides more useful data than experimental systems (such as static adhesion assays) which may fail to detect differences between the species which are small but functionally significant. Similarly, such assays may detect quantitative differences in ligand interactions which in fact have no biologically important consequences. Finally, in further support of such an experimental approach, it should be noted that even when a receptor/ligand interaction is preserved across the species barrier, its physiological effects are not always the same in different species (e.g., VLA-4 in hemopoiesis—see below).

Current experimental knowledge of several specific adhesion receptor/ligand interactions is outlined in the sections that follow.[1]

VCAM-1 (CD106)

Monoclonal antibodies (mAbs) have been raised against both human and porcine VCAM-1 and these can block the adhesion of porcine and human cells to one another in both directions. In more functional experiments, anti-porcine VCAM-1 inhibits the proliferation of human peripheral blood mononuclear cells (PBMCs) to phytohemagglutinin in the presence of porcine endothelial cells.[2] It ha s also been shown that the migration of human monocytes and natural killer (NK) cells across porcine endothelium is blocked by anti-porcine VCAM-1.[3] All these experiments suggest that VCAM-1 can interact functionally with its ligand across the species barrier in both species orientations.

The β1 (CD29) Integrins

Of the six heterodimeric β1 integrins (VLA-1-6, defined by the common β chain), VLA-4, VLA-5 and VLA-6 are important in rejection and hemopoiesis.

Anthony N. Warrens, Ph.D., F.R.C.P.
Departments of Renal Medicine
and Immunology
Imperial College School of Medicine
Du Cane Road
London, W12 0NN United Kingdom
Tel.: 44.20.8383.2307
Fax: 44.20.8383.2062
email: a.warrens@ic.ac.uk

. . . while blocking L-selectin might have an organ-protective effect following xenotransplantation, L-selectin-ligand interactions could prove to be important in xenogeneic tolerance induction.

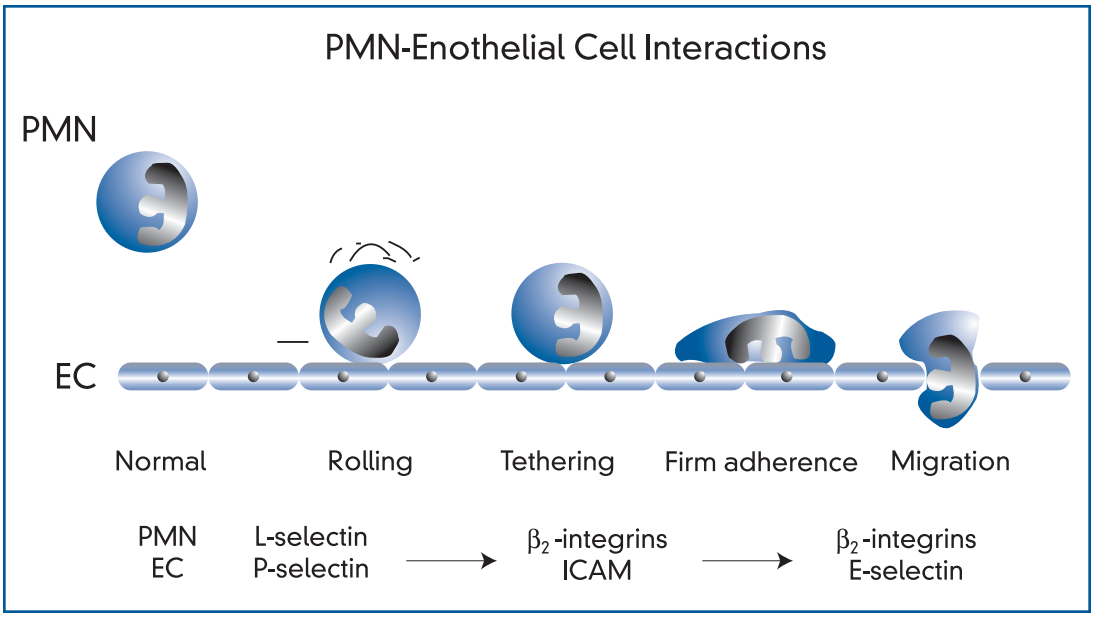

Figure 1. This cartoon shows the process whereby polymorphonuclear cells (PMNs) become increasingly adherent to, and subsequently migrate between, activated endothelial cells (ECs). The figure indicates which adhesion molecules are involved in which stages of the adhesion process.

VLA-4 (CD49d/CD29, α4β1). VLA-4 is known to bind to VCAM-1 and fibronectin. The pVLA-4 α-chain has a similar pattern of expression and molecular weight to the human molecule. In adhesion assay experiments, anti-human CD49d (which cross-reacts with porcine CD49d) has been shown to inhibit the adhesion of human PBMCs and NK cells to porcine endothelial cells, and of porcine PBMCs to human endothelial cells. Migration of human monocytes and NK cells across porcine endothelium in a transendothelial migration assay was also blocked by this mAb.[3] These data suggest that the interactions of VLA-4 with its ligand is preserved between pigs and humans in both species orientations. Surprisingly however, anti-CD49 has no effect on porcine hemopoiesis in long-term bone marrow culture assays (LTBMCs), although it markedly inhibits human hemopoiesis.[4] This is likely to represent different physiological VLA-4 usage rather than molecular incompatibility, since no effect was seen in the same species pig-to-pig positive control.

VLA-5 (CD49e/CD29, α5β1). VLA-5, which binds fibronectin, is involved in the intrathymic development of T cells. So far there is no evidence that pVLA-5 does interact with the human ligand. Depletion of VLA-5-positive cells had no effect on the adhesion of porcine PBMCs to human fibronectin. Also, anti CD49e mAb had no effect on porcine hemopoiesis in a LTBMC although the same mAb markedly inhibited human hemopoiesis.

VLA-6 (CD49f/CD29, α6β1). VLA-6, whose ligand is laminin, may have a role in thymic homing. Adhesion of porcine PBMCs to human laminin was inhibited by anti-VLA-6 in a static adhesion assay suggesting that pVLA-6 does indeed interact with its human ligand.[5]

The β2 (CD18) Integrins

Three β2-integrins have been identified: lymphocyte function-related molecule 1 (LFA-1), Mac-1 and p150.95. β2-integrins have been implicated in cell activation and differentiation, adhesion, extravasation and transendothelial migration, as well as in effector mechanisms. Anti-CD18 inhibits the adhesion of human neutrophils, PBMCs and NK cells to porcine endothelial cells in static adhesion assays. Migration of human monocytes across porcine endothelium is partially inhibited by anti-CD18. Anti-Mac-1 inhibits the adhesion of human monocytes to porcine endothelium.

LFA-1 (CD11a/CD18). LFA-1 is expressed on the surface of most leukocytes and binds to ICAM-1 (CD54), ICAM-2 (CD102) and ICAM-3 (CD50). There is conflicting evidence about the preservation of its interaction between pigs and humans. Anti-CD11a did not inhibit the adhesion of human monocytes to porcine endothelium in a static adhesion assay.[6] However, in a functional system, the pre-incubation of human PBMCs with anti-human LFA-1 decreased the proliferative response in a mixed lymphocyte reaction (MLR) with irradiated porcine cells.[7]

ICAM-1 (CD54)

LFA-1 is the dominant ligand for ICAM-1 but it also binds Mac-1 and CD43. A role for ICAM-1 is implicated in concordant xenograft rejection and interactions between hICAM-1 and pLFA-1 and between pICAM-1 and hLFA-1 may have a co-stimulatory effect. Therefore blocking ICAM-1 on the endothelial surface of a transplanted organ might improve the outcome of xenogeneic pig-to-human organ transplantation. Evidence of preserved ligand interactions across the species barrier comes from experiments in which adhesion of porcine PBMCs to human endothelial cells was inhibited by anti-human ICAM-1. It has also been shown that the pre-incubation of human PBMCs with anti-human ICAM-1 decreases their proliferation in an MLR with irradiated porcine cells.[7]

Evidence of preserved ligand interactions across the species barrier comes from experiments in which adhesion of porcine PBMCs to human endothelial cells was inhibited by anti-human ICAM-1.

CD44

CD44 is a ubiquitous cell surface protein that binds to hyaluronan. CD44 is expressed by virtually all hematopoeitic cells and is thought to play a role in thymic homing and thymocyte differentiation. Anti-CD44 mAb inhibits hemopoiesis in xenogeneic LTBMCs involving porcine hematopoeitic progenitors and human stromal layers.[4]

Selectins

Selectins are glycoproteins which, except for their short consensus repeats, are highly conserved between species.

CD62E. E-selectin is involved in the attachment of lymphocytes to vascular endothelium at sites of rejection; sialyl-Lewis x is the main ligand. Anti-E-selectin will inhibit adhesion of human PBMCs to porcine E-selectin and block the binding of a porcine E-selectin fusion protein to human keratinocytes.[8] Also, COS cells transfected to express porcine E-selectin will bind human polymorphonuclear cells in a way that is inhibitable by anti-porcine E-selectin.[9]

CD62L. L-selectin is expressed on most circulating lymphocytes and on hematopoeitic progenitor cells. Its ligands are GlyCAM and CD34. Roles have been suggested for L-selectin in both hemopoiesis and organ rejection. This suggests that, while blocking L-selectin might have an organ-protective effect following xenotransplantation, L-selectin-ligand interactions could prove to be important in xenogeneic tolerance induction. It has been shown that adhesion of human PBMCs and lymphocytes to porcine endothelial cells is inhibited by anti-human L-selectin in a static adhesion assay.[8]

CD62P. Like E-selectin, P-selectin binds to sialyl-Lewis x and is involved with the process of leukocyte infiltration. Evidence of a preserved ligand interaction across the species barrier is provided by experiments which have shown that adhesion of human PBMCs to porcine endothelium is inhibited by anti-P-selectin glycoprotein ligand.[8] Also, COS cells transfected to express porcine P-selectin bind to human neutrophils much more strongly than untransfected cells.[10]

Conclusion

As far as these molecules have been studied, the most remarkable conclusion that can be drawn is that there is a high degree of preservation of adhesion molecule-ligand interactions between human and porcine cells. Of all the molecules listed above, the only one for which there was no experimental evidence of an interaction was VLA-5. However it should be noted that most of the data referred to above is not from functional experiments and a great deal still remains to be done to identify the subtle differences in these interactions which can be expected to exist and their functional implications.

Abbreviations. PMN: Polymorphonuclear cell; EC: Endothelial cell: VCAM-1; Vascular cell adhesion molecule 1; ICAM: Intercellular adhesion molecule; VLA: Very late antigen; mAb: Monoclonal antibody; PBMC: Peripheral blood mononuclear cell; NK: Natural killer; LFA-1: Lymphocyte function-related molecule 1; MLR: Mixed lymphocyte reaction; SCR: Short consensus repeats.

REFERENCES

1. Warrens AN, Simon AR, Theodore PR, Sykes M. Human-porcine receptor-ligand compatibility within the immune system: Relevance for xenotransplantation. **Xenotransplantation 1999; 6(2):75-78**.
2. Mueller JP, Evans MJ, Cofiell R, Rother RP, Matis LA, Elliott EA. Porcine vascular cell adhesion molecule (VCAM) mediates endothelial cell adhesion to human T cells. Development of blocking antibodies specific for porcine VCAM. **Transplantation 1995; 60(11):1299-1306**.
3. Hauzenberger E, Hauzenberger D, Hultenby K et al. Porcine endothelium supports transendothelial migration of human leukocyte subpopulations: Anti-porcine vascular cell adhesion molecule antibodies as species-specific blockers of transendothelial monocyte and natural killer cell migration [see comments]. **Transplantation 2000; 69(9):1837-1849**.
4. Warrens AN, Simon AR, Theodore PR et al. Function of porcine adhesion molecules in a human marrow microenvironment. **Transplantation 1998; 66(2):252-259**.
5. Simon AR, Warrens AN, Yazzie NP et al. Cross-species interaction of porcine and human integrins with their respective ligands: Implications for xenogeneic tolerance induction. **Transplantation 1998; 66(3):385-394**.
6. Kwiatkowski P, Artrip JH, Ankersmit J et al. Importance of CD49d-VCAM interactions in human monocyte adhesion to porcine endothelium. **Xenotransplantation 1998; 5(1):67-74**.
7. Herrlinger KR, Eckstein V, Muller-Ruchholtz W et al. Human T-cell activation is mediate predominantly by direct recognition of porcine SLA and involves accessory molecule interaction of ICAM1/LFA 1 and CD2/LFA3. **Transplant Proc 1996; 28(2):650**.
8. Robinson LA, Tu L, Steeber DA et al. The role of adhesion molecules in human leukocyte attachment to porcine vascular endothelium: Implications for xenotransplantation. **J Immunol 1998; 161(12):6931-6938**.
9. Tsang YT, Stephens PE, Licence ST et al. Porcine E-selectin: Cloning and functional characterization. **Immunology 1995; 85(1):140-145**.
10. Rollins SA, Johnson KK, Li L, Birks C, Matis LA, Rother RP. Role of porcine P-selectin in complement-dependent adhesion of human leukocytes to porcine endothelial cells. **Transplantation 2000; 69(8):1659-1667**.