Determining Whether HLA-Sensitized Patients Will be Suitable Xenotransplant Candidates

Gilles Blancho, Nathalie Barreau and Jean-Paul Soulillou

Graft 2001; 4; 135

Determining Whether HLA-Sensitized Patients Will be Suitable Xenotransplant Candidates

Gilles Blancho, Nathalie Barreau and Jean-Paul Soulillou

Recent progress, especially the emergence of genetically modified pigs bearing human genes for complement regulatory molecules allowing prevention of hyperacute rejection (see related articles), has brought new hopes for the potential clinical application of xenotransplantation. One of the potential interests of xenotransplantation is to offer a therapeutic solution to highly-sensitized patients who cannot be easily treated by allotransplantation.

Highly Sensitized Patients

Some patients awaiting a graft are defined as "highly sensitized" when they have a high level of alloimmunization (they are also known as "hyper-immunized" patients). This alloimmunization, mainly due to previous transplantations, multiple transfusion, or pregnancies, is characterized in the laboratory by a reactivity against more than 80% of a representative panel of human cells (panel reactive antibody (Ab) = PRA > 80%). Human leukocyte antigen (HLA) molecules are the main targets against which this reactivity is directed and, in certain cases, some allorecognition specificities can be identified. Although both IgM and IgG HLA Ab can be identified, IfgG HLA Ab are considered as being more detrimental to graft outcome[1] with a risk of Ab-mediated rejection. Consequently, finding an appropriate organ is a major difficulty, and some patients are almost excluded from grafting because their alloimmunization is too extensive. If natural anti-porcine Ab can be controlled in the future, xenotransplantation could be a solution for these patients, providing HLA Ab do not recognize any antigen of the porcine organ, especially swine leukocyte antigens (SLA).

Arguments for a Probable Recognition of SLA by HLA Ab

Porcine SLA gene sequences have been shown to have a high level of homology with their human class I and class II counterparts.[2-4] Cross-recognition between SLA and HLA Ab has already been proven or suggested in vitro. In fact, Taylor et al[5] have shown that after depletion of xeno-reactive natural antibodies (XNA), the majority of IgG from highly-sensitized patients recognized pig cells, as determined by FACS analysis, whereas those from normal individuals did not. HLA class I Ab adsorption onto human platelets could dramatically reduce the binding of human Ab to pig lymphocytes in some cases suggesting that at least some IgG HLA could be responsible for a positive crossmatch against pig cells. Naziruddin et al[6] showed similar results with human IgG purified from highly-sensitized patients, which reacted in FACS and microtoxicity assays against a panel of porcine peripheral blood lymphocytes. Similarly, preadsorption onto human platelets reduced their binding to porcine cells and, moreover, they could prove that HLA IgG showed recognition of 45 kDa affinity-purified SLA class I via Western blotting. In our own experience, we have explored, ex vivo, the potential cross-reactivity of HLA Ab with porcine tissue.[7] It was of importance to investigate whether, in a system close to the in vivo situation, HLA Ab could bind to porcine tissue (and not only purified porcinecells) in the presence of SNA, that could have masked all binding sites. We showed that, despite the expected and rapid binding of SNA, the majority, but not all, HLA Ab was also retained within the tissue and could be eluted thereafter. Thus, in vitro and ex vivo evidence exists suggesting that HLA Ab could be another hurdle to the success of xenotransplantation in

Jean-Paul Soulillou, M.D., Ph.D.
Nephrology, Clinical Immunology and Transplant Unit
Hotel Dieu
30 Boulevard Jean Monnet
Nantes 44093 France

humans. Moreover, besides the Ab response, cellular components could also play a deletrious role, since it has been shown that human CD8 cytolytic T cell clones can directly recognize SLA class I molecules,[8] and human CD4 can recognize SLA class II molecules.[9,10]

However, further studies need to be conducted to clarify the feasibility of xenotransplantation in these patients, including:

1. a precise description of the cross-specificies in order to avoid positive crossmatch combinations,
2. the assessment of the function of these Ab, especially in the context of pig organs transgenic for complement regulatory molecules, presumable protected against complement-dependant Ab-mediated cytoxicity, and
3. preclinical studies on alloimmunized primates using transgenic pig organs, which, as demonstrated for XNA, might also resist anti-HLA injury more effectively.

. . . finding an appropriate organ is a major difficulty, and some patients are almost excluded from grafting because their alloimmunization is too extensive.

How Could Highly-Sensitized Patients Be Candidates for Xenotransplantation

With regards to a risky strategy, such as xenotransplantation, the issue concerning the choice of recipient is of fundamental importance. This particularly holds true for renal transplantation, where it seems inconceivable to propose such a strategy to patients, the majority of whom may have an acceptable quality of life under chronic hemodialysis. However, some highly sensitized patients, such as those with immunization against multiple MHC haplotypes, are simply excluded from grafting, unless an alternative strategy can be proposed. So far, no strategy aimed at removing their Ab has been successful, and xenotransplantation could thus be an alternative solution. Once safety (porcine endogenous retrovirus) and physiological aspects have been clarified, the first clinical trials could be conducted in renal xenotransplantation since there is the possibility of returning these patients to dialysis in the event of xenograft failure.

Highly-sensitized patients could be suitable recipients of xenografts in the future, once the virological risk (PERV) and the function of pig organs in a human context have been assessed and once an acceptable immunosuppression has been determined. It will probably be important to avoid incompatible combinations between HLA Ab and some SLA specificities that remain to be defined.

References

1. Chapman JR, Taylor CJ, Ting A et al. Immunoglobulin class and specificity of antibodies causing positive T cell crossmatches. Relationship to renal transplant outcome. **Transplantation 1986; 42:608-613**.
2. Sullivan J, Oettinger HF, Sachs DH et al. Analysis of polymorphism in porcine MHC class I genes: Alterations in signals recognized by human cytotoxic lymphocytes. **J Immunol 1997; 159:2318-2326**.
3. Hirsch F, Germana S, Gustafsson K et al. Structure and expression of class II alpha genes in miniature swine. **J Immunol 1992; 142:841-846**.
4. Gustaffson K, Germana S, hirsch F et al. Structure of miniature swine class II DRB genes: Conservation of hypervariable amino acid residues between distantly related mammalian species. **Proc Natl Acad Sci USA 1990; 87:9798-9802**.
5. Taylor CJ, Tang KG, Smith SI et al. HLA-specific antibodies in highly-sensitized patients can cause a positive crossmatch against pig lymphocytes. **Transplantation 1998; 65:1634-1641**.
6. Naziruddin B, Durriya S, Phelan D et al. HLA antibodies present in the sera of sensitized patients awaitng renal transplant are also reactive to sswine leukocyte antigens. **Transplantation 1998; 66:1074-1080**.
7. Barreau N, Godfrin Y, Bouhours JF et al. Interaction of anti-HLA antibodies with pig xenoantigens. **Transplantation 2000; 69:48-156**.
8. Shishido S, Naziruddin B, Howard T et al. Recognition of porcine major histocompatibility complex class I antigens by human $CD8^+$ cytolytic T cell clones. **Transplantation 1997; 64:340-346**.
9. Yamada K, Sachs DH, DerSimonian H. Human anti-porcine xenogeneic T cell response. Evidence for allelic specificity of mixed leukocyte reaction and for both direct and indirect pathways of recognition. **J Immunol 1995; 155:5249-5256**.
10. Shishido S, Naziruddin B, Xu XC et al. Indirect recognition of porcine xenoantigens by human $CD4^+$ T cell clones. **Transplantation 1998; 65:706-712**.