Suppressing Natural Antibody Production

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Perper and Najarian were among the first to recognize that both antibodies (Ab) and complement were involved in the hyperacute rejection of organs transplanted between widely disparate species, such as pig-to-dog.[1] Good et al characterized these antibodies in 1991, when they demonstrated that Ab in primates directed against Galα1-3Gal oligosaccharides (Gal), present on porcine vascular epithelium, were cytotoxic to pig cells.[2] It was determined that anti-Gal Ab accounts for more than 85% of circulating natural anti-pig Ab in humans. There is now clear evidence that anti-Gal Ab are responsible for complement-mediated hyperacute rejection of porcine vascularized organs transplanted into human or non-human primate recipients. In addition, even if complement activity is inhibited, for example, by the use of porcine organs transgenic for a complement regulatory protein or by the administration of complement regulatory agents, anti-Gal Ab still play a major role in acute humoral xenograft rejection (otherwise known as delayed xenograft rejection or acute vascular rejection) that may be less dependent on complement activation.[3]

Since it is now widely accepted that pigs (that express Gal epitopes in abundance) will most likely be the animals to provide organs for xenotransplantation in humans (who have high levels of anti-Gal Ab), resolving the problem caused by the presence of Gal-reactive Ab in humans is of prime importance to the future of xenotransplantation. In this brief review, we discuss current methods of depleting or inhibiting anti-Gal Ab and, furthermore, of suppressing production.

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Methods of Depletion of Anti-Gal Ab

Methods for depleting anti-Gal Ab can be considered as those that deplete anti-Gal Ab ex vivo, and those that deplete in vivo.

Ex vivo depletion of anti-Gal Ab. This can be achieved by:

- perfusing the recipient's blood or plasma through a donor (porcine) organ,
- performing plasma exchange (thus removing all antibodies), or perfusing the recipient's blood or plasma through an immunoadsorbent that is either
- nonspecific or
- specific for anti-Gal Ab.

Perfusing recipient blood through a donor organ is a technique that was initially studied in the 1960s and 1970s, and led to adsorption of the recipient's anti-pig antibodies on the perfused organ's vascular endothelium. This resulted in modestly prolonged survival (of up to a few days) of a subsequently transplanted organ from the same donor. Various organs have been used for antibody adsorption, but most data suggest that the pig liver and lung are better immunoadsorbants than the kidney or spleen.[4-6]

Plasma exchange—the (complete) removal of the subject's plasma (with replacement of volume by other fluids)—has also been studied in xenotransplantation. Most circulating Ab, including anti-Gal Ab, are removed with this technique but, due to the resulting (temporary) state of a-gammaglobulinemia and hypoproteinemia, the patient is at some risk for infection and coagulation disturbances. Although Alexandre et al were successful in using repeated plasma exchange to remove anti-ABO

blood group Ab from potential kidney allotransplant recipients,[7] and demonstrated that accommodation (discussed elsewhere in this issue) could result, in pig-to-baboon kidney transplantation, although graft prolongation (for up to 22 days) was achieved, accommodation did not develop.[8]

Nonspecific antibody adsorption differs from plasma exchange in that the plasma is passed through an immunoaffinity column containing proteins that remove immunoglobulins from the plasma. The remaining plasma is then returned to the patient, reducing the need for replacement fluids. The proteins (such as staphylococcal protein A or protein G) are efficient in removing Ab by binding to its Fc portion. Since the proteins are nonspecific, all Ab are removed, resulting again in hypogammaglobulinemia and predisposition for infectious complications. Palmer et al[9] reported some success in the use of protein A columns to deplete anti-HLA antibodies in potential renal allograft recipients, and this technique has been used by several groups in xenotransplantation studies.

Specific sorbents for anti-Gal Ab utilize columns of natural or synthetic Gal oligosaccharides. Significant hypogammaglobulinemia does not occur. Initial studies utilizing immunoaffinity columns of melibiose demonstrated significant reduction of cytotoxicity of baboon serum to pig kidney cells[10] and some prolongation of a pig heart transplanted into a baboon. With the advent of more specific synthetic Gal oligosaccharides, extracorporeal immunoadsorption of anti-Gal Ab can be successfully achieved, and anti-Gal Ab can be depleted for several days.[11-13] The return of anti-Gal Ab, however, has to date invariably led to rejection of transplanted porcine organs in baboons. More sustained depletion of anti-Gal Ab has proved much more difficult, and is perhaps the major barrier to successful xenotransplantation facing us at the present time.

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In vivo depletion of anti-αGal Ab. The other major approach to 'neutralize' anti-Gal Ab is to administer a large quantity of soluble antigen intravenously (IV). Many oligosaccharides terminating in Gal can inhibit the action of anti-Gal Ab.[14] Infusion of melibiose and/or arabinogalactan in very high concentrations was effective, at least partially, in eliminating cytotoxicity of baboon serum to porcine kidney cells.[10] Infusion in such high concentration, however, resulted in toxicity. The continuous infusion of highly-specific Gal oligosaccharides, however, did not prove toxic but, although this resulted in a greatly diminished serum cytotoxicity to pig cells, protection against rejection of a pig organ in a baboon was incomplete.[15,16]

Recent experience in our own laboratory with the infusion of bovine serum albumin conjugated to Galα1-3Gal (BSAGal) in naïve baboons, baboons receiving large numbers of porcine hematopoietic progenitor cells, or baboons receiving porcine kidney xenografts (in conjunction with extracorporeal immunoadsorption and costimulatory blockade with an anti-CD154 monoclonal antibody (mAb)), demonstrated complete or near complete depletion of anti-Gal Ab sustained for periods of up to 30 days (Teranishi, K, Gollackner B, unpublished data) (Fig. 1). The return of anti-Gal Ab following cessation of the BSAGal was more delayed than observed in controls that did not receive BSAGal. In addition to neutralizing anti-Gal Ab, this agent may, therefore, possibly tolerize anti-Gal Ab-producing B cells by binding to their antigen receptors. Further studies are underway.

An alternative approach to the infusion of synthetic oligosaccharides is the use of anti-idiotypic antibodies (AIA) directed against idiotypes expressed on anti-Gal Ab. AIAs recognize specific idiotypes that are antigenic determinants within the variable regions of immunoglobulins. AIAs are also directed against the B lymphocyte subpopulations that bear the same idiotypes as surface receptors. Koren et al produced AIAs directed against anti-Gal Ab, and demonstrated inhibition of serum cytotoxicity to pig cells both in vitro and in vivo.[17] Recently, we produced a polyclonal AIA by immunizing a pig with human anti-Gal Ab.[18] The purified final preparation contained 2% AIA. After repeated administration to a baboon (following repeated immunoadsorption of anti-Gal Ab), a delayed return of anti-Gal Ab and reduced cytotoxicity to pig cells was observed. Furthermore, at this time point, the baboon serum was able to partially inhibit the cytotoxicity of other highly cytotoxic sera. On the basis of earlier studies in rodents, we hypothesize that this inhibitory effect was due to the production of high-affinity noncytotoxic anti-Gal antibodies generated as a result of sensitization by AIA.

The IV infusion of concentrated human immunoglobulin (IVIg) has been used successfully in the treatment of certain autoimmune disorders, and more recently as therapy to reduce anti-HLA

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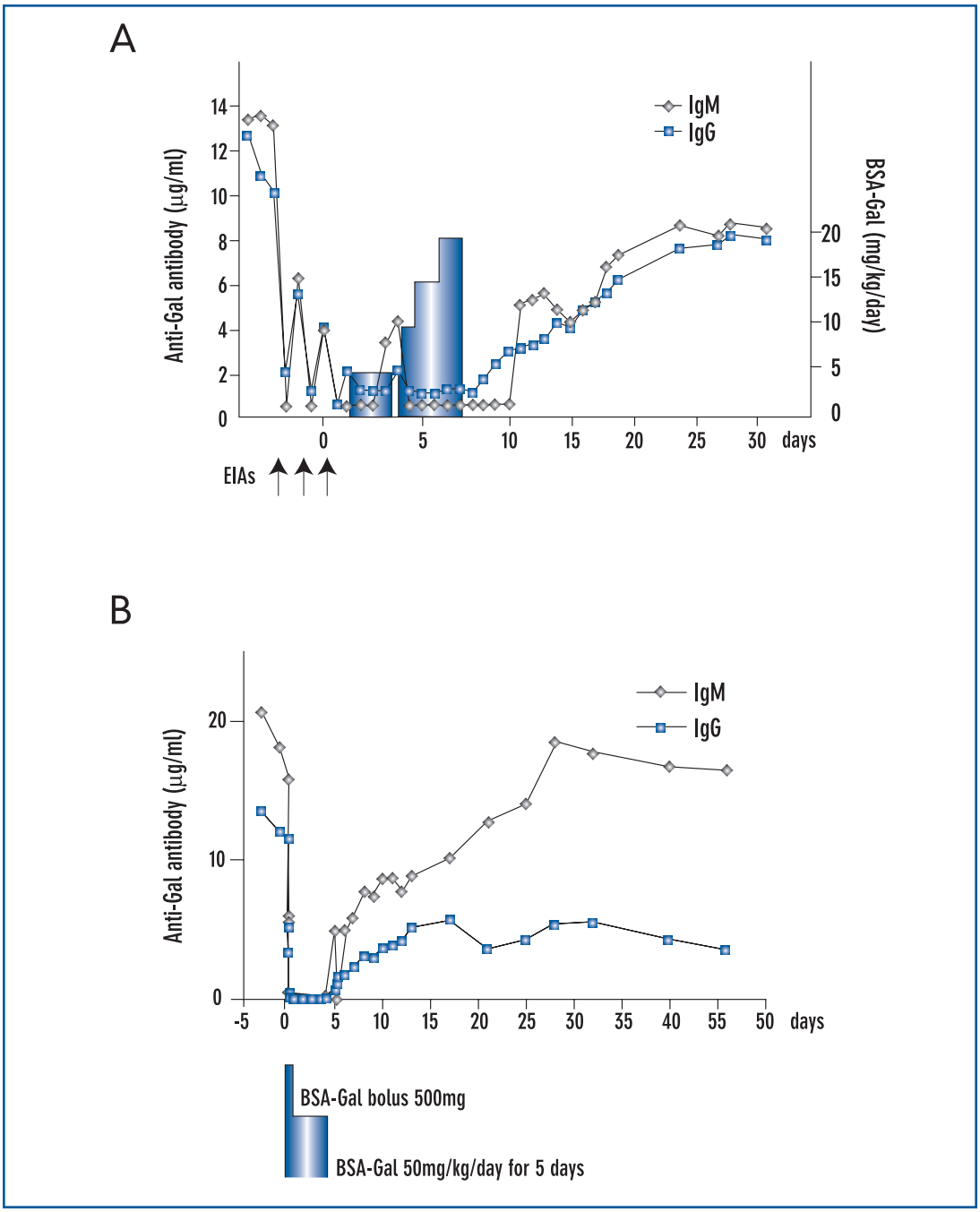

Figure 1. Panel A: Anti-Gal antibody levels in an otherwise unmodified baboon that underwent extracorporeal immunoadsorption (days -3, -2 and -1), followed by the continuous intravenous infusion of BSA-Gal at rates varying from 100-250 mg/kg/day for 8 days. Anti-Gal IgM and IgG are rarely measurable in the serum. In subsequent experiments, higher infusion rates have completely inhibited anti-Gal antibody. Panel B: Following the intravenous administration of bolus of BSA-Gal (500mg) to an otherwise unmodified baboon, no anti-Gal IgM or IgG can be measured in the serum. This state of absence of measurable anti-Gal antibody is maintained during the continuous intravenous infusion of BSA-Gal at 50mg/kg/day for 5 days (days 0-4). Once the infusion is discontinued, anti-Gal begins to be detected again in the serum (day 5).

antibodies in highly sensitized patients awaiting organ transplantation. Recent studies suggest that IVIg accelerates the physiological catabolism of IgG by saturating specific intracellular receptors, allowing degradation of IgG in proportion to its plasma concentration. If IVIg, depleted of anti-Gal Ab, is repeatedly infused, the level of anti-Gal Ab should steadily fall. Our preliminary data using

anti-Gal Ab-depleted IVIg in experiments involving the IV infusion of porcine peripheral blood progenitor cells into baboons indicate that significantly greater hematopoietic cell chimerism can be obtained than when IVIg is not administered.[19] We believe this beneficial effect is brought about, at least in part, by inhibition of macrophage function, and we have not observed any associated decrease in anti-Gal Ab levels. However, others have suggested that other mechanisms of action may be involved, such as an anticomplement effect.

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Methods of Suppression of Production of Anti-Gal Ab

Antibodies are produced by B lymphocytes and plasma cells. If it were possible to selectively suppress the cells responsible for the production of anti-Gal Ab, a major step would have been taken in achieving long-term xenograft acceptance. As this is not yet possible, present studies at our center have been directed towards temporarily destroying or suppressing all B and/or plasma cells by means of pharmacologic agents, irradiation, anti-B cell or antiplasma cell mAbs, or immunotoxins.

We have tested numerous pharmacologic agents, including cyclophosphamide, zidovudine, methotrexate, cladribine, mycophenolate mofetil, and melphalan, but no agent was successful in maintaining clinically significant depletion of anti-Gal Ab following immunoadsorption.[20,21]

In our assessment of specific anti-B cell mAbs, the most promising results were obtained with the chimeric antiCD20 mAb rituximab. Treatment of baboons with rituximab, in combination with 150 cGy total body irradiation, resulted in complete depletion of B cells in blood, bone marrow, and lymph nodes for >3 months. The rate of return of anti-Gal Ab following immunoadsorption, however, was not greatly reduced.[22]

We have also assessed the effect of an anti-CD38 mAb, targeting plasma cells, conjugated to the deglycosylated ricin A chain toxin, on the rate of return of anti-Gal Ab following immunoadsorption in baboons. Although this immunotoxin was effective in inhibiting anti-Gal Ab production in vitro (as measured by ELISPOT), the in vivo results were disappointing.[22] Inadequate internalization of the ricin A chain toxin was thought to be responsible, and studies are underway to develop better antiplasma cell-specific immunotoxins.

Conclusions and Future Investigations

Anti-Gal Ab plays a major role in both hyperacute rejection and acute humoral xenograft rejection of porcine vascularized organs. Sustained depletion of anti-Gal Ab is likely to be necessary if xenotransplantation is to find clinical application. Depletion of anti-Gal Ab can be achieved by extracorporeal immunoadsorption, but this effect has only been temporary. Pharmacologic agents or specific anti-B cell and/or antiplasma cell mAbs or immunotoxins have to date proven ineffective in achieving or maintaining such depletion. More recently, the continuous IV infusion of BSAGal has achieved excellent depletion of anti-Gal Ab, and studies are in progress to refine the use of this agent. A combination of extracorporeal immunoadsorption with BSAGal and/or an effective antiplasma cell agent may therefore be effective in achieving the goal of sustained depletion of anti-Gal Ab and contribute to long-term survival of porcine xenografts.

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