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Applications of gene therapy in transplantation

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Summary

The success of organ transplantation is limited by the availability of donor organs and the requirement for long-term immunosuppression. Use of animal organs would circumvent the organ shortage, and the swine is the most likely candidate to serve as donor for xenotransplantation. Establishing specific tolerance to donor organs would eliminate the need for chronic immunosuppression. Specific tolerance to transplantation antigens across major histocompatibility complex (MHC) barriers has been demonstrated by establishing mixed bone marrow chimerism in the recipient. An alternative approach to establishing chimerism is the use of gene therapy to transduce autologous bone marrow cells with a vector containing one or more MHC antigens. Previous studies in miniature swine have shown that tolerance can be established across allogeneic barriers when a single MHC antigen is shared between donor and recipient. A gene therapy approach is particularly attractive in xenotransplantation since the availability of MHC-inbred miniature swine as donors allows the use of a single vector for transferring swine MHC genes into recipient progenitor cells. In this article, we review the basics for using a gene therapy approach to achieve specific immune tolerance and describe the models being used to evaluate its efficacy in allogeneic as well as in xenogeneic organ transplantation.

I. Introduction

Gene transfer methodology for treating human disease was first used in 1990 under an FDA approved protocol for treating patients with adenosine deaminase deficiency (Reviewed in Anderson, 1992; Miller, 1992). Since then, over 100 protocols have been approved by the Recombinant Advisory Committee in the United States (Hodgson, 1995) for a variety of applications. Some of these applications have involved transplant of bone marrow cells which have been transduced with vectors carrying replacement genes for enzyme deficiencies or defects in hematopoietic cell function (Karlsson, 1991; Hoogerbrugge et al., 1996). Recent studies in large animal models suggest that gene therapy approaches can be used to establish tolerance to allogeneic antigens, enabling the transplant and long-term survival of solid organs between fully mismatched donor/recipient pairs (Emery et al., 1997). This application should be extremely useful in xenotransplantation where the donor species is well defined immunologically, such as with MHC inbred miniature swine, and the supply of organs is not limited (Sachs, 1994, Greenstein and Sachs, 1997).

II. Current status of clinical transplantation

In order to understand the potential application of gene therapy in solid organ transplantation, it may be helpful to review briefly some of the current issues in clinical transplantation. The development of new immuno-suppressive therapies in recent years has resulted in a dramatic increase in patient survival rates for the first five years following organ transplantation. Unfortunately, this increase in success has enhanced the problem of meeting the demand for available human donor organs. Each year, the demand for donor organs increases at a rate disproportionate to the number of human organs available (*United Network for Organ Sharing, Scientific Registry, March 1996).* Furthermore, the increase in early survival rates has not been sustained long-term (>5 years). Those

surviving long-term frequently experience a decrease in the quality of life as a result of life-long immunosuppression and episodes of chronic rejection. Prolonged immunosuppression often leads to increased infections, an increased incidence of cancer, and complications associated with drug toxicity. Such complications lead to repeat hospitalizations and increased healthcare costs. Generally speaking, it could be said that there are two major limitations on the current success of transplantation: the shortage of human donor organs available and the requirement for long-term immunosuppression.

A. Overcoming the problem of organ shortage

The first limitation, that of organ shortage, has not been diminished by increased education and presumed consent laws. It could be eliminated, however, by the use of animal organs. Several groups are currently pursuing the technology to enable xenotransplantation. The miniature swine appear to be the optimal donor for xenotransplantation for a variety of reasons (Sachs, 1994). Their size is comparable to that of a human. The physiology of swine organs and immune system is remarkably similar to that of humans (Cooper et al., 1991). Their reproductive patterns are such that organs are readily available. Miniature swine have a lower potential for zoonotic infections than do primates as witnessed by the fact that they have co-existed with humans for thousands of years. The large majority of pig diseases are known, have been characterized, and can be eliminated from source herds by barrier housing techniques (Michaels and Simmons, 1994; Fishman, 1994). Furthermore, the use of swine organs engenders less ethical concern than would the use of primate organs. Over ninety million pigs are used annually for food in the U.S. alone.

III. Avoiding long-term immunosuppression

The second limitation, long-term immunosuppression, could be eliminated by developing clinical protocols for the induction of specific immune tolerance to donor organs. It has been demonstrated that specific tolerance to transplantation antigens across major histocompatibility complex (MHC) barriers can be achieved by establishing allogeneic or xenogeneic mixed bone marrow chimerism in the recipient (Sharabi et al., 1990; Sharabi et al., 1992; Sykes et al., 1994; Kawai et al., 1995). Hematopoiesis gives rise to lymphoid precursors which migrate to the thymus. Within the thymic environment, T cells develop and undergo both positive and negative selection to maintain the so-called “protective” T cells while eliminating those which react with “self” antigens. This results in a tolerance to “self”. Mixed bone marrow chimerism can be established by engrafting pluripotent donor progenitor cells into an immunosuppressed recipient. In this case, both donor and recipient progenitors undergo selection in the thymus and become educated to recognize each other as "self". That implies that an organ derived from the same donor as the bone marrow and subsequently transplanted into the mixed marrow recipient will be treated as "self". Such tolerance induction has been observed in both rodents (Ilstadt, 1984, Sharabi and Sachs 1989) and large animals (Smith et al., 1992; Kawai et al., 1995) which have intact immune systems capable of reacting to foreign antigens. This is evidenced by the fact that organs from an unrelated or third party donor are rejected (reviewed in Sachs, 1995).

IV. Structural and functional similarities between porcine and human MHC

The general use of large animal models to explore the importance of MHC gene matching in graft survival has been difficult because of the lack of genetically defined animal models. The MHC inbred miniature swine are uniquely well suited for such studies because of the similarity of the pig MHC locus (SLA) to its human HLA counterpart and because of the availability of MHC recombinant strains (Pennington et al., 1981). Three partially inbred strains of miniature swine have been derived which are fully inbred at the SLA locus and which have the defined haplotypes *a*, *c*, and *d*. Furthermore, four additional SLA haplotypes (*f*, *g*, *h*, and *j*), with recombinations between the originally defined class I and class II loci, have been obtained and bred to homozygocity (**Figure 1**). Pulsed field analysis of the pig class II region allowed accurate mapping of the locus and showed similar class II gene distribution between pig and human (LeGuern, C., unpublished data). Porcine class I (Singer, 1982; Singer et al., 1987) and class II (Sachs et al., 1988; Pratt et al., 1990) genes have also been described.

The class II region encodes polymorphic cell surface molecules, composed of heterodimers consisting of an alpha and a beta chain, which are involved in the molecular control of immune responses. Three major class II subregions (DR, DQ and DP) have been identified in man to encode functional cell surface class II molecules whereas only two of them (DR and DQ) appear to be functional in miniature swine (Pratt et al., 1990). Analysis of class II cDNA clones corresponding to the DRA/B and DQA/B genes from two different SLA haplotypes (c and d) indicate that sequence variability is distributed similarly to that observed for the human class II sequences. As in human, the pig DRA sequence is

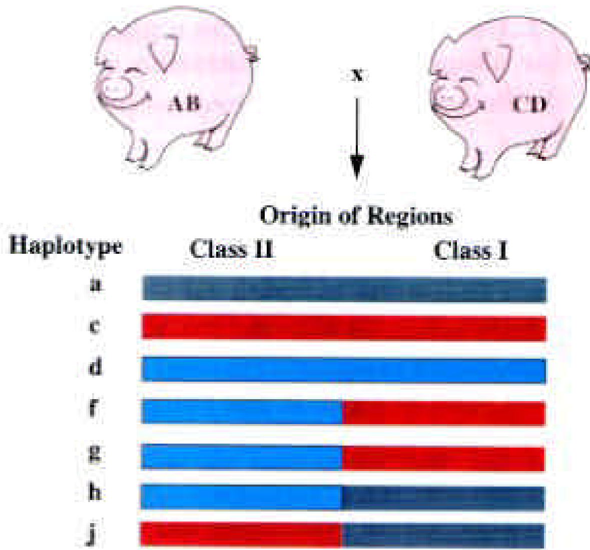

Figure 1. Origin of inbred miniswine.

conserved among various MHC haplotypes (Hirsch et al., 1992) whereas the DRB (Gustafsson et al., 1990a) and both the DQA (Hirsch et al., 1990) and DQB sequences (Gustafsson et al., 1990b) are polymorphic.

V. MHC class II genes and tolerance induction to transplanted organs

Transplantation studies on genetically defined miniature swine have demonstrated that sharing of MHC class II antigens between donor and recipient pairs is critical for insuring long-term renal allograft survival (Pescovitz et al., 1984a; Kirkman, 1979; Rosengard et al., 1992). In approximately 35% of miniature swine, long-term specific tolerance spontaneously develops to one-haplotype class I plus minor antigen disparate, but class II matched, renal allografts in the absence of exogenous immunosuppression (Pescovitz et al., 1983; Pescovitz et al., 1984a). If, however, the kidney recipients were treated with a short course of cyclosporine (CsA : 10 mg/kg i.v. for 12 days) starting the day of transplantation, they accepted long-term (> 100 days) two-haplotype class I mismatched renal allografts (i.e., gg dd) in 100% of the cases (**Figure 2**). Non-treated control animals rejected class II only matched grafts in 13.7 ± 0.9 days and cyclosporine-treated recipients showed a delayed but consistent rejection of fully mismatched grafts (reviewed in Gianello and Sachs, 1996) (**Figure 2**).

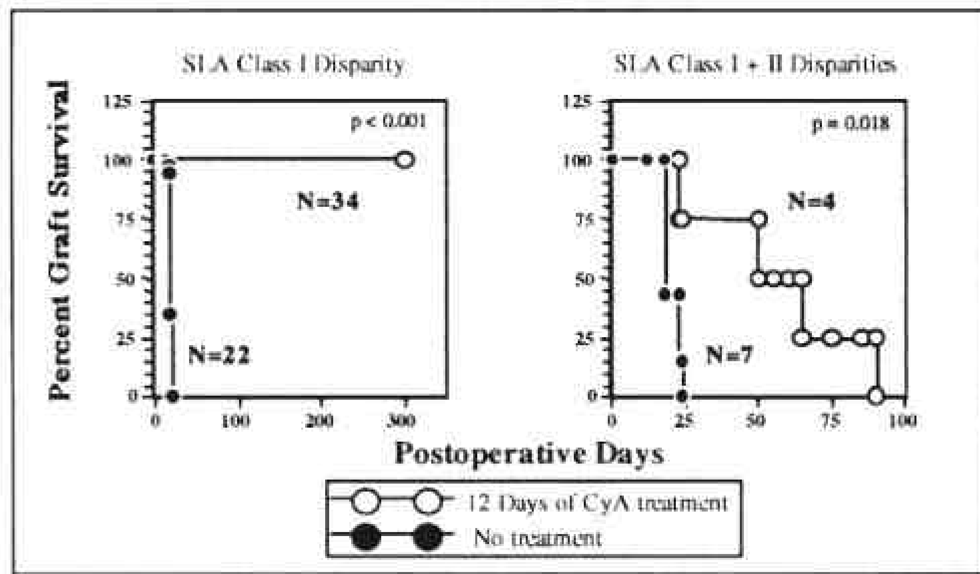

Figure 2. Effects of CsA on renal allograft survival across selective MHC barriers (Gianello and Sachs, 1996).

Importance of class II matching for the outcome of kidney allografts has also been reported in clinical transplantation (Opelz et al., 1991; Ichikawa et al., 1993). Rodent studies designed to look at the effects of class II matching on graft survival have yielded mixed results (Aizawa, 1984; Paris and Gunther, 1980). It should be noted, however, that the tissue distribution of class II gene expression on rodent vascular endothelium (Benson et al., 1985) differs from that found in humans (Daar et al., 1984) and in large animals (Pescovitz et al., 1984b) so that mechanisms of graft rejection may differ (Hart and Fabre, 1981a; Hart et al., 1981b). The singular importance of class II sharing between donor and recipient points to the likely feasibility of using a gene transfer approach clinically to provide sharing of class II. In other words, *if a recipient could be made to express the same class II as that of the donor organ, transplantation of that organ under the coverage of a short course of high dose CsA should result in prolonged graft survival.* The applicability of such an approach in allogeneic organ transplantation will depend on the number of class II genes required to provide for the genetic diversity of the human population. Rough estimates of five to six prototypic class II genes, corresponding to the major phylogenetic families of class II sequences for DRB and DQ, could be envisioned, although this has not yet been tested. The practicality of such an approach for xenotransplantation is obvious, especially when the organ donors are as genetically well defined as the MHC inbred miniature swine.

VI. Use of gene therapy in a miniature swine allogeneic transplantation model

A. Protocol for tolerance induction

An allogeneic miniature swine transplantation model has been developed to evaluate the efficacy of a gene therapy approach to provide sharing of class II on an MHC class I disparate background. A diagram of the experimental protocol is shown in **Figure 3**. Donor (SLA^d) and recipient (SLA^c) animals are chosen to differ at both class I and class II loci. BM is collected from the SLA^c recipient and transduced *in vitro* with a recombinant retrovirus vector for a class II^d gene matching that of the future kidney donor. The transduced marrow is then infused back into the recipient pig which has been conditioned with lethal irradiation. Following full reconstitution with the class II-engineered bone marrow, the SLA^c recipient receives a fully mismatched SLA^d kidney transplant, which is matched only to the class II^d transgene previously introduced through the retrovirus, under a 12 day course of CsA (**Figure 3**). Serum creatinine levels are then monitored as a read-out for kidney function (Emery et al., 1997).

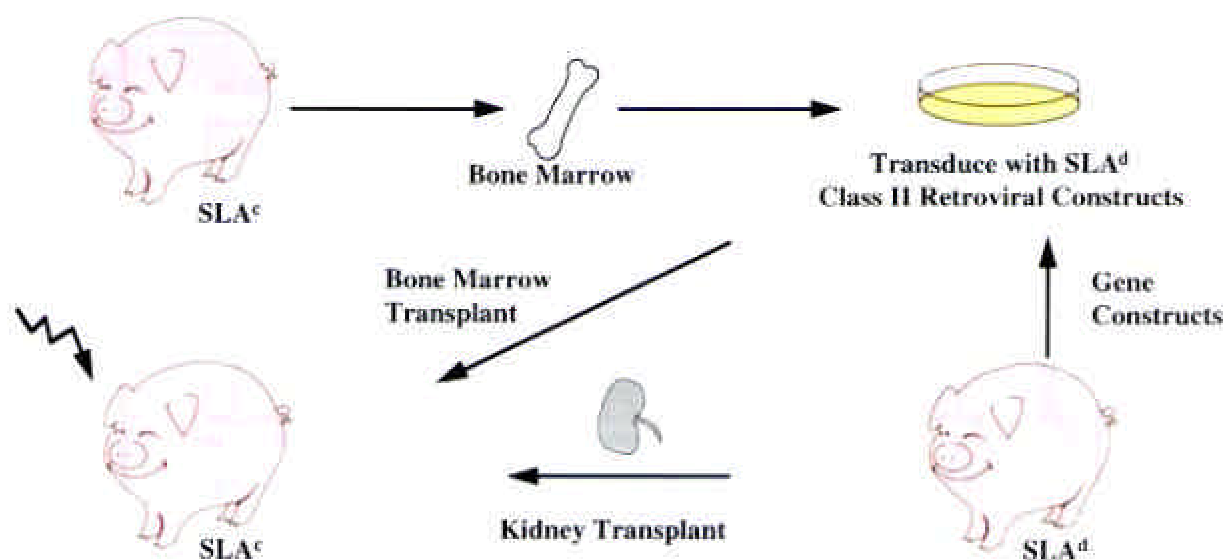

Figure 3. Tolerance induction by class II gene transduction of bone marrow.

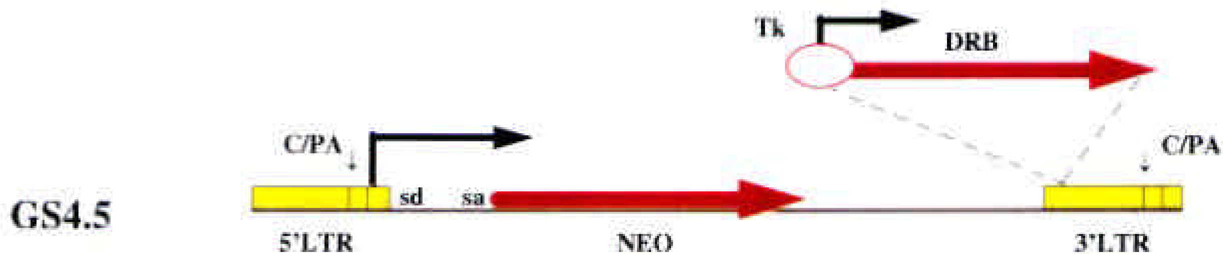

Figure 4. Retrovirus vector for SLA class II DR allogeneic gene transfer.

B. Construction of recombinant vectors for pig Class II

The utility of retroviral vectors in mediating gene transfer for the introduction of new genetic material into the hematopoietic compartment has now been demonstrated by a number of groups in rodents (Gilboa et al, 1986; Bodine et al, 1989; Sykes et al, 1993; Fraser et al, 1995; Mayfield et al., 1997) as well as in large animals (Emery et al., 1993; Banerjee et al., 1997a) and humans (Lu et al., 1993; von Kalle et al., 1994; Conneally et al., 1996). Since retroviral vectors have a high efficiency of chromosomal integration once inside of a cell, they insure transmission of the gene to future progeny of the targeted cell. It appears, however, that stable genome integration of recombinant proviruses requires cell division (Harel et al., 1981, Miller and Miller, 1990), a prerequisite not fulfilled by mostly quiescent hematopoietic early progenitors and stem cells. Although the requirements for long-term expression of allogeneic class II in this model have not been determined, stable expression of the trangene(s) would seem to be preferable, based on the results of allotransplant studies between recombinant haplotype pigs as described above (Rosengard et al., 1992).

An initial construct, named GS4.5, was engineered to express both the neomycin phosphotransferase drug-resistance gene (Neo) as well as the pig DRB^d allelic cDNA (**Figure 4**) (Shafer et al., 1991). Since the DRA nucleotide sequence is non-polymorphic, it was assumed that the recipient endogenous DRA^c chain would associate with the donor DRB^d product for expression of allogeneic class II heterodimers. The SLA-DRB^d cDNA transgene is under the control of the thymidine kinase (Tk) promoter. The expression cassette was inserted into the 3' long terminal repeat (LTR) of the N2A double-copy vector (Hantzopoulos et al, 1989) in the same transcriptional orientation as that of the retrovirus. Recombinant retrovirus particles were first produced in the ecotropic cell line GP+E86 (Markowitz et al., 1988) and then transferred to the amphotropic packaging cell line PA317 (Miller and Buttimore, 1986). Extensive selection of clones resulted in a stable producer cell line which yielded virus-containing supernatants at a titer of 3 x 10^6 G418-resistant colony forming units (CFU) per ml. A highly sensitive marker-rescue assay (Miller and Rosman, 1989) was used to confirm the absence of replication-competent helper virus (Emery et al., 1993) in all preparations of amphotropic DRB-virus.

C. Transduction of swine cells

To demonstrate that the GS4.5 vector could transduce and express recombinant SLA class II in or on cells of swine origin, primary swine fibroblast cultures were incubated with high-titered virus supernatant without selection for three to four days and assayed by Northern blotting using probes for either Neo or DRB. The DRB-specific transcript pattern observed was similar to that resulting from RNA derived from the producer cell line indicative of correct expression of the integrated DRB-provirus (not shown). Whole swine BM cells were then harvested and cultured in the presence of viral supernatants at an estimated multiplicity of infection of 3 to 5. To assess for bone marrow (BM) transduction, a colony assay for swine colony-forming-unit-granulocyte-macrophage (CFU-GM) was developed. Titration studies were first performed to test the plating efficiency of swine CFU-GM with increasing concentrations of the neomycin drug analog G418. In these studies (Emery et al., 1993), colony formation was inhibited at G418 concentrations of 1 mg/ml. Transduced BM cells were then plated in methylcellulose cultures in the presence or absence of 1.2 mg/ml active G418 to assess the efficiency of transduction of CFU-GM. In addition, they were also plated directly into long-term bone marrow cultures (up to 5 weeks) followed by CFU-GM assay to determine if early progenitors were also transduced. Transduction efficiencies estimated from the initial CFU-GM cultures ranged from 4% to 14% in 9 experiments. Only slightly lower numbers were obtained in the CFU-GM assays of the long-term cultures. These ranges of efficiency were later confirmed by PCR assay using DR-specific probes (Emery et al., 1993). These data indicate that early

progenitors were transduced and predict that engraftment of these cells *in vivo* should result in long-term presence of the gene.

D. Addition of growth factors to optimize transduction of bone marrow cells

At the time these initial experiments were performed, pig-specific cytokines were not available. Cytokines from man and mouse were therefore screened for cross-reactivity with pig BM cells. Mouse c-kit ligand (KL) and human GM-CSF were stimulatory for pig cells but, as expected, mouse or human IL-3 was not. Surprisingly, PIXY321, a fusion molecule consisting of human IL-3 and GM-CSF (gift from Dr. D.E. Williams, Immunex Corp.), exhibited more activity than did similar molecular concentrations of GM-CSF alone, suggesting that the fused IL-3 portion of the molecule was stimulating pig BM cells. When KL and PIXY321 were added to cultures during transduction, the transduction efficiency was increased almost 3-fold (Emery et al., 1997).

Table 1: MLR Analysis of Lymphocyte Responses from Animals 10736 and 10807 (Emery et al., 1997)

Responder Cells	Blocking Ab	% Relative Response
Naive cc	Anti-DR	41
	Anti-DQ	50
	Anti-DR + DQ	10
10736	Anti-DR	52
	Anti-DQ	15
	Anti-DR + DQ	1
Naive cc	Anti-DR	52
	Anti-DQ	73
	Anti-DR + DQ	18
10807	Anti-DR	75
	Anti-DQ	79
	Anti-DR + DQ	12

E. DR matching “turns off” alloreactive T cells

The putative effect of *in vivo* DRB transgene expression on T cell reactivity was assessed *in vitro* on target cells expressing class II molecules related to the transgene products. Since there are no recombinant class II haplotypes which separate DR from DQ, it was not possible to test directly the DR-dependent reactivity of T cells harvested from engineered animals. Therefore, mixed lymphocyte experiments were performed in the presence of either anti-DR or anti-DQ specific antibodies (**Table 1**). Cells from either control (#10807) or experimental (#10736) pigs were used as responder cells in a mixed lymphocyte response (MLR) to cells bearing the same DR allele as the donor DRB transgene. Antibodies specific for either DR or DQ were added to the cultures. When the anti-DR antibody was added either to cells from the control or experimental pig, no effect on proliferation was observed. When anti-DQ was added, no effect was observed on the response of the control pig; however, the response of the experimental pig to the allogeneic cells was inhibited. Control blocking MLR experiments performed with both antibodies confirmed complete inhibition. These data indicate that the immune cells from pig #10736, which received autologous BM cells engineered with the allogeneic DRB transgene, were unresponsive to the donor DR antigen (Emery et al., 1997), and support the use of gene therapy to induce specific immune tolerance and long-term graft survival by enabling the expression of donor class II determinants in a class I, class II-disparate recipient.

Table 2: Summary of Experimental Animals

Animal Number	Host Haplo-type	DR Vector Allele	Cyto-kines*	Ktx Donor Haplo type
Experimental:				
10660	c	d	-	d
10680	d	c	-	c
10697	d	c	-	c
10736	c	d	+	d
Control:				
10807	c	c	-	d

*BMC were treated (+) or not treated (-) with cytokines at the time of transduction (Emery et al., 1997).

F. Kidney survival in miniature swine engrafted with Class II targeted bone marrow cells

Autologous BM cells from five miniature swine were transduced in the presence or absence of cytokines and then infused back into the original pigs which underwent a split dose of 10 Gy given on two consecutive days just

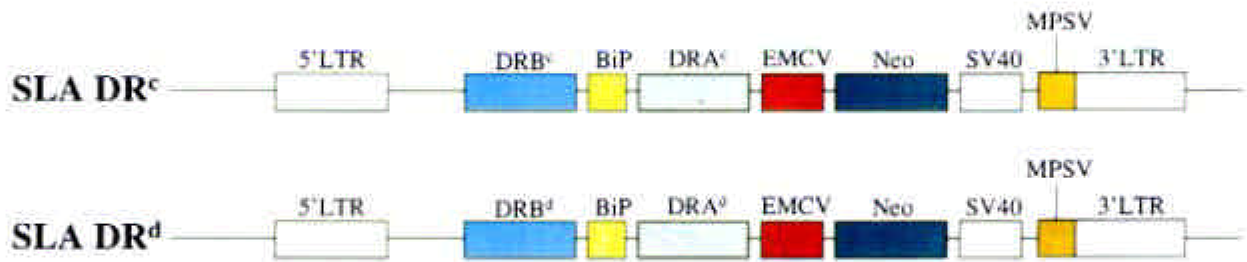

Figure 5. Construction of polycistronic retroviral vectors (Banerjee et al, 1997).

prior to BM transplant (**Table 2**). Three pigs (#10660, 10680, and 10697) received autologous BMC transduced with allogeneic class II in the absence of cytokines. One (#10736) was reconstituted with DRB-transduced BMC in the presence of cytokines, and, finally, a control animal (#10807) received BM cells transduced with syngeneic class II in the absence of cytokines. Each animal was infused with approximately 9 x 10^7 transduced cells per kg of body weight. Engraftment occurred within 12 ± 4 days post BMT based on the criteria of a white blood cell count of 1000/mm^3. Progenitor colony formation was estimated on aliquots of BM taken prior to infusion and from serial rib biopsies taken post-infusion. The transduction efficiencies prior to infusion ranged from 6.5% to 25.9% with the highest being associated with the bone marrow transduced in the presence of cytokines. The initial levels of G418 resistant CFU-GMs were maintained for at least 12-22 weeks post BM transplant after which they declined substantially (Emery, D.W., 1997). Following full reconstitution of the recipient with the DRB engineered BM cells (4-6 months post BM transplant), each animal was challenged with a fully allogeneic kidney only DR matched to the introduced transgene. According to the protocol presented in **Figure 3**, a 12 day treatment with CsA was administered, beginning at the time of allogeneic kidney transplant. Function of renal allografts was measured by serum creatinine levels. The control pig # 10807, which expressed a syngeneic DRB transgene in BM derived cells, underwent an early rejection episode which was followed by progressive renal failure. It eventually died 120 days post-transplant. Of the three pigs reconstituted with non-cytokine treated BM cells, one rejected its transplant on day 8 while still under CsA coverage; the other two survived 22 and 40 days, respectively. In contrast, the pig whose BM was transduced in the presence of growth factors, and who showed a high initial transduction rate (25.9% G418^r CFU), accepted its kidney long-term and showed no sign of cellular rejection. It exhibited normal kidney functions until it was sacrificed on day 995 post-BM transplant (Emery et al., 1997).

VII. Use of gene therapy in a pig to primate xenotransplant model

Since matching of class II genes between graft donor and recipient, through gene therapy appeared to control T cell dependent responses in the miniswine allotransplantation model, similar studies have been initiated in a pig to primate xenotransplantion model to assess the extent to which class II sharing may also provide specific unresponsiveness to xenogeneic antigens. Transfer and expression of porcine class II DR antigens into monkey bone marrow cells was first accomplished. In contrast to the allotransplantation studies in the miniature swine in which the DR*a* chain sequence is conserved, expression of pig DR in other species requires that both the DRA and DRB genes be transferred.

A. Construction of Vector for Pig Class II

In order to insure transfer of the DRA and DRB genes into the same cell, a recombinant polycistronic vector was generated (Banerjee et al., 1997a) (**Figure 5**). This vector enables the expression of multimeric proteins from a single transcript by using internal ribosomal entry sites (IRES) for independent initiation of translation from internal cistrons. The plasmid pL7gCAT (gift from Dr. B. Seed, Massachusetts General Hospital, Boston, MA) was derived from the pLN backbone (Miller and Rosman, 1989). Derivatives of this vector which initially contained three copies of the immunoglobulin heavy chain binding protein IRES (BiP) (Macejak, 1990) were found to be unstable in transduction experiments. Thus, two of the three BiP cassettes were eliminated and the remaining cellular-derived BiP was used in combination with the viral IRES derived from the encephalomyocarditis virus (EMCV) (Jang, 1988). In addition, the murine myeloproliferative sarcoma virus (MPSV) enhancer sequence (Johnson et al., 1989) was incorporated into the 5' end of the 3' LTR in order to increase the tropism of

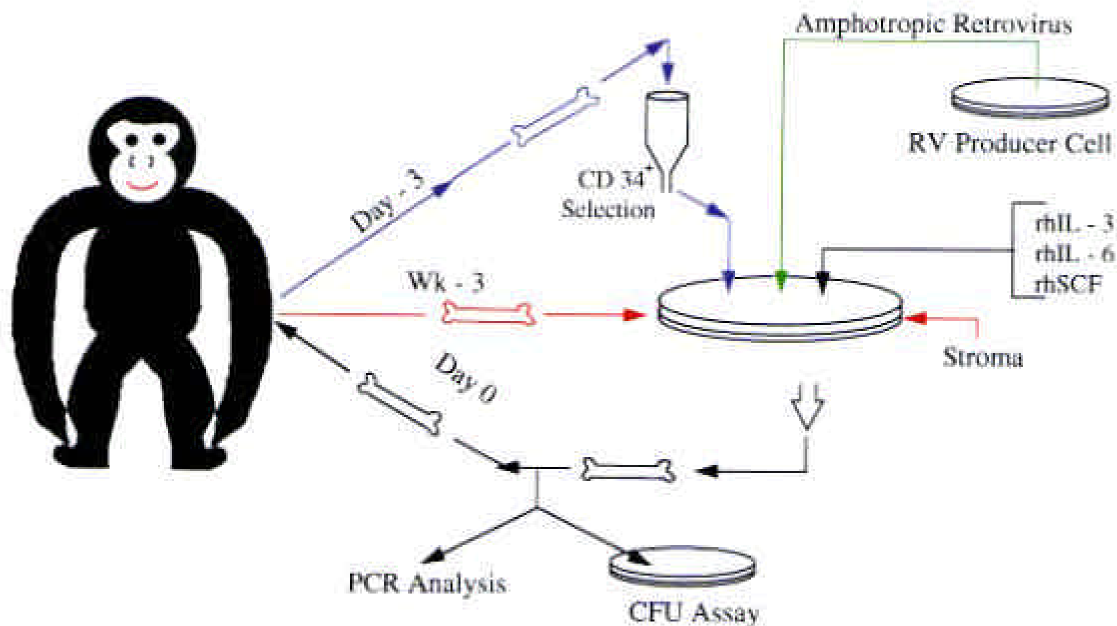

Figure 6. *Ex vivo* transduction of primate bone marrow cells with recombinant retrovirus for SLA gene transfer.

vector expression toward hematopoietic tissues as reported (Stocking, 1994). An enhancerless SV40 origin of replication sequence was also inserted upstream of the 3' LTR to allow testing of preliminary recombinant constructs using transient expression in COS cells (Aruffo and Seed, 1987). Porcine DRB and DRA sequences were generated by PCR amplification of cDNA clones (Gustafsson 1990a, Hirsch et al. 1992). The sequence for resistance to neomycin (Neo) was inserted downstream of the DRA cDNA to allow for selection with G418. High titer virus producer clones (> 1x 10^6 pfu/ml) were derived by transfection/transduction of appropriate ecotropic/amphotropic packaging lines according to standard protocols (Banerjee, 1997a). Analysis of selected virus producer clones demonstrated stability of particle expression over an eight to ten week period. These clones were cultured in roller bottles and production was monitored by G418 resistant CFU titers. The viral titer averaged approximately 4 x 10^7 CFU/ml during eight weeks of culture. No replication-competent retroviruses were detected by S+L- assay on PG4 cells (Banerjee et al. 1997a). Virus producer clones were ultimately selected for their ability to express cell surface class II heterodimers (not shown).

B. Transduction of non-human primate cells

Bone marrow was harvested from a Cynomolgus monkey and enriched for CD34$^+$ cells by selective binding to a Ceprate column (CellPro Inc.) (**Figure 6**). The enriched population was then plated onto autologous stromal cells which had been established 3 weeks prior to transduction. Cultures were exposed twice to retrovirus containing culture supernatants in the presence of polybrene and the human recombinant cytokines SCF, IL-3 and IL-6 for 18 hours. Aliquots were tested for transduction by plating into CFU assays in the presence of G418. All showed G418^r colonies (15.5% +/- 5.6%). Transduced cells were then infused back into the monkey from which they were originally taken.

Both DNA- and RT-PCR assays were performed to confirm transduction and expression of the transgene according to the scheme detailed in **Figure 7**. Two sets of DRB and DRA specific primers were used in a nested

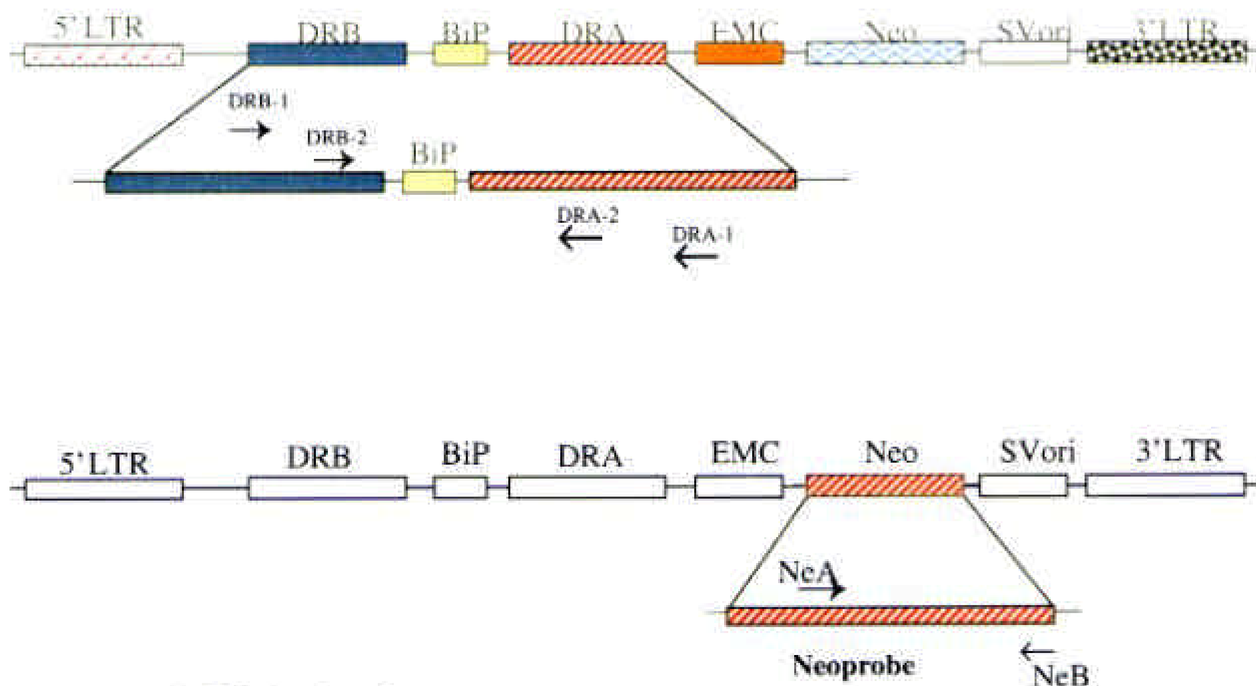

Figure 7. Primers for PCR detection of construct.

PCR reaction to generate a fragment that initiated in the DR sequences and spanned the IRES. Primers specific for the Neo resistance gene have also been generated. In addition, PCR primers specific for the beta-actin gene were used as controls in parallel PCR reactions for monitoring the quality of the cDNA templates. All bone marrow cultures transduced with the pig DR vector showed a clear PCR positive signal when assayed *in vitro* (not shown).

C. Engraftment of transduced cells

In contrast to miniature swine recipients which received lethal irradiation, a non-myeloablative conditioning regimen which made use of T cell depleting reagents was applied to monkey recipients of transduced bone marrow cells. The protocol included non-lethal total body irradiation (TBI) of 300 Rads, thymic irradiation of 700 Rads, and anti-thymocyte globulin (ATGAM) on days -3, -2, and -1. Administration of CsA was initiated on day 0 and continued for 28 days. Recombinant human GM-CSF was given subcutaneously from day 0 to day 14.

Peripheral blood and BM aspirates were collected periodically and either assayed directly by PCR or plated into CFU assays from which colonies were assayed by PCR. RT-PCR and DNA-PCR products generated using the nested primers were detected in all of the peripheral blood samples assayed through week 56 post-BM transplantion indicating that transcription of pig DR cDNA persisted long-term in peripheral blood cells of the monkey. Furthermore, the amplification of PCR products of predicted size indicated that no apparent genomic rearrangement had occurred within the region of the proviral genome containing the DR sequences. The frequency of expression was estimated by assaying colonies derived from BM aspirates taken at 4 weeks and 25 weeks post-BM transplantation. Frequencies for these time points were estimated to be approximately 2% and 1%, respectively (Banerjee, et al. 1997b). These data show that long-term stable engraftment was achieved in the Cynomolgus monkey.

VIII. Conclusions

The potential for using gene therapy to establish specific immune tolerance and to enable long-term graft survival has been demonstrated in the miniature swine allotransplantion model. Studies have shown that sharing of a single class II DR gene between a kidney donor and the recipient results in specific immune unresponsiveness for organs expressing the shared gene. These promising results led us to believe that a gene therapy approach could be adapted to clinical transplantation. In the long-term, this approach could also be valuable in

xenotransplantation in order to control the T cell dependent anti-xenogeneic immune responses.

Xenotransplantation, however, is complicated by additional issues such as managing the natural antibodies which exist in primates and which react with the tissues of discordant species (i.e., pig) to cause hyperacute rejection. The pilot primate experiment described here shows that long-term expression of class II genes can be achieved in non-human primates using a non-lethal, clinically acceptable protocol and points to the appropriateness of a gene therapy approach in achieving shared antigen expression across allogeneic and xenogeneic barriers. It has yet to be determined if tolerance to xenogeneic antigens can be achieved by sharing only class II. Thus, the possibility of transferring additional pig genes such as class I genes is currently under investigation. It is our conviction that the benefits of having a genetically well-defined unlimited supply of organs justify the additional research required to facilitate implementation of xenotransplantation.

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