Preventing the Induced Anti-Pig Antibody Response

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In pig-to-primate organ xenotransplantation, hyperacute rejection can be prevented by various methods, including depletion of the recipient's anti-pig or anti-Galα1-3Gal (Gal) antibody (Ab), the administration of anticomplement agents, such as cobra venom factor or soluble complement receptor 1, or the use of donor pigs transgenic for a human complement regulatory protein, such as human decay-accelerating factor (hDAF) (all reviewed in ref. 1). However, the transplanted organ is still rejected within days or weeks by a process variously termed delayed xenograft, acute vascular, or acute humoral xenograft rejection. Induced high-affinity anti-Gal IgG[2] and possibly Ab directed against new porcine (non-Gal) antigenic determinants are considered to play a major role in acute vascular rejection. In animal models, transplantation of porcine cartilage, kidneys, hearts, or bone marrow cells has resulted in a marked increase (of 100- to 300-fold) in anti-pig or anti-Gal IgG and, to a lesser extent, IgM. Transgenic porcine hDAF organs transplanted into nonhuman primates survive longer than organs from nontransgenic pigs, but do not show truly long-term survival despite intensive immunosuppressive therapy. In diabetic patients transplanted with fetal pig islets, an induced response to Gal has been observed with an increase in Gal-reactive IgG of approximately 300-fold.

A large number of pharmacologic immunosuppressive agents have been tested, either singly or in combination, and have failed to prevent an induced antibody response to a transplanted pig organ or to transplanted pig cells (e.g., hematopoietic cells, islets). Furthermore, these drugs have usually failed to prevent the induced response to either Gal epitopes or to non-Gal epitopes, against which there were previously no natural antibodies.

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An Anti-CD154 mAb Prevents an Induced Antibody Response in Pig-to-Baboon Hematopoietic Cell Transplantation

The costimulatory pathway of CD40 and the T cell ligand CD154 (or CD40L) is crucial for effective activation of T cells to antigen and plays an important role in establishing T cell-dependent B cell activity. Blockade of this pathway alone or in combination with blockade of the B7/CD28 pathway effectively prolongs survival of skin and organ allografts in rodents and of kidney allografts in monkeys. At our center, the addition of costimulatory blockade has been shown to facilitate the establishment of mixed chimerism and tolerance to skin allografts in mice when combined with a nonmyeloablative regimen alone. In xenotransplantation, costimulatory blockade has allowed prolonged survival of rat-to-mouse skin and heart grafts, as well as pig-to-mouse skin grafts.

We have recently infused high doses of porcine peripheral blood mobilized progenitor cells (PBPC) (2-4 x 10^{10} cells/kg) into baboons undergoing a nonmyeloablative conditioning regimen. This regimen provided profound immunosuppression, as it comprised splenectomy, whole body irradiation (300 cGy), thymic irradiation (700 cGy), cyclosporine, mycophenolate mofetil, cobra venom factor, and the extracorporeal immunoadsorption of anti-Gal antibodies. Nevertheless, the infusion of porcine hematopoietic cells resulted in the emergence of both high levels of anti-Gal (with increase in IgM and IgG of 3- to 6-fold and 100-fold, respectively) and of anti-pig Ab directed against new determinants (non-Gal) within 20 days of PBPC transplantation (Fig. 1A).[3,4]

Subsequently, we administered murine anti-human CD154 monoclonal antibody (mAb) to

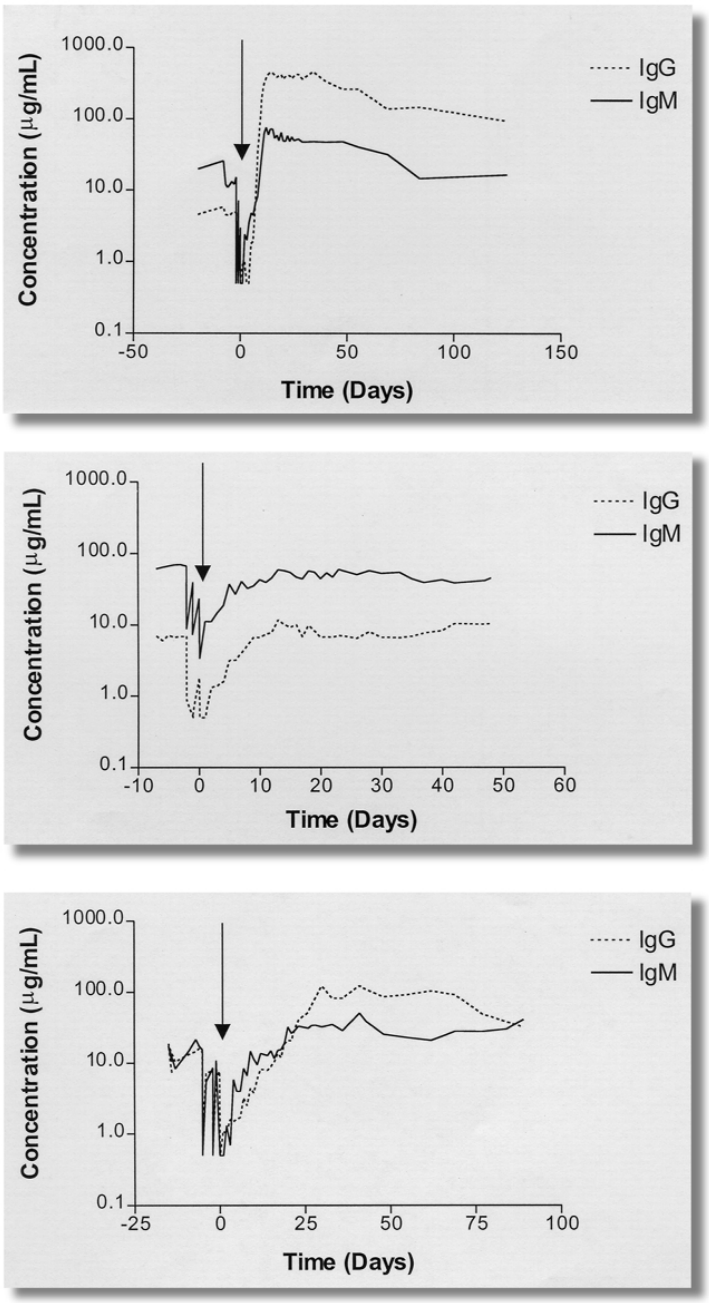

Figure 1. AntiGal IgM and IgG levels in representative baboons transplanted with porcine hematopoietic cells and conditioned with a nonmyeloablative regimen. The arrow on day 0 indicates the first day of porcine hematopoietic cell transplantation, which was administered after the final extracorporeal immunoadsorption of the preparative regimen. (A) In a baboon not receiving anti-CD154 monoclonal antibody (mAb), a 4-fold increase of anti-Gal IgM and 100-fold rise in anti-Gal IgG occurred by day 20, indicating sensitization to porcine antigens. This baboon also developed new antibody to non-Gal pig antigens (not shown). (B) In a baboon treated with anti-CD154 mAb, there was no increase in anti-Gal IgM or IgG over pretreatment levels, indicating that no sensitization occurred. No new antibody to non-Gal pig antigens developed (not shown). (C) In a baboon treated with anti-CD154 mAb and macrophage depletion (medronate liposomes as pretreatment on days –2 and –1), anti-Gal antibody levels showed a 3-fold increase of anti-Gal IgM and 10-fold increase of anti-Gal IgG over baseline within 20 days after pig leukocyte infusion. No new antibody to non-Gal pig antigens developed in this baboon (not shown), but new antibody was seen in another baboon receiving this treatment.

When attempts were made to prolong pig hematopoietic cell chimerism by reducing the activity of the macrophage phagocytic system by deleting macrophages with medronate liposomes, the beneficial effect of anti-CD154 mAb on preventing the induced antibody response was partially abrogated.

baboons pretreated with the same nonmyeloablative regimen and transplanted with PBPC. This regimen prevented sensitization to all pig antigens, including Gal (Fig. 1B).[3,4] Although there was a return of Gal-reactive IgM and IgG to pretransplant levels, there was no increase of either immunoglobulin class above those levels. Furthermore, the development of Ab to other pig antigens (anti-non-Gal Ab) was prevented.

These results support the hypothesis that the induced anti-pig antibody response is T cell-dependent, as anti-CD154 mAb therapy, which is directed against T cells but not B cells, prevented a humoral response. The pre-existing natural anti-pig antibody production would seem to be T cell-independent, as baseline synthesis of anti-Gal Ab was not inhibited.

When attempts were made to prolong pig hematopoietic cell chimerism by reducing the activity of the macrophage phagocytic system by deleting macrophages with medronate liposomes, the beneficial effect of anti-CD154 mAb on preventing the induced antibody response was partially abrogated. Although the induced anti-

body response was not as significant as in baboons not receiving anti-CD154 mAb, the removal of macrophages was followed by an attenuated antibody response (Fig. 1C).[5] This suggested that macrophages were essential if costimulatory blockade was to be efficient in this model. It also suggested that it was the indirect pathway of antigen recognition that was involved, and this was confirmed by subsequent in vitro studies involving the baboon mixed lymphocyte reaction.[5]

An Anti-CD154 mAb Prevents an Induced Antibody Response in Pig-to-Baboon Organ Transplantation

We have also tested costimulatory blockade in pig-to-baboon organ transplantation.[6] The aim of the study was to monitor the survival of porcine kidneys transplanted into baboons treated with a non-myeloablative regimen and a murine anti-human CD154 mAb. Kidneys were obtained from miniature swine or hDAF pigs, and two induction protocols (based on either whole body irradiation or cyclophosphamide) were compared. Anti-CD154 mAb was administered in every case.

In all baboons, the humoral response showed reappearance of anti-Gal IgM below baseline (pretransplant) levels, with no or low return of anti-Gal IgG. Miniature swine kidneys required excision within 6-13 days for acute vascular rejection or the development of disseminated intravascular coagulation. hDAF kidneys functioned for 28-29 days, but then required excision for acute vascular rejection or disseminated intravascular coagulation. IgM deposition was detected in all excised organs, but complement (C3) depletion was absent, and IgG deposition was absent or minimal. No baboon showed a rebound of anti-Gal Ab immediately after graft excision, in contrast to the usual experience following pig-to-primate organ transplantation,[7] suggesting that anti-CD154 mAb prevented T cell-dependent sensitization to pig antigens.

One native kidney remained in these baboons at the time of transplantation, and therefore long-term survival after donor kidney excision was possible. In the long-term survivors, anti-Gal Ab levels remained below pretransplant level while anti-CD154 mAb was being administered. Later, however, there was a slow increase to above pretransplant levels (IgM 2- to 3-fold, IgG 13- to 20-fold). This may have been related to the continuing presence of cuffs of porcine aorta, inferior vena cava and ureter that remained after porcine kidney excision. Induced antibody directed against these porcine remnants was suppressed while anti-CD154 mAb was being continued but, once this agent was discontinued, an antibody response developed.

Comment

The employment of anti-CD154 mAb (or possibly other agents that block costimulation) has considerable potential in the field of discordant xenotransplantation. By preventing the production of high-affinity Gal-reactive IgG and Ab to new pig determinants, anti-CD154 mAb therapy may reduce the requirement for other, more toxic, forms of immunosuppressive therapy.

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