Matching the Results Obtained in Concordant Primate Xenotransplantation Models

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Graft 2001; 4; 96

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Xenotransplantation has traditionally been classified as either concordant or discordant, depending on the divergence or phylogenetic distance between the species combination. A discordant xenograft in which hyperacute rejection (HAR) is expected to occur refers to transplantation between widely disparate species, such as pig-to-nonhuman primate or human. Concordant xenotransplantation, without HAR, refers to transplantation between closely related species, such as baboon-to-monkey or human.

Concordant Xenotransplantation: Promising Results

In the early 1990s, baboons were considered ideal donor candidates for human recipients due to their genetic and functional similarity. During this period, the combination of baboon (*Papio anubis*) and cynomolgous monkey (*Macaca fasicularis*) was widely used to investigate optimal immunosuppressive protocols for future clinical xenotransplantation.[1,2] In this concordant model, untreated xenografts survived only 6 days with no evidence of HAR. The addition of traditional immunosuppressive agents, such as cyclosporine (CsA), did not prolong xenograft survival because of their inability to inhibit the vigorous humoral rejection.[1] However, the combination of CsA and total body radiation or anti-B cell immunosuppressants (i.e., cyclophosphamide (CyP), mycophenolate mofetil (MMF) or rapamycin (Rap)) significantly prolonged xenograft survival in both the cardiac and renal models (Table 1). Unfortunately, the enthusiasm for using primates as donors has faded due to ethical concerns and the threat of xenozoonosis.

Discordant Xenotransplantation: Difficulties Arise

Economic, ethical and practical factors have positioned the pig as the lead candidate as an organ source in future clinical xenotransplantation. The development of transgenic pigs expressing human complement regulatory genes, such as hDAF (human decay accelerating factor) and/or hCD59, has revolutionized the field and allowed researchers to overcome the barrier of HAR. Based on the current literature, most of the world's experience in this field derives from two groups: David White's (Imutran/Novartis) and Jeffrey Platt's (Duke-Mayo/Nextran-Baxter). These groups have not only demonstrated that HAR can be prevented but that transgenic porcine organs, such as the kidney, can also support primate life until xenograft rejection occurs.[3,4] Despite aggressive immunosuppression, most of the recipient primates unfortunately still die 30 to 40 days post-transplant, with most deaths attributed to delayed xenograft rejection, otherwise known as acute vascular rejection (Table 2). The benefit of currently available immunosuppressive protocols, with proven effectiveness in concordant models, has not yet been firmly established in discordant xenograft models.

Concordant and Discordant Xenotransplantation: Why the Disparity in Results?

The initial hypothesis that transgenic pig organs would be functionally concordant for human transplantation has proved incorrect. Figure 1 shows a comparison of the immune responses in concordant and discordant xenotransplantation. The immune response following concordant

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Table 1 | **Results of Baboon-to-Monkey Concordant Xenotransplantation**

Author	Organ	Treatment	Mean graft survival	Reference
Myburgh (1994)	Kidney	TLI,RATG	171 days (21-293)	2
Roslin (1992)	Heart	TLI,CsA,MP	225 days (70-540)	5
Zheng (2000)	Kidney	CsA,CyP,RAP,MP	302 days (149-380)	6

* abbreviations: TLI, total lymphoid irradiation; RATG, rabbit anti-human thymocyte globulin; CsA, cyclosporine; MP, methylprednisolone, CyP, cyclophosphamide; RAP, rapamycin.

Table 2 | **Results of Transgenic Pig-to-Primate Discordant Xenotransplantation**

Author	Primate	Organ	Treatment	Mean graft survival	Reference
Ostlie(1999)	Monkey	Kidney	CsA,CyP,CS,RAD	31 days (9-71)	3
Vial (1999)	Monkey	Kidney	CsA,CyP,ERL080	29 days (2-51)	4
Lin (1998)	Baboon	Heart	ID,CsA,CyP,CS	11 days (1-29)	7
Vial (1999)	Baboon	Heart	CsA,CyP,MMF,CS	14 days (2-39)	8

* abbreviations: ID, immune absorption with column; CsA, cyclosporine; CyP, cyclophosphamide; CS, corticosteroid; MMF & ERL080, mycophenolate mofetil; RAD, Novartis rapamycin.

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xenotransplantation is similar to that following allotransplantation where T cells play an essential role in both the cellular and humoral responses. In this setting, the humoral immune response is mediated by antibody induction, which is T cell-dependent. Therefore, in concordant models, humoral rejection can be effectively inhibited by currently available immunosuppressants (i.e., CsA) in combination with radiation or anti-B-cell agents (i.e., CyP). In contrast, discordant xenotransplantation activates both the innate and acquired immune systems. In this model, natural antibodies, complement, natural killer cells and macrophages—in addition to T-cells—are likely to play important roles in the pathogenesis of xenograft rejection. It is probable that currently available immunosuppressive agents, such as CsA, which primarily target T cells, are less effective in inhibiting the vigorous potentially T cell-independent humoral immune response following discordant xenotransplantation. Furthermore, the molecular incompatibility between pigs and primates involving traditionally non-immune system effectors, such as thrombomodulin, may provide a further hurdle to overcome following discordant xenotransplantation.

Future Directions

Attempts at transplanting animal organs into humans date back to 1905 and, although unsuccessful to date, the increasing shortage of suitable human donor organs has renewed interest in this field. The current results of pig-to-primate transplants are not promising enough to justify clinical trials. However, the significant contribution from the development of transgenic pigs should not be underestimated and the rapid advancement of genetic engineering techniques has brought xenotransplantation to the doorstep of clinical application. The future of xenotransplantation depends on results from the following fields:

1. the further modification of pigs, such as by Gal knock-out or by upregulation of "protective" genes;
2. the development of novel immunosuppressive agents that target the innate immune system and B cells; and
3. the development of clinically applicable methods to induce donor-specific tolerance.

These daunting tasks will only be achieved by multi-center and multi-disciplinary collaborations between universities and industries because of the field's scientific complexity and the high cost associated with its research. As Abdul Daar recently commented, "There can be few subjects in biomedicine more interesting or more challenging than xenotransplantation. The renewed interest has reached fever pitch. The stakes are high, the list of stakeholders is large, there are many unknowns, and the field is evolving rapidly".[9] In our center, a monkey survived 1,073 days after receiving a baboon liver. We believe that the development of protocols allowing a series of primates to survive

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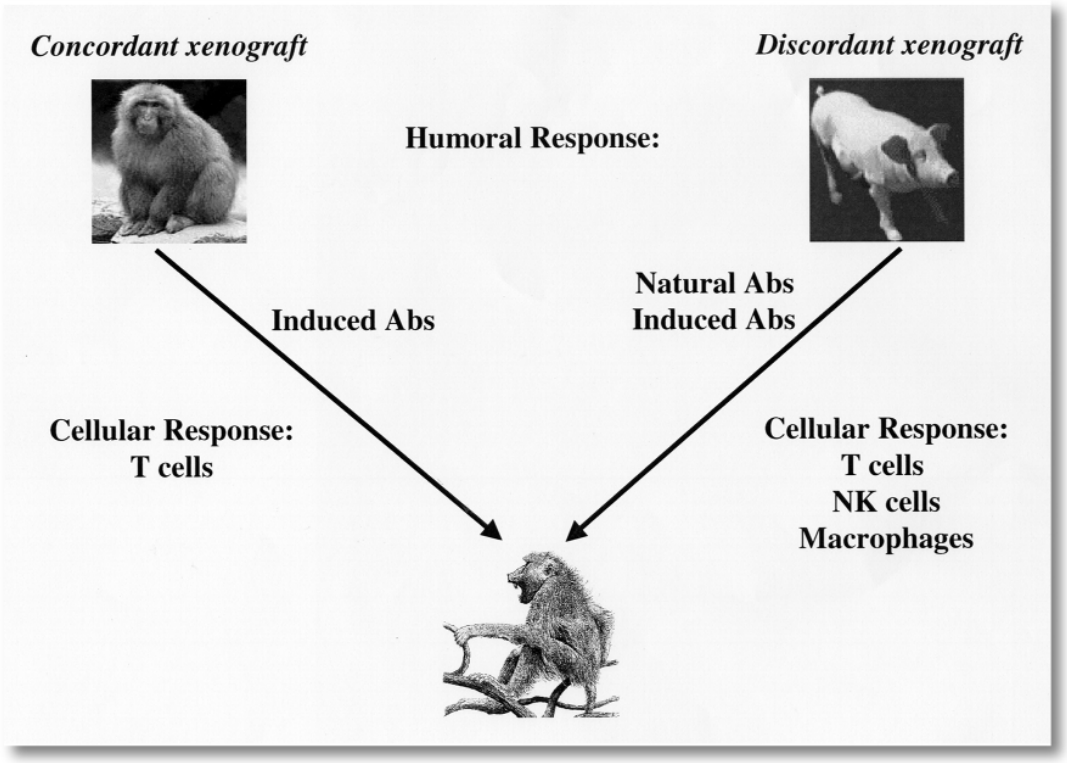

Figure 1. Comparison of immune responses following concordant and discordant xenotransplantation.

for one year following xenografting of pig organs would justify the leap into the clinical arena. This is the dream of many thousands of patients, currently awaiting human organs, who continue to suffer from end-stage organ failure.

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