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On the intersections of basic and applied research in xenotransplantation*

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Abstract

I am very grateful to the Council and members of the International Xenotransplantation Association for this Honorary Membership. In accepting this prestigious award, I pay tribute to my mentors Antonio Oriol i Anguera, Carlos Martinez, Robert A. Good, and Hans Müller-Eberhard for their guidance and friendship as I was beginning my travels in biomedical research. I also thank the many gifted collaborators, students, and technical personnel, as well as the agencies and taxpayers, who funded our research and made our scientific contributions possible. Here I briefly mention some of these contributions, including early work on the immunobiology of the thymus, my short incursion in the immunology of Chagas disease, and what have been the dominant themes of my career: the mechanisms of complement injury, the role of complement in pathophysiology, and induction of cytoprotection in the vascular endothelium. I emphasize our contributions on the role of complement as related to understanding and overcoming xenograft injury, a work that has been personally very rewarding. Now it is exciting to see that the field of xenotransplantation research is moving forward vigorously, a time of great optimism suggesting that many potential clinical applications of xenotransplantation will come to fruition in the near future.

Keywords

accommodation; complement; cytoprotection; endothelial cells; xenotransplantation

Introduction

I am deeply honored and grateful to receive this important award. I accept it on behalf of the many collaborators, fellows, and students who made possible my contributions to biomedical science and, specifically, to advance the field of xenotransplantation. The interaction with these talented and dedicated people has been a source of immense stimulation and joy throughout my professional life. Of course, the significance of this

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biennial award is defined by the very important contributions of the previous awardees: Thomas Starzl, John Najarian, Guy Alexandre, Ian McKenzie, Carl Groth, and David Cooper. They have been true pioneers in leading the efforts on studies in xenotransplantation and have carried out work that often fell at the intersection of basic and applied research. In this brief recount of my career in biomedical research, I intend to narrate some of my efforts, especially those contributions that led to my participation in the quest to understand and overcome xenograft rejection. This field is advancing vigorously, giving us reasons to believe that many potential clinical applications will be realized in the not too distant future.

Photo 1.
Agustin P. Dalmaso.

Medical School often succeeds in fostering a potential scientist

While attending Medical School at the University of Cordoba in Argentina, I soon felt attracted to understand the mechanisms of disease. At that time, however, knowledge about many of those mechanisms was often rudimentary, and at times, instead of focusing on learning what I needed to prepare for an exam, I occasionally found myself pondering questions addressing ways to solve that lack of knowledge. For this reason, while a student, I applied to compete for a coveted position as a teaching assistant in the Department of Physiology, which at the time was under the leadership of Professor Antonio Oriol i Anguera. Professor Oriol was born in Spain in a small town near Barcelona. He obtained a medical degree and trained in physiology and physical chemistry, and began his professional career in Spain, but emigrated to Argentina at the time of the Spanish civil war. Professor Oriol had a brilliant mind, was an outstanding lecturer and a prolific writer who contributed incisive essays in areas of philosophy and culture, usually at their intersection with biology and medicine. I have always been extremely grateful to Professor Oriol for his influence in nurturing in me a strong desire to include research in my career plans and become an academic physician. It may also be of interest to the xeno research community that Rafael Oriol, who has made important contributions to our field, is one of Antonio Oriol i Anguera's two sons.

Immunobiology of the thymus

After a short stint as an instructor in Physiology at the University of Cordoba, I accepted a fellowship at the University of Minnesota (U. of M.) to work in the laboratories of Carlos Martinez and Robert A. Good. It was a magnificent opportunity to learn immunology in one of the leading laboratories in the world. Arriving in Minneapolis to find patches of residual snow in the middle of April was just a prelude of what would come the following winter! Coming from Cordoba, Argentina, my wife and I had never seen snow before, so this experience was very exiting but also somewhat foreboding.

The work in the laboratory with Carlos and his small group was extremely gratifying. Carlos was a rigorous scientist who had trained in Argentina with the Nobel Prize winner Bernardo Houssay and then moved to Minneapolis to work on cancer biology with John Bittner. Carlos acquired expertise in mouse genetics, which led him to start collaborating with Bob Good in immunological tolerance of tissue allografts. His untimely death at the age of 52 yr was a tragic loss for his family and colleagues.

Bob was a professor of Pediatrics and, at the time, the unquestioned authority in immune deficiencies, with a consuming interest in defining the mechanisms underlying those diseases, including the role of the thymus in immunobiology. I began my work with studies on tolerance but quickly moved to investigate the role of the thymus. The paper of J.F. Miller demonstrating the profound effect of thymectomy in newborn mice on their immunological capacity [1] as well as our work in the Good's group showing that thymectomy of young rabbits and newborn mice would lead to significant immunologic impairment [2] stimulated me to continue working with renewed enthusiasm in this area [2,3]. Among the many fundamental contributions of Bob Good and his collaborators was

the definition of the origin of T and B lymphocytes [4]. For me, those were three wonderful years of exciting work at a time that represented the beginnings of cellular immunology. However, I deemed it important for a more thorough training to learn approaches that would allow me to tackle questions of molecular immunology.

Complement

I was very fortunate to be offered a position in the laboratory of the late Hans Müller-Eberhard, who was moving from Rockefeller University to the Scripps Research Institute in La Jolla, California. In 1963, the young Institute was already well known internationally, with a very strong Immunology/Experimental Pathology group. Hans' goal was to identify and characterize the serum proteins that form the human complement system and establish their reaction mechanism. When Hans arrived at Scripps, he had already purified the serum proteins, C1q, C3, and C4, to the acclaim of many or the disbelief of a few who felt complement proteins did not exist in relatively high amounts in serum. The work atmosphere in the laboratory was outstanding and, of course, La Jolla was a wonderful place to raise my young family. Hans had learned biochemistry of plasma proteins first in his native Germany and then in Sweden from the leading protein researchers of the time. I learned much from him and also from my very good friend the late Ulf Nilsson, another Research Fellow in the laboratory, who had great expertise in immunochemistry and protein purification. I participated with enthusiasm in studies of the reaction mechanism of C3, C4, and C5 [5,6], the selective denaturalization of these proteins by chaotropic agents [7], and the characterization of C3 and C4 dissociated from complement-cell complexes [8] and began studies on mechanisms of cell killing by the membrane attack complex [7].

During my last years in the United States, I felt a strong commitment to return to Argentina, thinking that I could contribute to the development of Immunology in my country of origin. So, against the strong advice of Hans and others at Scripps, I decided to apply to the National Research Council of Argentina for a position as a Career Investigator. Before leaving Scripps, I wrote my first grant application to the NIH, which helped me fund studies on the immunology of Chagas' disease in Argentina.

Chagas' disease

The reason I had chosen to work on Chagas' disease was that this parasitic disease was, and continues to be, widespread in Latin America, from Southern Mexico to Northern Argentina, and like many other parasitic diseases, little was known in the mid-sixties about its immunology and pathogenesis. In addition, I had planned to perform studies on regulatory mechanisms of cell killing by complement. In Buenos Aires, I was received with enthusiasm by those able to facilitate developing my project and was offered to be the Head of the Immunology Division in the Department of Medicine of the University of Buenos Aires. I soon found collaborators, postdoctoral fellows, and students and was able to organize a functioning laboratory. The talent and effort of Maria M. de Bracco, Rosalia Lelchuk, and Alberto Diaz were fundamental for the success we were able to achieve. We carried out studies on antibodies and immunoglobulins in patients with Chagas' disease [9], the lymphocyte proliferation in response to the antigens of *Trypanosome cruzi*, the agent of

Chagas' disease [10], and mechanisms of *T. cruzi* killing by antibody and complement [11]. Work on complement included studies on the effects of alkali metal cations on cell killing by the membrane attack complex of complement [12,13].

Despite reasonably good progress and very significant opportunities to expand my work, I felt that organizing a larger research enterprise would require overcoming the many obstacles of political instability and a heavy bureaucracy, and, most importantly, enduring the consequences of the tragic internecine strife that would continue in Argentina for many years.

Pathophysiologic role of complement

I had the privilege of being offered a position at the University of Minnesota as a Clinical Pathologist at the Minneapolis Veterans Affairs Medical Center that would allow time for research. So, after four interesting years in Buenos Aires, we began packing to return to the USA. At the beginning of the 70s, immunology was growing stronger at the University of Minnesota; Bob Good was still there heading a large group, and Edmond Yunis was making important contributions in human histocompatibility. For many years in Minnesota, I worked with collaborators and students on various aspects of the complement system. Among basic studies were the demonstration that complement reduces the fluidity of the cell membrane [14–16] and measurements of the functional size of the complement lesion in red cell membranes [17–19].

On the other hand, as when I was a medical student, I continued to be attracted to questions at the intersection of basic and applied research. Our studies on the role of complement in pathophysiology were indeed very gratifying, as illustrated by the following examples. With Ron Falk and Alfred Michael, we developed and used an antibody that reacted only with C9 incorporated into the membrane attack complex of complement with no reactivity against native C9, which allowed the demonstration of deposits of membrane attack complexes in diseased tissues, implying that these complexes may be pathogenic [20,21]. We found this to be the case in renal lesions of diabetes, hypertension, and systemic lupus erythematosus. With Gloria Niehans, we demonstrated weak or no association in the expression of complement inhibitory proteins DAF, CD46, and CD59 in cancers of the breast, lung, colon, and kidney [22]. Together with Harry Jacob, we showed that C5a was the mediator that caused pulmonary vascular leukostasis during hemodialysis with the membranes used at the time [23,24]. In a study with Ed Janoff, we discovered that polymeric IgA activates complement that in turn stimulates phagocytes to kill *Streptococcus pneumoniae* [25].

Xenotransplantation and the vascular endothelium

In 1987, the late Fritz Bach, then at the U. of M., began to explore the possibility of establishing a research program to address mechanisms of xenograft rejection and approaches that could prevent it and asked me to join the effort with studies on the role of complement in rejection. Fritz was a brilliant and passionate scientist who was very gifted at establishing and implementing a new program such as this. Other members of the group were Greg Vercellotti, who had pioneered research on vascular injury and induction of cytoprotection of endothelial cells; Jeffrey Platt, who at the time was interested in vascular

immunopathology, especially in the context of renal allotransplantation; and the transplant surgeons R. Morton Bolman and Arthur Matas, who had a strong interest in various areas of transplant pathophysiology.

I very vividly remember my first conversation with Fritz regarding the formulation of the plans for xeno research. My first reaction was one of great enthusiasm because of the tremendous potential represented by clinical xenotransplantation, despite recognizing that such a project presented what I thought were herculean challenges. I told Fritz that this project would allow me to explore a fascinating idea that presented itself at the time of the discoveries a few years earlier of complement regulatory proteins of cell membranes that I thought could be used to prevent complement activation on the vascular endothelium of a xenograft. Fritz told me that, as a cellular immunologist, he knew little about complement and had not yet heard of such things as membrane complement inhibitors. So I presented to the nascent xeno group a couple of talks about complement and my hypothesis about using the membrane complement inhibitors in experiments that might provide proof-of-principle for application to xenotransplantation. This work was soon performed, demonstrating that introduction of human decay-accelerating factor into the cell membrane of pig endothelial cells was able to prevent killing of the cells by human complement [26].

This was followed by the work of Emanuele Cozzi, David White and collaborators that resulted in the production and testing of pigs transgenic for human decay-accelerating factor as donors for primate recipients [27], which represented the first important advance toward clinical applications of xenotransplantation. This fundamental area continued being explored in other laboratories testing transgenes for all membrane-associated complement inhibitors in various models of xenotransplantation.

Other early contributions of our xeno group were studies on the role of innate humoral immunity in rejection, leading to establishing the predominant role of IgM anti-pig antibodies and the classical complement pathway in hyperacute rejection in the pig heart-to-rhesus monkey model [28], the role of C5a in causing loss of endothelial cell-associated heparan sulfate [29,30], and the role of endothelial cell-bound iC3b-mediating recipient neutrophil adhesion [31]. The xeno group at the U. of M. was moving along successfully but unfortunately Fritz Bach and Jeff Platt decided to accept offers from other institutions, and Greg Vercellotti, who remained at U. of M., changed his research focus to other areas of endothelial cell biology and inflammation. So I continued pursuing my interest in xenotransplantation with Chip Bolman and Art Matas and carried out some collaborative work with Fritz and Jeff after they left the U. of M.

With Jeff Platt, we demonstrated the effectiveness of C1-inhibitor to prevent the loss of heparan sulfate and the killing of endothelial cells incubated in vitro with human serum [32]. The other collaborators and I were able to extend graft survival of a pig heart in baboons to several days using antibody removal, complement inhibition, and immunosuppression, but the grafts were lost due to vascular rejection [33]. We also demonstrated the effectiveness of the anti-C5 monoclonal antibody to protect a pig heart from injury caused by perfusion with human blood [34]. Using similar models, we showed the protective effect of the complement inhibitor CD59 expressed by hearts and lungs from CD59-transgenic pigs [35]. We found

that a soluble hybrid molecule derived from human decay-accelerating factor and membrane cofactor protein called CAB-2 was very effective to prevent hyper-acute rejection of a pig heart in rhesus monkeys [36].

One of the protective mechanisms that had been observed in ABO-incompatible organ allografts in humans is accommodation and we thought that the concept might be applied to xenotransplantation [37]. After leaving the U. of M., Fritz Bach and his group did extensive work on the mechanisms of accommodation in rodent models. Using one such model, my collaborators and I demonstrated that, if T-cell-mediated rejection was prevented, the only obstacle to the development of accommodation after grafting a hamster heart into a rat was the membrane attack complex of complement [38]. This study placed the inhibition of the membrane attack complex as a means to facilitate induction of accommodation in xenotransplantation.

During the last several years, I have been interested in inducing protection against inflammatory and coagulant injury in the porcine vascular endothelium. The goal of these studies is based on the hypothesis that induction of intrinsic cytoprotection in the vascular endothelium of an organ graft contributes to graft survival, either through facilitating the development of accommodation or other mechanisms. To achieve this goal the contribution of John Grehan and Sylvester Black was fundamental, as was that of Barbara Benson, who also contributed with great dedication and insight to many previous projects of my laboratory. We had begun these studies using antibodies or lectins that bind alpha galactosyl, the dominant antigen of pig endothelial cells [39,40]. With this approach, however, the protective response was preceded by a transient pro-inflammatory response, making it difficult to test the approach in an animal model. Potentially, much more useful was our observation that the interleukins IL-4 and IL-13 were effective to induce cytoprotection without causing inflammation [41]. Then, we could show that transduction of IL-4 into pig iliac arteries resulted in the production of IL-4 and protection of the artery from the hyperacute injury that occurs when the artery is perfused with human blood [42]. This injury is caused by the membrane attack complex of complement, and the endothelial protection requires activation of PI3K/Akt, SREBP-1, and phospholipid synthesis [43,44]. More recently, we discovered that IL-4 also upregulates expression of the inter-cellular junction protein claudin-5 and that this upregulation is required for the protection from killing by complement.

Prospects for xenotransplantation in medical practice and contributions of xenotransplantation research to other fields

The first major achievement to overcome xenograft rejection was the generation of transgenic pigs for complement regulatory proteins [27]. This technical breakthrough elicited great enthusiasm, and a very large number of studies were performed to assess the efficacy of the transgenes in preventing rejection. Study after study proved that while hyperacute rejection was avoided, there was little or no protection against vascular rejection. Now we have a better understanding of molecular incompatibilities between pig and human [45] and the major advance represented by the availability of alpha-Gal knockout pigs. More recently, we have witnessed great progress in genetic engineering of alpha-Gal KO pigs to

express multiple transgenes, including a complement inhibitor, an anti-coagulant, and one or more genes that contribute to intrinsic protection. Organs and tissues from these animals are being tested in non-human primates, and organ survivals of many months are often achieved. The research continues to optimize the engineering of these pigs in terms of effective levels of transgene expression and the most appropriate combination of transgenes [46,47] and, clearly, success is not that far away. Therefore, it is not surprising to notice a new era characterized by the enthusiasm of young investigators joining this scientific field. It is obvious to perceive a shared vision of the IXA members and those who will soon join IXA, that fine-tuning of various approaches and further pre-clinical testing will soon provide xenogeneic cells and organs for a variety of patients [48–50].

Over two decades ago, when I embarked in our common endeavor of understanding and overcoming xenograft rejection, I expected that achieving this goal would not be the only fruit of our labor. Of course, achievement of clinical xenotransplantation was and remains our major objective and, once reached, most likely will be the major consequence of our efforts. However, even before xenotransplantation has reached the stage of clinical use, there are many areas that have seen advances derived from work on xenotransplantation, from which I will mention only a few. Important technical advances have been made in the preparation of transgenic pigs, which undoubtedly will be applied to large animal studies for a variety of purposes. The studies of pig endogenous retroviruses and other microbiological issues will have applications to several areas of veterinary medicine [51,52]. In my view, and perhaps most importantly, the extensive studies on the biology and immunology of pig vascular endothelium is greatly extending the knowledge necessary to prevent and treat vascular diseases, not only the vasculopathies of organ transplantation, but also ischemia–reperfusion, atherosclerosis, and other pathologies [42,53,54].

Conclusion

In my estimation, research in xenotransplantation is progressing very well indeed. This outlook is very different from the time when many believed that translating to clinical medicine what was being learned experimentally about xenotransplantation was just an unachievable utopian vision. If we compare the papers and lectures that are being presented at this meeting with those from previous meetings, it is clear that we are rapidly moving forward and that there are good reasons to be optimistic. This is not entirely surprising given the great strides that have been made in basic sciences and the success of interdisciplinary research in biology and medicine, which provide the foundations for our endeavor. In this regard, an optimistic outlook was properly encapsulated by the Millennium Project in the 2011 “State of the Future”, predicting that “The coming biological revolution may change civilization more profoundly than did the industrial or informational revolutions” [55]. Given the collective intellect, fortitude, and determination of the xeno community, I am confident that the success of xenotransplantation will be part of this change. I feel privileged to have participated in the development of what, 10 or 20 yr from now, will likely be called the “early stage of xenotransplantation”, which was comprised of work mostly at the intersections of basic and applied research. It is with great pleasure that I again thank the Council and members of the Association for having chosen me to receive this prestigious award, which I value immensely.

References

1. Miller JF. Immunological function of the thymus. *Lancet*. 1961; 2:748–749. [PubMed: 14474038]
2. Good RA, Dalmaso AP, Martinez C, et al. The role of the thymus in development of immunologic capacity in rabbits and mice. *J Exp Med*. 1962; 116:773–796. [PubMed: 13949053]
3. Dalmaso AP, Martinez C, Sjodin K, Good RA. Studies on the role of the thymus in immunobiology; reconstitution of immunologic capacity in mice thymectomized at birth. *J Exp Med*. 1963; 118:1089–1109. [PubMed: 14112268]
4. Cooper MD, Raymond DA, Peterson RD, et al. The functions of the thymus system and the bursa system in the chicken. *J Exp Med*. 1966; 123:75–102. [PubMed: 5323079]
5. Dalmaso AP, Muller-Eberhard HJ. Interaction of autologous complement with red cells in the absence of antibody. *Proc Soc Exp Biol Med*. 1964; 117:643–650. [PubMed: 14244915]
6. Muller-Eberhard HJ, Dalmaso AP, Calcott MA. The reaction mechanism of beta-1C-globulin (C'3) in immune hemolysis. *J Exp Med*. 1966; 123:33–54. [PubMed: 4159289]
7. Dalmaso AP, Muller-Eberhard HJ. Hemolytic activity of lipoprotein-depleted serum and the effect of certain anions on complement. *J Immunol*. 1966; 97:680–685. [PubMed: 5926456]
8. Dalmaso AP, Muller-Eberhard HJ. Physicochemical characteristics of the third and fourth component of complement after dissociation from complement-cell complexes. *Immunology*. 1967; 13:293–305. [PubMed: 6054730]
9. Lelchuk R, Dalmaso AP, Inglesini CL, et al. Immunoglobulin studies in serum of patients with American trypanosomiasis (Chagas' disease). *Clin Exp Immunol*. 1970; 6:547–555. [PubMed: 4097822]
10. Tschudi EI, Anziano DF, Dalmaso AP. Lymphocyte transformation in Chagas disease. *Infect Immun*. 1972; 6:905–908. [PubMed: 4629392]
11. Anziano DF, Dalmaso AP, Lelchuk R, Vasquez C. Role of complement in immune lysis of *Trypanosoma cruzi*. *Infect Immun*. 1972; 6:860–864. [PubMed: 4564786]
12. De Bracco MM, Dalmaso AP. Effect of the cationic environment on immune haemolysis of high potassium and low potassium sheep erythrocytes. *Immunology*. 1969; 17:559–569. [PubMed: 5352363]
13. Dalmaso AP, Giavedoni EB, Lelchuk R, DeBracco MM. Role of intracellular and extracellular cationic composition in immune lysis of mammalian erythrocytes. *J Immunol*. 1973; 111:527–535. [PubMed: 4717992]
14. Giavedoni EB, Dalmaso AP. The induction by complement of a change in KSCN-dissociable red cell membrane lipids. *J Immunol*. 1976; 116:1163–1169. [PubMed: 1254965]
15. Mason RP, Giavedoni EB, Dalmaso AP. Complement-induced decrease in membrane mobility: introducing a more sensitive index of spin-label motion. *Biochemistry*. 1977; 16:1196–1201. [PubMed: 191062]
16. Giavedoni EB, Mason RP, Dalmaso AP. Complement-induced modifications in membrane fluidity: studies with resealed and glutaraldehyde-treated erythrocyte membrane ghosts. *J Immunol*. 1978; 120:2003–2007. [PubMed: 207778]
17. Giavedoni EB, Chow YM, Dalmaso AP. The functional size of the primary complement lesion in resealed erythrocyte membrane ghosts. *J Immunol*. 1979; 122:240–245. [PubMed: 570203]
18. Giavedoni EB, Chow YM, Dalmaso AP. Complement lysis of resealed red cell membrane ghosts pretreated with glutaraldehyde. *J Immunol*. 1979; 122:1643–1648. [PubMed: 109512]
19. Dalmaso AP, Benson BA, Falk RJ. Complement channels in membranes: inhibition with a monoclonal antibody to a neoantigen of polymerized C9. *Biochem Biophys Res Commun*. 1984; 125:1013–1019. [PubMed: 6517929]
20. Falk RJ, Dalmaso AP, Kim Y, et al. Neoantigen of the polymerized ninth component of complement. Characterization of a monoclonal antibody and immunohistochemical localization in renal disease. *J Clin Invest*. 1983; 72:560–573. [PubMed: 6348093]
21. Falk RJ, Dalmaso AP, Kim Y, et al. Radioimmunoassay of the attack complex of complement in serum from patients with systemic lupus erythematosus. *New Engl J Med*. 1985; 312:1594–1599. [PubMed: 4000197]

22. Niehans GA, Cherwitz DL, Staley NA, et al. Human carcinomas variably express the complement inhibitory proteins CD46 (membrane cofactor protein), CD55 (decay-accelerating factor), and CD59 (protectin). *Am J Pathol.* 1996; 149:129–142. [PubMed: 8686736]
23. Craddock PR, Fehr J, Dalmasso AP, et al. Hemodialysis leukopenia. Pulmonary vascular leukostasis resulting from complement activation by dialyzer cellophane membranes. *J Clin Invest.* 1977; 59:879–888. [PubMed: 856872]
24. Craddock PR, Hammerschmidt D, White JG, et al. Complement (C5a)-induced granulocyte aggregation in vitro. A possible mechanism of complement-mediated leukostasis and leukopenia. *J Clin Invest.* 1977; 60:260–264. [PubMed: 874088]
25. Janoff EN, Fasching C, Orenstein JM, et al. Killing of *Streptococcus pneumoniae* by capsular polysaccharide-specific polymeric IgA, complement, and phagocytes. *J Clin Invest.* 1999; 104:1139–1147. [PubMed: 10525053]
26. Dalmasso AP, Vercellotti GM, Platt JL, Bach FH. Inhibition of complement-mediated endothelial cell cytotoxicity by decay-accelerating factor. Potential for prevention of xenograft hyperacute rejection. *Transplantation.* 1991; 52:530–533. [PubMed: 1716798]
27. Cozzi E, Tucker AW, Langford GA, et al. Characterization of pigs transgenic for human decay-accelerating factor. *Transplantation.* 1997; 64:1383–1392. [PubMed: 9392299]
28. Dalmasso AP, Vercellotti GM, Fischel RJ, et al. Mechanism of complement activation in the hyperacute rejection of porcine organs transplanted into primate recipients. *Am J Pathol.* 1992; 140:1157–1166. [PubMed: 1580328]
29. Platt JL, Vercellotti GM, Lindman BJ, et al. Release of heparan sulfate from endothelial cells. Implications for pathogenesis of hyperacute rejection. *J Exp Med.* 1990; 171:1363–1368. [PubMed: 2139104]
30. Platt JL, Dalmasso AP, Lindman BJ, et al. The role of C5a and antibody in the release of heparan sulfate from endothelial cells. *Eur J Immunol.* 1991; 21:2887–2890. [PubMed: 1936126]
31. Vercellotti GM, Platt JL, Bach FH, Dalmasso AP. Neutrophil adhesion to xenogeneic endothelium via iC3b. *J Immunol.* 1991; 146:730–734. [PubMed: 1987283]
32. Dalmasso AP, Platt JL. Prevention of complement-mediated activation of xenogeneic endothelial cells in an in vitro model of xenograft hyperacute rejection by C1 inhibitor. *Transplantation.* 1993; 56:1171–1176. [PubMed: 8249120]
33. Leventhal JR, Sakiyalak P, Witson J, et al. The synergistic effect of combined antibody and complement depletion on discordant cardiac xenograft survival in nonhuman primates. *Transplantation.* 1994; 57:974–978. [PubMed: 8154049]
34. Kroshus TJ, Rollins SA, Dalmasso AP, et al. Complement inhibition with an anti-C5 monoclonal antibody prevents acute cardiac tissue injury in an ex vivo model of pig-to-human xenotransplantation. *Transplantation.* 1995; 60:1194–1202. [PubMed: 8525509]
35. Kroshus TJ, Bolman RM III, Dalmasso AP, et al. Expression of human CD59 in transgenic pig organs enhances organ survival in an ex vivo xenogeneic perfusion model. *Transplantation.* 1996; 61:1513–1521. [PubMed: 8633381]
36. Salerno CT, Kulick DM, Yeh CG, et al. A soluble chimeric inhibitor of C3 and C5 convertases, complement activation blocker-2, prolongs graft survival in pig-to-rhesus monkey heart transplantation. *Xenotransplantation.* 2002; 9:125–134. [PubMed: 11897005]
37. Bach FH, Turman MA, Vercellotti GM, et al. Accommodation: a working paradigm for progressing toward clinical discordant xenografting. *Transplant Proc.* 1991; 23:205–207. [PubMed: 1990513]
38. Suhr BD, Black SM, Guzman-Paz M, et al. Inhibition of the membrane attack complex of complement for induction of accommodation in the hamster-to-rat heart transplant model. *Xenotransplantation.* 2007; 14:572–579. [PubMed: 17991145]
39. Dalmasso AP, Benson BA, Johnson JS, et al. Resistance against the membrane attack complex of complement induced in porcine endothelial cells with a Gal alpha(1–3)Gal binding lectin: up-regulation of CD59 expression. *J Immunol.* 2000; 164:3764–3773. [PubMed: 10725736]
40. Grehan JF, Levay-Young BK, Benson BA, et al. Alpha Gal ligation of pig endothelial cells induces protection from complement and apoptosis independently of NF-kappa B and inflammatory changes. *Am J Transplant.* 2005; 5:712–719. [PubMed: 15760394]

41. Grehan JF, Levay-Young BK, Fogelson JL, et al. IL-4 and IL-13 induce protection of porcine endothelial cells from killing by human complement and from apoptosis through activation of a phosphatidylinositol 3-kinase/Akt pathway. *J Immunol.* 2005; 175:1903–1910. [PubMed: 16034134]
42. Black SM, Grehan JF, Rivard AL, et al. Porcine endothelial cells and iliac arteries transduced with Aden-oIL-4 are intrinsically protected, through Akt activation, against immediate injury caused by human complement. *J Immunol.* 2006; 177:7355–7363. [PubMed: 17082655]
43. Black SM, Schott ME, Benson BA, et al. Interleukin-4 induces lipogenesis in porcine endothelial cells, which in turn is critical for induction of protection against complement-mediated injury. *Transplant Proc.* 2008; 40:638–640. [PubMed: 18374150]
44. Black SM, Schott ME, Batdorf BH, et al. IL-4 induces protection of vascular endothelial cells against killing by complement and melittin through lipid biosynthesis. *Eur J Immunol.* 2010; 40:803–812. [PubMed: 20017192]
45. Navarro-Alvarez N, Yang YG. CD47: a new player in phagocytosis and xenograft rejection. *Cell Mol Immunol.* 2011; 8:285–288. [PubMed: 21258362]
46. Fisicaro N, Londrigan SL, Brady JL, et al. Versatile co-expression of graft-protective proteins using 2A-linked cassettes. *Xenotransplantation.* 2011; 18:121–130. [PubMed: 21496119]
47. Deng W, Yang D, Zhao B, et al. Use of the 2A peptide for generation of multi-transgenic pigs through a single round of nuclear transfer. *PLoS ONE.* 2011; 6:e19986. [PubMed: 21603633]
48. Pierson RN III, Dorling A, Ayares D, et al. Current status of xenotransplantation and prospects for clinical application. *Xenotransplantation.* 2009; 16:263–280. [PubMed: 19796067]
49. Cooper DK, Ayares D. The immense potential of xenotransplantation in surgery. *Int J Surg.* 2011; 9:122–129. [PubMed: 21059418]
50. Schuurman HJ. Xenotransplantation: from the lab to the clinic. *Clin Transplant.* 2011; 25:E415–E421. [PubMed: 21535153]
51. Wynyard S, Garkavenko O, Elliot R. Multiplex high resolution melting assay for estimation of Porcine Endogenous Retrovirus (PERV) relative gene dosage in pigs and detection of PERV infection in xenograft recipients. *J Virol Methods.* 2011; 175:95–100. [PubMed: 21545811]
52. Denner J. Infectious risk in xenotransplantation – what post-transplant screening for the human recipient? *Xenotransplantation.* 2011; 18:151–157. [PubMed: 21696444]
53. Black SM, Benson BA, Idossa D, et al. Protection of porcine endothelial cells against apoptosis with interleukin-4. *Xenotransplantation.* 2011; 18:343–354. [PubMed: 22168141]
54. Chatelais M, Devalliere J, Galli C, Charreau B. Gene transfer of the adaptor Lnk (SH2B3) prevents porcine endothelial cell activation and apoptosis: implication for xenograft's cytoprotection. *Xenotransplantation.* 2011; 18:108–120. [PubMed: 21496118]
55. Glenn JC, Gordon TJ, Florescu E. State of the future: The Millenium Project. 2011