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Inducing Tolerance by Mixed Hematopoietic Cell Chimerism

Leo Buhler, David H. Sachs and David K.C. Cooper

Our laboratory has previously reported methods of inducing specific immunologic tolerance to allografts and concordant xenografts by mixed hematopoietic chimerism in rodents and nonhuman primates.[1-3] In attempts to extend this approach to a discordant pig-to-baboon combination, extracorporeal immunoadsorption of anti-Galα1-3Gal (Gal) antibody (Ab) has prevented hyperacute rejection but has to date been ineffective in preventing acute vascular (delayed xenograft, acute humoral xenograft) rejection following organ transplantation.[4,5] Furthermore, the conditioning regimen that has been successful in allograft models[2] has been insufficient to achieve consistent hematopoietic cell chimerism detectable by flow cytometry in the pig-to-nonhuman primate model.[4-6]

Potential Barriers

Several possible reasons for this lack of success can be identified. These include:

1. the return and continuing presence of anti-Gal antibody and the possible induction of antibody against new porcine epitopes,
2. the effects of the macrophage phagocytic system which removes pig hematopoietic cells rapidly from the baboon circulation, and
3. the detrimental effect of the baboon microenvironment on porcine hematopoietic cell survival.

Initially, following the conditioning regimen, when porcine bone marrow cells were transplanted into baboons in small numbers (2-20 x 10^8 cells/kg) with no organ graft, no anti-pig Ab other than anti-Gal Ab was subsequently produced by the recipient baboons.[7] However, more recently, we have infused high doses of porcine peripheral blood mobilized progenitor cells (PBPC) (2-4 x 10^{10} cells/kg) in baboons undergoing the same conditioning regimen, with the emergence of both high levels of anti-Gal and of anti-pig Ab directed against new (non-Gal) determinants within 20 days of PBPC transplantation.[6] We concluded, therefore, that the transplantation of large numbers of pig cells resulted in sensitization to Gal (with sensitization being defined as an increase in anti-Gal IgM and/or IgG over baseline titers) and sometimes also to new porcine (non-Gal) antigens.

Inclusion of Anti-CD154 mAb Therapy

Costimulatory blockade has been shown to facilitate the establishment of mixed chimerism and subsequent tolerance to skin allografts in mice when combined with a nonmyeloablative regimen. In xenotransplantation, anti-CD154 monoclonal antibody (mAb) has been shown to delay T cell-mediated cellular rejection of porcine skin grafts in mice and also to block T cell-dependent induced Ab production in the pig-to-baboon model.

To improve the engraftment of porcine hematopoietic cells in primates, we have infused high doses of PBPC in baboons undergoing a nonmyeloablative conditioning regimen in combination with murine anti-human CD154 mAb treatment.[6,8] Initial pig chimerism was detectable for 5 days (maximum 73%) by flow cytometry.[8] Two of 3 baboons showed reappearance of pig cells on days 11 and 16, respectively. In one, in which no anti-Gal IgG could be detected for 30 days, pig cells were documented in the blood by flow cytometry on days 16-22 (maximum 6% on day 19) (Fig. 1)

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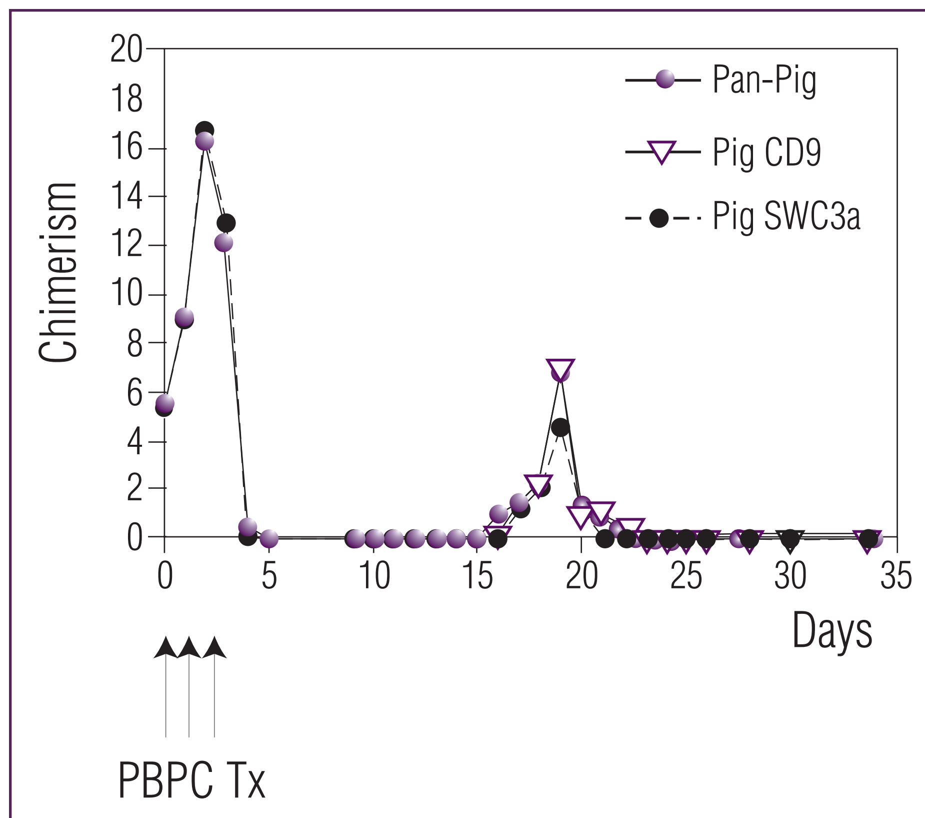

Figure 1. Pig cell engraftment detected by flow cytometry in a baboon conditioned with a nonmyeloablative regimen and CD154 blockade, with initial chimerism during infusion of up to 16% and subsequent reappearence of pig monocytes (staining for pan-pig, pig CD9 and pig SWC3a, a marker for granulocytes and monocytes) from days 16 to 22.

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and pig colony forming cells were documented on days 19-33. Microchimerism was continuous by polymerase chain reaction up to 33 days.

Factors Potentially Contributing to Chimerism

Several other factors may have contributed to the increased levels of macro- and microchimerism in this study. These include:

1. the source of the hematopoietic cells,
2. the dosage of hematopoietic growth factors, and
3. the immunosuppressive therapy.

We did not anticipate any difference in the levels of chimerism in relation to the genotype of the pig from which the hematopoietic cells were obtained. The initial level of chimerism was higher when cells from miniature swine of SLAcc haplotype[9] had been infused compared with cells of the SLAdd haplotype, and these, in turn, gave higher initial chimerism than cells from outbred large white Landrace cross-pigs transgenic for human decay accelerating factor (generously provided by Imutran, a Novartis Pharma company). None of these differences, however, reached statistical significance. The reason for any possible difference is unknown, but could be related to the structure of SLA molecules and the homology between SLA and primate MHC molecules. However, it is unclear whether a higher degree of MHC homology would allow greater chimerism or, in contrast, induce an accelerated clearance of xenogeneic cells from the circulation.

The importance of species-specific hematopoietic growth factors to achieve xenogeneic bone marrow cell engraftment has been shown in human-to-mouse and pig-to-mouse bone marrow transplantation experiments, where long-term maintenance of xenogeneic hematopoiesis required donor-specific growth factor therapy. The dosages of porcine

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hematopoietic growth factors[10] were therefore increased in an attempt to promote the porcine cells to engraft and differentiate in the baboon bone marrow microenvironment. Our results suggest that higher levels of porcine hematopoietic growth factors might be a factor in the transient engraftment of pig cells in baboons.

Combination Therapy with Anti-CD154 mAb and Cyclosporine

Anti-CD154 mAb therapy resulted in a humoral unresponsiveness to Gal and non-Gal antigens of pig cells.[6] In baboons receiving a combination of anti-CD154 and cyclosporine, no sensitization to Gal or non-Gal antigens was observed while cyclosporine was being administered, and anti-Gal IgG remained at extremely low levels. During this period, transient pig cell engraftment and differentiation of pig monocytes were observed. As IgG deposition on the cells could induce antibody-dependent cell-mediated cytotoxicity, the absence of these antibodies might be important for pig cell engraftment in baboons. Even though anti-Gal IgM antibody levels returned to baseline during this time period, the complement-mediated cytotoxicity associated with them was neutralized by cobra venom factor.

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Combination Therapy with Anti-CD154 mAb and Macrophage Depletion

The subsequent loss of pig cell chimerism could be related to the innate immune response, as macrophages and NK cells have been shown to be a major hurdle to xenogeneic stem cell engraftment in rodents. When macrophages are depleted by the infusion of medronate-liposomes, chimerism is increased and is slightly prolonged. However, in the present study, depletion of macrophages resulted in a detrimental effect in that the prevention of sensitization by anti-CD154 mAb was lost.[11]

Comment

Although several factors may have been involved, our data suggest that anti-CD154 mAb therapy significantly prolonged microchimerism in all baboons receiving miniature swine hematopoietic cells. These results suggest that there is no absolute barrier to pig hematopoietic cell engraftment in primates, and that this engraftment may be facilitated if the return of anti-Gal IgG can be prevented.

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