

Th1/Th2 XENOGENIC ANTIBODY RESPONSES ARE ASSOCIATED WITH RECIPIENT DENDRITIC CELLS

NOBUYUKI KANAI, M.D.,¹⁻³ WEI-PING MIN, M.D.,²⁻⁶ THOMAS E. ICHIM, Ph.D.,^{1,2} HAO WANG, M.D.,^{2-5*} and ROBERT ZHONG, M.D.¹⁻⁶

We characterized dendritic cells (DC) phenotypically and functionally between C57BL/6 (Th1-prone) and BALB/c (Th2-prone) mouse recipients in an *in vivo* sensitization model. Two strains of mice were presensitized with Lewis rat splenocytes as xenogeneic antigens. We found that BALB/c recipients mounted a significantly higher total IgG response to the xeno-antigens when compared with C57BL/6 recipients, 10 days after rat splenocyte infusion. A Th2-mediated antibody response with high ratio of IgG1/IgG2a was seen in the BALB/c recipients, while a Th1 antibody response with low ratio of IgG1/IgG2a was detected in C57BL/6 recipients. CD11c⁺ DC isolated from C57BL/6 recipients possessed increased expression of CD8 α ⁺ (DC-1 type). The administration of bone marrow derived-DC from IL-12 knockout mice into C57BL/6 recipients induced a shift of Th-mediated anti-xenogeneic antibody responses from Th1 to Th2 domain. Our findings suggest that DC could play an important role to regulate the balance of Th1/Th2 cytokine profiles and rejection patterns in xenotransplantation. © 2007 Wiley-Liss, Inc. *Microsurgery* 27:234–239, 2007.

Xenotransplantation is one of the novel approaches presently being sought to solve the problems of the severe organ shortage. Pig donors possess several advantages for this purpose, including defined phenotype and physiological similarities with human organs, and the ability to raise the animals under germ-free conditions.¹ Unfortunately, xenotransplantation is limited by aggressive immune responses when cells are transplanted across species. In discordant transplantation, a severe immunological attack termed hyperacute rejection (HAR) occurs immediately following xenografting. This problem has been sufficiently solved through the use of human decay accelerating factor (hDAF) transgenic pigs.² Nevertheless, hDAF transgenics still evoke acute vascular rejection (AVR). Concordant models of xenotransplantation, such as rat to mouse, do not induce HAR, however, AVR is still present.³ Therefore, a better understanding of the mechanisms of AVR is important and necessary in the future development of xenotransplantation as a therapeutic option.

The Th1/Th2 paradigm has been conventionally used to categorize immune responses into cell mediated (associated with IFN- γ and IL-12) and antibody mediated

(associated with IL-4 and IL-10) reactions.⁴ Although there is controversy in allotransplantation, Th1 responses are classically associated with accelerated graft rejection, whereas Th2 responses are associated with enhanced survival and in some cases of tolerance.⁵ In the context of concordant xenotransplantation, we have previously made the surprising observation that Th1 responses are associated with prolonged xenograft survival, whereas Th2 responses are associated with accelerated rejection.⁶ When Lewis rat cardiac allografts were placed into the Th1-prone C57BL/6 recipients, there was a prolonged survival when compared with Th2-prone BALB/c recipients. Ablation of the Th1 cytokines IL-12 or IFN- γ from C57BL/6 recipients endowed them with accelerated rejection.

Another characteristic of Th1/Th2 responses is the antibody isotype produced. Although Th1 responses are usually cellular mediated, antibodies albeit at lower levels are also produced. In the murine system, Th1-associated antibody responses are of the IgG2a isotype, whereas Th2-associated responses are of the IgG1 isotype.⁷ It is believed the IgG2a allows for more potent complement activation, as well as opsonization and antibody dependent cellular cytotoxicity (ADCC) than IgG1.⁸ Thus the antibody responses during Th1 activation synergize with the activated T cells to allow more effective clearance of the pathogen. We have confirmed that animals predisposed to Th1 responses produce higher titers of anti-xeno IgG2a in comparison to xenograft recipients predisposed to Th2 responses.⁹

Control of the naive T cell into Th1/Th2 polarization is maintained at the level of dendritic cells (DC), which provide T cells with costimulatory signals, such as CD80/CD86, and soluble signals, such as IL-10/IL-12. The combination of these signals programs the differentiation path of the naive T cell. For example, lack of CD80/86, will cause default of the T cell into Th2 differentiation.¹⁰

¹Robarts Research Institute, London, ON, Canada

²Multi-Organ Transplant Program, London Health Sciences Centre, London, ON, Canada

³Department of Surgery, The University of Western Ontario, London, ON, Canada

⁴Lawson Health Research Institute, London, ON, Canada

⁵Department of Microbiology and Immunology, The University of Western Ontario, London, ON, Canada

⁶Department of Pathology, The University of Western Ontario, London, ON, Canada

*Correspondence to: Dr. Hao Wang, Department of Surgery, London Health Sciences Centre-University Hospital, 339 Windermere Road, P.O. Box 5339, London, Ontario, Canada N6A 5A5. E-mail: hwang1@uwo.ca

Received 11 March 2007; Accepted 14 March 2007

Published online 3 May 2007 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/micr.20342

A strong IL-12 signal from the DC will cause Th1 differentiation. Accordingly, cytokines produced by DC also act as autocrine signals, for example, IL-12 acts as an autocrine signal during the stimulation of DC maturation, whereas IL-10 also acts in an autocrine manner in immature DC to maintain their immaturity.¹¹ DC from IL-12 knockout mice have been previously described as Th2 inducing,¹² and may serve as an ideal tool for modifying Th1/Th2 responses in vivo. Indeed we have previously demonstrated that silencing the IL-12 p35 gene, using siRNA, gives rise to Th2-promoting DC that secrete high levels of IL-10 and induce the differentiation of naive T cells into high IL-4 and low IFN- γ secretors.¹³

In the current study, we sought to examine immunological reactions during concordant anti-xeno responses, using a model of splenocyte sensitization. Lewis rat splenocytes were injected into BALB/c or C57BL/6 mice, and recipient anti-xeno antibody responses were evaluated for titer and isotype using a previously described assay.⁶ We demonstrate that: 1) The xeno-sensitization model is a simple and reliable system for investigating anti-xeno antibody responses without needing to perform the procedure of organ transplantation; 2) The Th1-prone strain of C57BL/6 mice produced lower overall anti-xeno antibodies but higher IgG2a in comparison with the Th2-prone strain of BALB/c mice; and 3) Administration of Th2-promoting IL-12 knockout DC into the C57BL/6 mice resulted in enhanced overall anti-xeno antibody titers and caused higher production of the Th2-associated IgG1 isotype.

MATERIALS AND METHODS

Animals

Lewis rats (RT1) 200–250 g, BALB/c (H-2d), and C57BL/6 (H-2b) 25–30 g mice were purchased from The Jackson Laboratory (Bar Harbor, Maine). IL-12p40 knockout C57BL/6 mice (C57BL/6-IL-12btm1jm) were kindly provided by Dr. D.J. Kelvin. Animals were housed under pathogen-free conditions at the Animal Care Facility, University of Western Ontario. They were cared for in accordance with the guidelines established by the Canadian Council on Animal Care.¹⁴

Presensitized Model

Splenocyte suspensions were prepared by harvesting spleen from Lewis rats in RPMI 1640 medium supplemented. BALB/c and C57BL/6 mice were presensitized with an intravenous injection of 20×10^6 rat splenocytes in phosphate buffer saline (PBS). 10–14 days after injection of rat splenocytes, whole blood was collected, sera were purified by centrifugations, and stored at -70°C until use.

Isolation of Mouse Splenocytes and Bone Marrow DC Culture

For purification of splenic DC, spleens were harvested from BALB/c and C57BL/6 mice (nontreated and presensitized), mechanically dissociated, subjected to hypotonic erythrocyte lysis, and resuspended in PBS (GIBCO Life Technologies, Burlington, Ontario, Canada) with 10% FCS (GIBCO), 0.5% EDTA (GIBCO). Purification of CD11c⁺ DC was performed using magnetic activated cell sorting (MACS; Miltenyi BioTech, Germany) according to the manufacturer's instructions. Bone marrow derived-DC (BM-DC) were generated as originally described by Inaba et al.¹⁵ with modifications.¹⁶ By day 4–6 of culture, nonadherent cells and loosely adherent proliferating DC aggregates were seen. After 6–7 day DC culture, BM-DC from IL-12KO mice were collected and used for FACS analysis, and then were injected into C57BL/6 mice.

Flow Cytometry

As described by Kodaira et al.,¹⁷ 1×10^6 cells were incubated in buffer (PBS with 2% FBS and 0.1% sodium azide) were stained with the following mAbs and analyzed by FACScan, using Cell Quest software (Becton Dickinson, San Jose, CA). FACS cell sorter was used for cell sorting (Becton Dickinson). The mAbs for cell staining, including anti-mouse H-2b, or H-2d, anti-DEC205, anti-mouse CD11c, anti-mouseCD8 α , anti-mouse IL-12 (BD Biosciences, San Diego, CA), were conjugated with either FITC, or PE, or Cy. Xeno-reactive antibody (xAb) levels (mouse anti-rat Immuno-globulin) were also measured using flow cytometry, as described by Wang et al.⁶ Cells were stained with the following FITC-coated antibodies: anti-mouse IgM, IgG, IgG1, IgG2a conjugated with FITC (BD Biosciences, San Diego, CA), and then analyzed by flow cytometry. Antibody titers were quantified as mean fluorescent intensity.

Statistical Analysis

The data were reported as mean \pm SD. The serum antibody levels and the phenotype of DC were analyzed, using student's *t*-test and the one-way ANOVA. Differences with *P* values less than 0.05 were considered significant.

RESULTS

Different Anti-Xeno Antibody Responses Between Two Different Strains of BALB/c and C57BL/6 Mice

Our previous work suggests that anti-xenogenic responses are more potent when the recipient possesses a Th2 predisposition (unpublished data). In light of this, we choose to investigate whether the heightened antibody

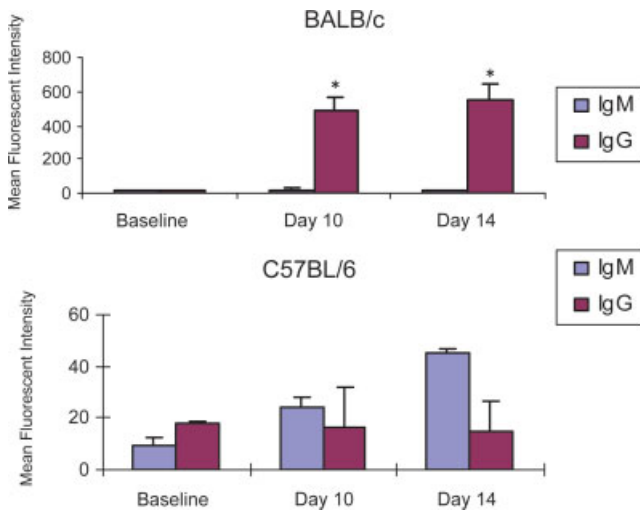


Figure 1. Comparison of anti-xeno antibody responses between BALB/c and C57BL/6 strains. After presensitization with xenogeneic antigen, spleen cells of BALB/c and C57BL/6 recipients were analyzed by flow cytometry as described in the Materials and Methods section. BALB/c recipients showed significantly higher total IgG response compared with C57BL/6 in 10 and 14 days after splenocyte infusion. The bars show the mean \pm SD of three independent experiments. (* $P < 0.05$). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

response in the Th2-prone mouse strain also occurs in a model of presensitization. The immune responses to xenoantigens can be directed toward both MHC, and non-MHC elements, since in contrast to allotransplantation, almost every non-species conserved protein can be immunogenic. We therefore intend to use a model system that will allow the detection of antibody responses to all antigens, in which the recipient is recognizing the antigens on the xenotransplanted cells.

The rat splenocyte used to sensitize murine recipients is a convenient, easily accessible tissue that possesses many characteristic xenoantigens that would be found on other organs.¹⁷ Both BALB/c and C57BL/6 mice tolerated the intravenous infusion of 20×10^6 rat splenocytes without developing anaphylaxis or alteration of behavior. Overall levels of anti-xeno antibodies were approximately 10-fold higher in the BALB/c as opposed to the C57BL/6 recipient, both on day 10 and day 14 after sensitization (Fig. 1). The BALB/c responses consisted predominantly of IgG antibody, while the C57BL/6 response was characterized by elevated IgM. This may suggest that in the C57BL/6 recipient, the xeno-antigens increased production of natural antibodies, which are typically known to be of the IgM class.¹⁸ In contrast, the IgG predominance in the BALB/c recipients, along with the robustness of the response seems to indicate that class switching had occurred in responses to the xeno-antigenic challenge.

Different Isotype of Anti-Xeno Antibody Between BALB/c and C57BL/6 Mouse Strains

Antibody responses mediate effector functions through several mechanisms, including Th1 associated functions such as complement fixation and ADCC, as well as, Th2 associated functions such as direct inactivation of toxins, or prevention of pathogen adhesion to host epithelium. In regard to the IgG class, IgG2a is more effective at the Th1-mediated functions, whereas IgG1 is predominant at the Th2-mediated functions. We therefore investigated the isotypic profiles of the anti-IgG responses that are occurring as a result of xeno-sensitization. The BALB/c strain consistently produced higher levels of IgG1 at both days 10 and 14 after sensitization. In contrast, the antibody response in C57BL/6 recipients was characterized by elevated levels of IgG2a, predominantly on day 14 postimmunization (Fig. 2). These data suggest that this xeno-sensitization model can serve as a practical and convenient surrogate for whole organ transplantation in the investigation of xenograft rejection. This notion is supported by the fact that the present observations mimicking our previous findings in a whole organ xenotransplant model in terms of antibody titer, class of antibody, and isotype of antibody.⁶

Different Phenotypes DC Between BALB/c and C57BL/6 Strains

Since DC are associated with stimulation of immune responses and control both T and B cell function, we assessed the possibility that DC changes were occurring in the spleen as a result of xeno-sensitization. Using magnetic purification, splenic cells were purified for the DC marker CD11c. When these cells were assessed for production of the Th1 promoting cytokine IL-12, measurements of IL-12 by intracellular staining revealed that there was no difference between xenosensitized or naive BALB/c or C57BL/6 mice. As $CD8\alpha^+CD11c^+$ DC were reported to be Th1-promoting DC, we decided to examine whether there was any alteration in the numbers of these cells before and after xenosensitization and whether this alteration would be different between BALB/c and C57BL/6 strains. At day 10 postxenosensitization, there was an increase in the number of $CD8\alpha^+$ DC in the spleens of both recipient strains, but with a significantly higher increase ($P < 0.05$) in the C57BL/6 mice (Fig. 3). Although this observation is only correlative, it prompted us to ask whether DC may play a role in the ability of the recipient to respond to various xenoantigenic challenges.

Alteration of Anti-Xeno Responses by Administration of Th2-Promoting DC

Due to the critical role of DC in orchestrating both T cell and B cell responses, we asked whether preadminis-

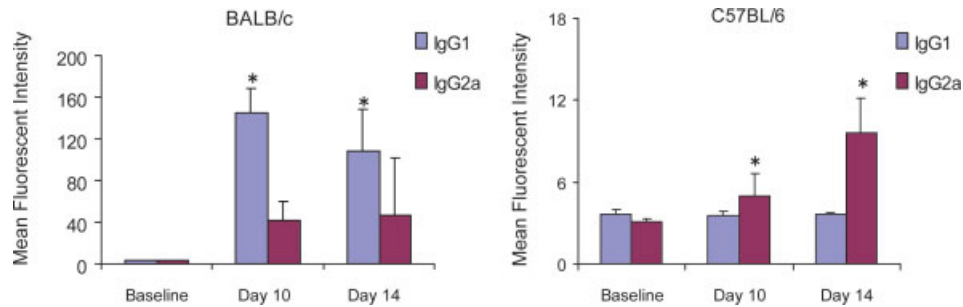


Figure 2. Comparison of IgG isotypes of anti-xeno antibody between BALB/c and C57BL/6. A Th2 mediated antibody response (high ratio of IgG1/IgG2a) was seen in the BALB/c recipients, while a Th1 antibody response (low ratio of IgG1/IgG2a) was seen in C57BL/6 recipients, after presensitization with xenogeneic antigen on day 10 and 14. The bars show the mean \pm SD of three independent experiments. (* $P < 0.05$). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

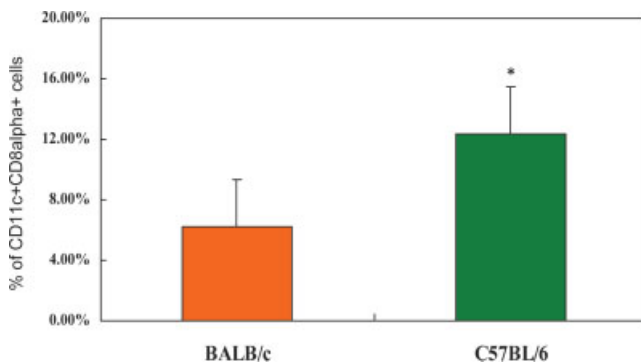


Figure 3. Phenotypes of DC in BALB/c and C57BL/6 mice. DC isolated from C57BL/6 recipients on day 10 after presensitization with xenogeneic antigen showed significantly increased expression of CD11c+CD8 α + (DC-1 type) cells (* $P < 0.05$ by student's *t*-test). The data show mean \pm SD and are representative of three independent experiments. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

tration of syngeneic Th2-promoting DC before xenosensitization could alter the profile of the anti-xeno response in C57BL/6 recipients. The low levels of antibodies, as well as propensity to produce anti-xeno IgM and IgG2a are characteristic of a Th1-prone recipient. By administering Th2-promoting DC before the xenosensitization, we would anticipate that the exogenous DC would possess characteristics that overcome the endogenous Th1-predisposition of the C57BL/6 recipient. Previously we have reported that silencing the IL-12 gene, using siRNA on DC, gives rise to a population of Th-2 promoting DC that are active both in vitro and in vivo. In the present study we choose IL-12 knockout DC as a prototypic Th2-promoting DC based on previous reports that these DC can stimulate Th2 responses¹⁹ and secrete higher levels of IL-10.²⁰ Intravenous administration of 10^6 CD11c+ BM-DC from either IL-12 knockout or wild-type mice was well tolerated, with no adverse effects observed (data not shown). When wild-type DC were administered to C57BL/6 recipients 3 days before xenosensitization, the

anti-xeno IgM and IgG levels were approximately identical to xenosensitized recipients that did not receive DC infusion. In contrast, administration of IL-12 knockout DC resulted in a marked upregulation of the IgG antibody production ($P < 0.05$) (Fig. 4A).

This suggests that the IL-12 knockout DC possessed a potent immune modulating function that could have been the cause of class-switching of the anti-xeno response. Additional experiments were performed to evaluate whether administration of the IL-12 knockout DC altered the isotype of the response. Administration of wild-type DC resulted in a minute but significant upregulation of IgG1 anti-xeno antibodies. In contrast, when IL-12 knockout DC were administered a marked (>5-fold) increase in IgG1 was observed ($P < 0.05$) (Fig. 4B). These results suggest that administration of the Th2 promoting IL-12 knockout DC can potentially switch the anti-xeno antibody response from characteristic Th1-response produced by C57BL/6 mice into a Th2-like response as typically mounted by BALB/c recipients. Additionally, the observation of this antibody switch confirms that Th2 promoting DC possesses a predominant role in the initiation of immune response because of their ability to overcome the inherent Th1-bias of C57BL/6 mice.

DISCUSSION

We have previously demonstrated that silencing of the IL-12 gene in DC using siRNA renders these cells high IL-10 producers, and endows the ability to stimulate Th2 responses both in vitro and in vivo.¹³ We have also demonstrated that in the context of xenotransplantation the Th1/Th2 predisposition of recipients results in altered antibody responses to the xenograft.⁶ In the present study, we have determined two issues, that being: 1) whether this newly developed xenosensitization model is much easier and more convenient for xenotransplant studies and provides similar results to whole organ transplantation in terms of immunological responses; and 2) whether

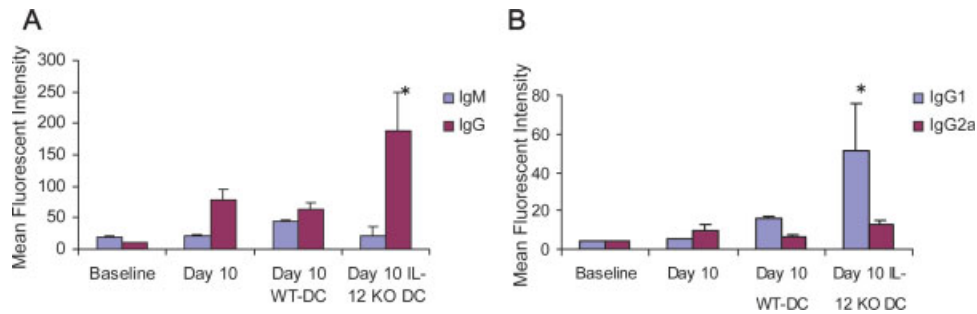


Figure 4. (A) Alteration of anti-xeno responses by administration of Th2-promoting DC. The administration of bone marrow derived DC from IL-12 knockout mice into C57BL/6 recipients induced significantly higher IgG production on day 10 compared with C57BL/6 injected DC from wild type mice. The data represent mean \pm SD and are representative of three independent experiments. (* $P < 0.05$). **(B)** IgG isotypes of anti-xeno antibody of C57BL/6 after Th2-

promoting DC injection. The administration of bone marrow derived DC from IL-12 knockout mice into C57BL/6 mice showed significantly higher response of IgG1. These data from Figures 4 and 5 suggest a shift in Th1 to Th2 pattern of anti-xenogeneic antibody production in C57BL/6 mice. The bars show mean \pm SD of three independent experiments. (* $P < 0.05$). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

administration of IL-12 knockout DC predominate over the inherent Th1 predisposition of C57BL/6 recipients and alter antibody responses, thereby indicating the potential effects of DC transfer in modulation of xeno-immune responses.

We found that murine recipients tolerate rat splenocyte infusions without toxicity or adverse effects. Furthermore, the Th1 associated antibody profile of low overall antibody production, little class switching, and IgG2a isotype predominance was detected in the xenosensitized C57BL/6 recipients. In the meanwhile, the converse was observed in the Th2-prone BALB/c recipients. The verification that this xenosensitization model yields similar results to the whole organ xenotransplant model implies this model can be used for future studies of immunological responses to xenoantigens without the need of the complex procedure of whole organ xenotransplants.

DC control of immunological functions is established both for T cells and B cells. DC production of soluble (i.e., IL-10, IL-12) and contact-dependent (i.e., CD40, CD80, CD86, PD-1L) signals stimulate/inhibit T cell activation, as well as control whether the naive T cells will differentiate into Th1/Th2 or T regulatory cells.²¹ These T cells then interact with B cells to dictate the antibody response that will ensue. DC can also directly modulate B cell activation through secretion of factors such as BLYS.²² It has been demonstrated that DC from BALB/c mice preferentially stimulate Th2 responses, whereas DC from C57BL/6 mice preferentially stimulate Th1 responses.²³ The second goal of this study was to determine whether priming of an antibody response to xenoantigens could be altered by the preadministration of cytokine-biased DC. The IL-12 knockout DC were indeed able to raise the amount of class-switching that was occurring in response to the xenoantigen, as well as influence the isotypic profile of the responses, making it Th2-

like. Our results possess several implications. First, the fact that the DC were administered before the xenogenic challenge implies that they were interacting with the xenoantigen directly, or biasing other immunological cells that have already been activated. Studies are ongoing to determine whether the IL-12 knockout DC can be administered concurrently with xenogenic challenge and even after challenge to induce the antibody profile. Second, because we were able to increase both the titer and isotype of antibody using the IL-12 knockout DC, this strategy may be useful in other contexts (i.e., in vaccination) to elicit a desired immunological response. Finally, the verification of IL-12 as a key target, and the DC as a key cell in modulating immune responses in vivo strengthens the basis of ongoing efforts for immune modulation using these components. In conclusion, our successful achievement of immunomodulation in the xenogenic setting demonstrates that DC manipulation is a feasible and simple strategy for programming systemic antibody responses.

ACKNOWLEDGMENTS

The authors would like to thank Xuyan Huang for technical assistance, Drs. David White and Gill Strejan for critical discussion, and Mrs. Sharon Mutch for secretarial support.

REFERENCES

- Platt JL. New directions for organ transplantation. *Nature* 1998;392 (Suppl):11–17.
- Kanai N, Platt JL. Xenotransplantation of the liver. In: Holland K, editor. *Clinics in Liver Disease*, Vol. 4, No. 3. Philadelphia, PA: WB Sanders; 2000. pp 731–746.
- Zhang Z, Bedard E, Luo Y, Wang H, Deng S, Kelvin D, Zhong R. Animal models in xenotransplantation. *Exp Opin Invest Drugs* 2000;9:2051–2068.

4. Kourilsky P, Truffa-Bachi P. Cytokine fields and the polarization of the immune response. *Trends Immunol* 2001;22:502–509.
5. Bishop DK, Wood SC, Eichwald EJ, Orosz CG. Immunobiology of allograft rejection in the absence of IFN- γ : CD8⁺ effector cells develop independently of CD4⁺ cells and CD40-CD40 ligand interactions. *J Immunol* 2001;166:3248–3255.
6. Wang H, DeVries ME, Deng S, Khandaker MH, Pickering JG, Chow LH, Garcia B, Kelvin DJ, Zhong R. The axis of interleukin 12 and γ interferon regulate acute vascular xenogeneic rejection. *Nat Med* 2000;6:549–555.
7. Pulendran B, Smith JL, Caspary G, Brasel K, Pettit D, Maraskovsky E, Maliszewski CR. Distinct dendritic cell subsets differentially regulate the class of immune response in vivo. *Proc Natl Acad Sci USA* 1999;96:1036–1041.
8. Shyur SD, Raff HV, Bohnsack JF, Kelsey DK, Hill HR. Comparison of the opsonic and complement triggering activity of human monoclonal IgG1 and IgM antibody against group B streptococci. *J Immunol* 1992;148:1879–1884.
9. Dujovny N, Varghese A, Shen J, Yin D, Ji S, Ma L, Finnegan A, Chong AS. Acute xenograft rejection mediated by antibodies produced independently of TH1/TH2 cytokine profiles. *Am J Transplant* 2002;2:526–534.
10. Guermonprez P, Valladeau J, Zitvogel L, Thery C, Amigorena S. Antigen presentation and T cell stimulation by dendritic cells. *Annu Rev Immunol* 2002;20:621–667.
11. Corinti S, Albanesi C, Sala AL, Pastore S, Girolomoni G. Regulatory activity of autocrine IL-10 on dendritic cell functions. *J Immunol* 2001;166:4312–4318.
12. Schijns VECJ, Haagmans BL, Wierda CMH, Kruithof B, Heijnen IAFM, Alber G, Horzinek MC. Mice lacking IL-12 develop polarized Th1 cells during viral infection. *J Immunol* 1998;160:3958–3964.
13. Hill JA, Ichim TE, Kusznierek KP, Li M, Yan X, Zhong R, Claims E, Bell DA, Min WP. Immune modulation by silencing IL-12 production in dendritic cells using small interfering RNA. *J Immunol* 2003;171:691–696.
14. Olfert ED, Cross BM, McWilliam AA. Responsibility for the care and use of experimental animals. In: Olfert ED, Cross BM, McWilliam AA, editors. *Guide to the Care and Use of Experimental Animals*, Vol. 1. Ottawa, Canada: Association of Universities and Colleges of Canada; 1993. p 1.
15. Inaba K, Inaba M, Romani N, Aya H, Deguchi M, Ikehara S, Murmatsu S, Steinman RM. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J Exp Med* 1992;176:1693–1702.
16. Min WP, Gorczynski R, Huang XY, Kushida M, Kim P, Obataki J, Lei J, Suri RM, Catral MS. Dendritic cells genetically engineered to express Fas ligand induce donor-specific hyporesponsiveness and prolong allograft survival. *J Immunol* 2000;164:161–167.
17. Kodaira Y, Ikuta K, Tanaka S, Yokomuro K. Antigen-driven clonal accumulation of peritoneal gammadelta T cells in vivo. *Immunol Invest* 1999;28:137–48.
18. Yasutomi M, Ito M, Hayashi S, Ohtsuka S, Namii Y, Uchida K, Yokoyama I, Takagi H. Establishment of a concordant xenogeneic splenocyte injection model for the dynamic study of the marginal zone in the spleen. *J Heart Lung Transplant* 1998;17:452–459.
19. Platt JL. Xenotransplantation. *Sci Med* 1996;3:62–71.
20. Mattner F, Magram J, Ferrante J, Launois P, Di Padova K, Behin R, Gately MK, Louis JA, Alber G. Genetically resistant mice lacking interleukin-12 are susceptible to infection with *Leishmania major* and mount a polarized Th2 cell response. *Eur J Immunol* 1996;26:1553–1559.
21. Moser M, Murphy KM. Dendritic cell regulation of TH1-TH2 development. *Nat Immunol* 2000;1:199–205.
22. Jonuleit H, Schmitt E, Steinbrink K, Enk AH. Dendritic cells as a tool to induce anergic and regulatory T cells. *Trends Immunol* 2001;22:394–400.
23. Litinsky MB, Nardelli B, Hilbert DM, He B, Schaffer A, Casali P, Cerutt A. DCs induce CD40-independent immunoglobulin class switching through BLYS and APRIL. *Nat Immunol* 2002;3:822–829.