Understanding the Mechanisms of Acute Cellular Rejection

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This could be the greatest challenge in discordant pig-to-primate xenotransplantation. When recipient primates are pre-treated to avert hyperacute rejection, or donor grafts are genetically modified to resist complement mediated damage, grafts are still rejected at times varying from 3 days to three months. It is already clear that cell-mediated xenograft rejection will involve cellular components not found in allograft rejection (Fig. 1). Thus in allograft rejection, rejection is initiated by $CD4^+$ T cells, the effector mechanisms being a mixture of $CD8^+$ T cells, macrophages, and cytokines mediating direct damage to graft parenchyma (Fig. 1). To date, in vivo models of porcine-to-primate xenotransplantation have described an infiltration of NK cells and monocytes. In addition, in vitro studies have shown strong responses of human $CD4^+$ T cells and neutrophils to porcine endothelium, which must therefore be added to the likely repertoire of human anti-pig cell-mediated responses (Fig. 1). NK cells have rarely been described after human solid organ allotransplantation, and neutrophils are only found associated with antibody-mediated hyperacute rejection. It is not clear why T cells and neutrophils have not yet been seen in large numbers in pig-to-primate models; it may be that non-human primates behave differently to humans, or that grafts do not survive long enough for the response to become apparent. In addition to different types of cells, it is clear that the nature of ligand/receptor interactions differs between and allogeneic and xenogeneic systems, giving the responses different sensitivities to currently used immunosuppressive reagents. This review will update what is known about the nature of interactions between human T cells, NK cells, monocytes, and neutrophils and porcine endothelium.

In vitro studies have shown direct activation of purified human $CD4^+$ T cells by porcine endothelial cells (EC).[1,2] It is clear that human $CD4^+$ T cells recognize swine leukocyte antigen (SLA) class II, and the second signal is provided by CD86, which is constitutively expressed in porcine EC.[1,2] This is in contra-distinction to human EC which activate allogeneic $CD4^+$ T cells, but do not express CD80 or CD86 molecules. This is an important species difference which may have implications for xenotransplantation. It explains why the proliferative response and production of IL-2 is greater when T cells respond to porcine EC compared to allogeneic EC[1,2] and why this response is less sensitive to inhibition by cyclosporine than the allogeneic T cell/EC response.[3] In allotransplantation, IL-2 is produced as a result of direct stimulation of recipient $CD4^+$ T cells by B7-expressing graft-derived dendritic cells. In xenotransplantation, the abundance of CD86 EC will be so much greater than dendritic cells in the graft that one could predict a stronger IL-2 response. However, these conclusions are derived from in vitro data, and the limitations that this implies. A number of groups are now testing the effects of human leukocyte populations to infiltrate or reject porcine tissues transplanted into immune-deficient mice. These informative studies confirm that $CD4^+$ T cells are essential for rejection of porcine skin grafts and pancreatic islets.[4,5] Cytotoxic $CD8^+$ T cells have been cloned against porcine EC and shown to recognize both SLA class I and processed peptides.[6] How essential cytotoxic $CD8^+$ T cells are for xenograft rejection in vivo is not known but the experimental model described above[4,5] will be useful in answering these questions. Human T cells will also be primed against porcine antigens via the indirect pathway and it may be

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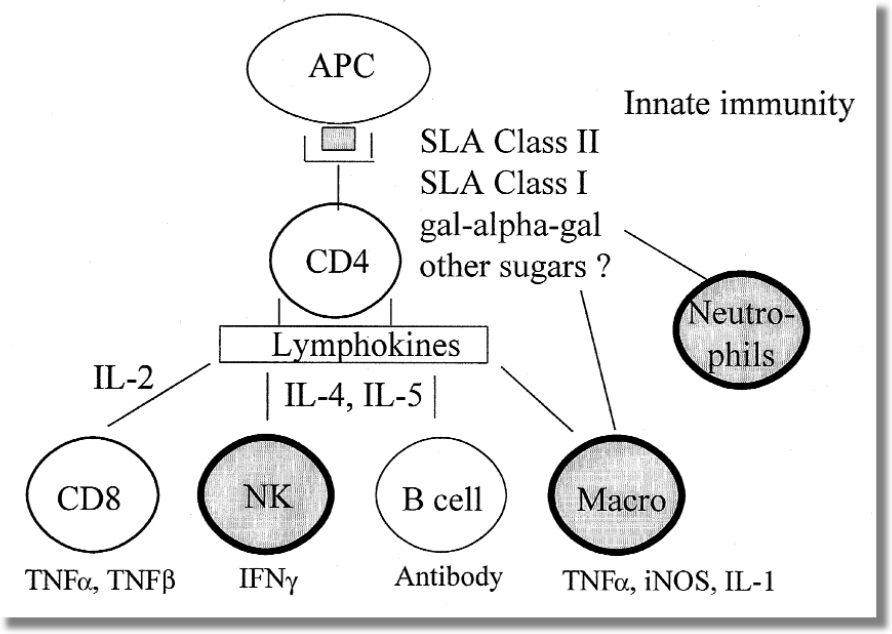

Figure 1. A diagrammatic comparison of the immune components of allogeneic and xenogeneic (pig-to-primate) cell mediated rejection. Allo-rejection is initiated by $CD4^+$ T cells, and effector mechanisms include $CD8^+$ T cells, cytokines, macrophages and B cells. In xenograft rejection, $CD4^+$ T cells are involved, but so are NK cells, neutrophils and macrophages. The unshaded circles denote mechanisms common to both, the shaded circles denote mechanisms more important in xenograft rejection. Innate immunity refers to the possibilty that macrophage/monocytes and neutrophils may recognise pig cells by pattern-recognition receptors.

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that porcine cells will present a larger array of non-HLA polymorphisms than human cells.

That NK cells can contribute to primate rejection of porcine xenografts is clear from both in vivo and in vitro studies. Allogeneic and normal autologous cells are not susceptible to NK-mediated cytotoxicity because they express certain MHC class I molecules that give a negative signal to NK cells through specific inhibitory receptors. In vitro studies have shown that isolated NK cells lyse porcine EC and lysis is increased by IL-2-activated NK cells.[7,8] The most potent way of NK cells lysing porcine EC is probably in the presence of human IgG xenoreactive antibody via FcRIII on NK cells. It has been shown that treatment of porcine EC with TNFα protects against NK cell-mediated lysis, an effect which is reversed in the presence of antibodies against SLA class I antigens.[8] It is clear, therefore, that strategies to enhance porcine endothelial expression of SLA or HLA class I molecules may reduce human NK activity against porcine xenografts.

Monocytes are a prominent feature of pig-to-primate xenograft rejection. They are also a prominent feature of allograft rejection where it is assumed they are secondary to either damage caused by specific $CD4^+$ T cell recognition of foreign HLA or are part of the response of ischemic injury. Whether monocytes have a more primary role in damaging xenografted tissue is not known. In vitro, human monocytes adhere to resting porcine EC in significantly greater numbers than to human EC,[9] an effect that is partially mediated by CD49d-VCAM interactions. Adherence of monocytes to porcine EC results in upregulation of endothelial E-selectin, IL-8 and monocyte chemotactic protein, all of which will act to enhance further adhesion and migration of mononuclear cells. The precise molecules responsible for interaction between porcine EC and monocytes are not known, but we know that monocytes express pattern recognition receptors which recognize repeating sugar residues (e.g., mannose residues) found on common bacteria. This raises the possibility that the enhanced role of monocytes in xenograft rejection may relate to different sugars expressed on pig EC. Similarly, neutrophils express pattern recognition receptors and show enhanced adhesion to porcine EC compared to human EC.[10] Enhanced adhesion is accompanied by activation as assessed by calcium influx. Neutrophils have been observed histologically in xenografts undergoing early hyperacute rejection, in the presence of human IgG. However, al-Mohanna et al[10] suggest that they may also play a role in cell mediated rejection, but in vivo data are really needed to confirm this hypothesis.

In conclusion, mainstay immunosuppression for allogeneic cell-mediated rejection depends on use of calcineurin inhibitors to inhibit TCR-mediated signals. It is clear that many components of the cell-mediated xenograft response will not respond to existing drugs, and it is likely that we shall have to develop even more ingenious methods of preventing and treating this complication..

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