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# Determining the Histologic and Immunopathologic Features of Acute Humoral Xenograft Rejection

*Akira Shimizu and Robert B. Colvin*

Hyperacute rejection in discordant xenotransplantation can be prevented by strategies such as depletion of anti-Gal $\alpha$ (1-3)Gal (Gal) anti-body, depletion or inhibition of complement, or the use of organs from pigs transgenic for human complement regulatory proteins [reviewed in ref. 1]. However, a discordant organ xenograft, such as heart, kidney, liver, or lung transplanted from pig to non-human primate, is still rejected within several days, and this form of rejection has been termed acute humoral xenograft rejection (AHXR), acute vascular rejection or delayed xenograft rejection.<sup>2-4</sup> Recently, the role of various effector mechanisms contributing to AHXR has become apparent. Here, we discuss the immunologic basis and histopathology of AHXR.

## Apoptotic Cell Death During AHXR

Both antibody and cellular immunity are believed to be major factors in the pathogenesis of AHXR. Antibody and complement-mediated cytotoxicity has been implicated as a cause of membrane attack complex (C5b-9)-mediated cell-lysis.<sup>5</sup> Recent studies demonstrate that the C5b-9 injury triggers intracellular apoptotic signals, which mediate apoptotic DNA fragmentation in target cells.<sup>6</sup> Cell-mediated cytotoxicity has been implicated as a cause of perforin-mediated cell lysis, perforin + granzyme-mediated apoptosis, and Fas-triggered apoptosis in target cells.<sup>7</sup> Apoptotic DNA fragmentation can be detected histopathologically by the terminal deoxynucleotidyl transferase-mediated dUTP-biotin DNA nick end-labeling (TUNEL) method.<sup>8</sup> This technique can also detect cells sometimes undergoing necrosis, as DNA degradation also occurs during necrosis. This assay, therefore, has the advantage of allowing the detection of damaged cells induced by

antibody and complement-mediated or cell-mediated immune injury.

## Endothelial Responses During AHXR

In AHXR, immune injury may lead to apoptosis or necrosis in endothelial cells.<sup>4</sup> The remaining endothelial cells are activated, induced possibly by cytokine stimulation (particularly IL-1, TNF $\alpha$ , and IFN $\gamma$ , complement or thrombin). The pathogenesis of AHXR might be linked to the activation of endothelial cells in the grafts.<sup>9,10</sup> The responses of activated endothelial cells include:

- shift to a procoagulant state, with downregulation of surface thrombomodulin and/or heparan sulfate, induction of plasminogen activator inhibitor-1, expression of tissue factor, and formation of small intracellular gaps, consistent with fibrin deposition; and
- induction of leukocyte adhesion molecules, including E-selectin and ICAM-1, and production of chemokines such as MCP-1, and cytokines which promote the influx and activation of inflammatory cells. Indeed, recent studies, using immunostaining techniques, demonstrate the expression of several molecules on activated endothelial cells.<sup>3</sup>

Morphologically, activated endothelial cells are characterized by cell hypertrophy, increased cellular organelles, and loss of fenestration. These morphological features are similar to those seen in high endothelium in postcapillary venules in lymphoid tissue that are the entry site for leukocytes into the lymph node.<sup>11</sup> Activated endothelial cells exhibit postcapillary venule-like transformation, and this phenotypic changes of endothelial cells may

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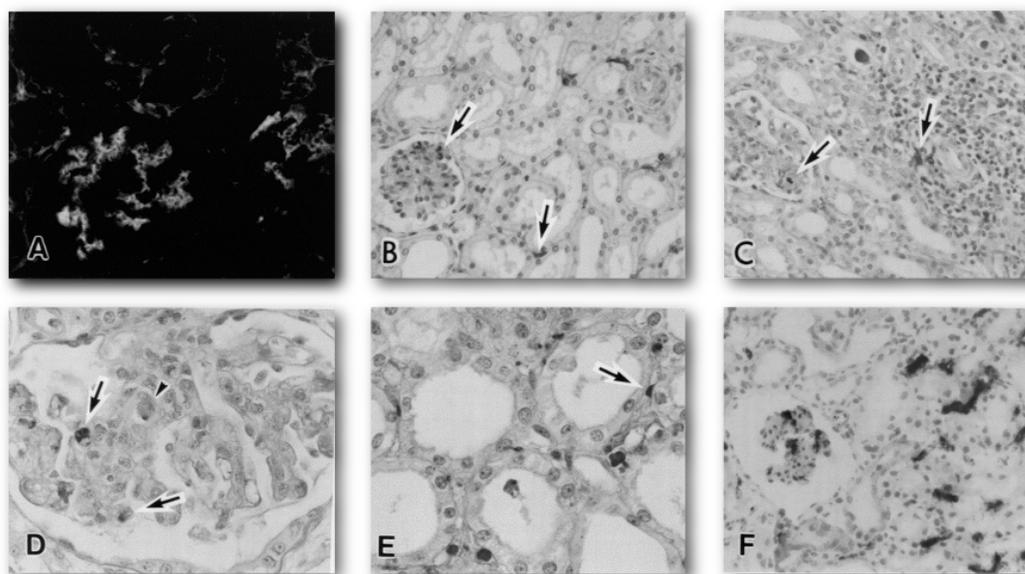


Figure 1. Acute humoral xenograft rejection (AHXR) in pig-to-nonhuman primate renal xenografts. A) IgM deposition is evident in glomeruli and peritubular capillaries on day 9. B) On day 5, the earliest detectable change in AHXR is glomerular and peritubular capillary endothelial cell death ( $\rightarrow$ ) as defined by TUNEL. C) On day 9, the number of TUNEL<sup>+</sup> dead cells ( $\rightarrow$ ) increases in glomeruli and peritubular capillaries. D) On day 9, numerous TUNEL<sup>+</sup> endothelial cells ( $\rightarrow$ ) are observed in glomeruli with morphological features of thrombotic microangiopathy. ( $\blacktriangledown$ ) indicate thrombus in glomerular capillary. E) On day 9, numerous TUNEL<sup>+</sup> endothelial cells ( $\rightarrow$ ) are observed in peritubular capillaries. (B-E; TUNEL method). F) Marked platelet accumulation is present in glomeruli and peritubular capillaries on day 11 (CD62P stain).

Xenoreactive antibodies not directed against the Gal epitope might also contribute, and cytotoxic T cells might add to its severity.

contribute to enhance influx of inflammatory cells into the grafts.

#### Histopathology of AHXR

Our recent study demonstrates immunological destruction of microvasculature in AHXR in pig-to-nonhuman primate renal xenografts.<sup>4</sup> Kidneys from Massachusetts General Hospital miniature swine were transplanted into cynomolgus monkeys or baboons that received regimens aimed to induce mixed chimerism and tolerance.

The early phase of AHXR (days 0-12) is characterized by focal deposition of IgM, IgG, and C3 (Fig. 1A), and scanty neutrophil and macrophage infiltrates. The first abnormality recognized is glomerular and peritubular capillary endothelial cell death as defined by TUNEL (Fig. 1B). Damaged endothelial cells undergo apoptosis and, later, frank necrosis. The progressive phase develops around day 6, and is characterized by progressive deposition of IgM, IgG, C3, and prominent infiltration of cytotoxic T cells and macrophages, with a small number of NK cells. Thrombotic microangiopathy develops in the glomeruli and peritubular capillaries, with TUNEL<sup>+</sup> endothelial cells (Fig. 1C-E), platelet aggregation (Fig. 1F), fibrin-platelet thrombi formation (Fig. 1D, F) and destruction of the capillary

network. The number of TUNEL<sup>+</sup> cells in the microvasculature correlates with the progressive deposition of antibody.<sup>4</sup> The degree of platelet aggregation correlates with the number of TUNEL<sup>+</sup> damaged endothelial cells. Only rare damaged arterial endothelial cells and tubular epithelial cells are observed, with rare endothelialitis and tubulitis. In the advanced phase of AHXR, interstitial hemorrhage and infarction occur.

In this model, after extracorporeal immunoadsorption, anti-Gal IgM and IgG recover to pre-transplant levels or higher by day 7.<sup>12</sup> The progressive deposition of antibody is associated with return of circulating anti-Gal antibody and possibly antibodies to new non-Gal porcine determinants. The progressive deposition of antibody correlates with the development of endothelial cell injury. Cellular elements also appear to play a significant role in the development of AHXR.<sup>4</sup> The cellular response is characterized by a massive infiltration of CD3<sup>+</sup> T cells, together with a similar intense macrophage infiltrate, and relatively small numbers of NK cells. Many CD3<sup>+</sup> cells have cytotoxic granule-associated protein (GMP-17). Many cytotoxic T cells infiltrate the graft. Similar frequencies of CD4<sup>+</sup> and CD8<sup>+</sup> T cells are seen in the graft, suggesting that class II-restricted T cell-mediated reactions play a role in AHXR.

### Summary

Natural anti-Gal antibody would appear to play an important role in the pathogenesis of AHXR.<sup>1-4,9,10</sup> Xenoreactive antibodies not directed against the Gal epitope might also contribute,<sup>13</sup> and cytotoxic T cells might add to its severity.<sup>4</sup> AHXR is characterized by the development of a thrombotic microangiopathy with endothelial cell apoptosis and necrosis, followed by destruction of the microvasculature, platelet aggregation, and formation of fibrin-platelet thrombi. The first abnormality recognized in AHXR is antibody deposition and endothelial labeling with TUNEL in the microvasculature. A recent report demonstrates that deposition of C4d in the peritubular capillary microvasculature is a useful adjunct in the diagnosis of humoral-mediated allograft rejection, and is more specific and sensitive than immunofluorescence for IgG, IgM, or C3.<sup>14</sup> C4d deposition may therefore prove to be of value in the diagnosis of AHXR. The TUNEL assay may also have a practical value in the diagnosis of xenograft rejection.

### REFERENCES

1. Lambrigts D, Sachs DH, Cooper DKC. Discordant organ xenotransplantation in primates, World experience and current status. **Transplantation** 1998; **66**:547-561.
2. Platt JL, Lin SS, McGregor CGA. Acute vascular rejection. **Xenotransplantation** 1998; **5**:169-175.
3. Bach FH, Winkler H, Ferran C, et al. Delayed xenograft rejection. **Immunol Today** 1996; **17**:379-385.
4. Shimizu A, Meehan SM, Kozlowski T, et al. Destruction of the microvascular capillary endothelium in pig-to-nonhuman primate renal grafts. **Lab Invest** 2000; **80**:815-830.
5. Hebert LA, Cosio FG, Birmingham DJ. The role of the complement system in renal injury. **Semin Nephrol** 1992; **12**:408-427.
6. Shimizu A, Masuda Y, Kitamura H, et al. Complement-mediated killing of mesangial cells in experimental glomerulonephritis: Cell death by a combination of apoptosis and necrosis. **Nephron** 2000; **86**:152-160.
7. Doherty PC. Cell-mediated cytotoxicity. **Cell** 1993; **75**:607-612.
8. Gavrieli Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. **J Cell Biol** 1992; **119**:493-501.
9. Lawson JH, Platt JL. Molecular barriers to xenotransplantation. **Transplantation** 1996; **62**:303-310.
10. Hancock WW. Delayed xenograft rejection. **World J Surg** 1997; **21**:917-923.
11. Ronkonen R, Turunen JP, Rapola J, et al. Characterization of high endothelial-like properties of peritubular capillary endothelium during acute renal allograft rejection. **Am J Pathol** 1990; **137**:643-651.
12. Kozlowski T, Shimizu A, Lambrigts D, et al. Porcine kidney and heart transplantation in baboons undergoing a tolerance induction regimen and antibody adsorption. **Transplantation** 1999; **67**:18-30.
13. McCurry KR, Parker W, Cotterell AH, et al. Humoral responses in pig-to-baboon cardiac transplantation: Implications for the pathogenesis and treatment of acute vascular rejection and for accommodation. **Hum Immunol** 1997; **58**:91-105.
14. Collins AB, Schneeberger EE, Pascual MA, et al. Complement activation in acute humoral renal allograft rejection: Diagnostic significance of C4d deposits in peritubular capillaries. **J Am Soc Nephrol** 1999; **10**:2208-2214.