T-Cell-Based Immunosuppressive Therapy Inhibits the Development of Natural Antibodies in Infant Baboons

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Background. We set out to determine whether B-cell tolerance to A/B-incompatible alloantigens and pig xenoantigens could be achieved in infant baboons.

Methods. Artery patch grafts were implanted in the abdominal aorta in 3-month-old baboons using A/B-incompatible (AB-I) allografts or wild-type pig xenografts (pig). Group 1 (Gp1) (controls, n=6) received no immunosuppressive therapy (IS) and no graft. Gp2 (n=2) received an AB-I or pig graft but no IS. Gp3 received AB-I grafts+IS (Gp3A: n=2) or pig grafts+IS (Gp3B: n=2). IS consisted of ATG, anti-CD154mAb, and mycophenolate mofetil until age 8 to 12 months. Gp4 (n=2) received IS only but no graft.

Results. In Gp1, anti-A/B and cytotoxic anti-pig immunoglobulin-M increased steadily during the first year. Gp2 became sensitized to donor-specific AB-I or pig antigens within 2 weeks. Gp3 and Gp4 infants that received anti-CD154mAb made no or minimal anti-A/B and anti-pig antibodies while receiving IS.

Discussion. The production of natural anti-A/B and anti-pig antibodies was inhibited by IS with anti-CD154mAb, even in the absence of an allograft or xenograft, suggesting that natural antibodies may not be entirely T-cell independent. These data are in contrast to clinical experience with AB-I allotransplantation in infants, who cease producing only donor-specific antibodies.

Keywords: ABO-incompatible, Antibodies, Natural, Immunosuppressive therapy, Baboon, Infant, Xenotransplantation.

(Transplantation 2012;93: 769–776)

A major hurdle in transplantation (Tx) is the long waiting time to obtain a donor organ. This problem is particularly striking in infants with congenital heart defects, who often require heart Tx at a young age (*1*). The pig could provide an alternative source of organs if the immunologic barriers could be overcome (*2*). The initial barrier is related to the presence of natural (preformed) antibodies (Abs) in the recipient directed to antigens on the vascular endothelium of the pig organ (*3*, *4*). Ab binding initiates activation of the complement cascade, resulting in hyperacute rejection (*5*, *6*).

E.M.D. is the recipient of fellowships from the Ter Meulen Fund of the Royal Netherlands Academy of Arts and Sciences and the Stichting Professor Michael van Vloten Fund, The Netherlands. The work on xenotransplantation in the Thomas E. Starzl Transplantation Institute of the University of Pittsburgh is supported in part by NIH grants no. U01 AI068642, no. R21 A1074844, and no. U19 A1090959, and by Sponsored Research Agreements between the University of Pittsburgh and Revivicor, Inc., Blacksburg, VA. The baboons were provided by the University of Oklahoma Health Sciences Center, which is supported in part by NIH P40 sponsored grant RR012317-09.

The authors declare no conflicts of interest.

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E.M.D. and C.M. designed and performed the experiments, analyzed data, were responsible for in vivo management of infant baboons, and wrote the manuscript; C.E.L. and H.H. performed experiments and contributed to the manuscript; G.J.E. and B.E. carried out transplants and managed infant baboons; C.E., D.R.M., and D.J.v.d.W. performed experiments and managed infant baboons; N.M. carried out transplants; L.H.R. performed histological studies; R.W. and R.F.W. supervised in vivo management of infant baboons; M.E. designed experiments and supervised transplantations; L.J.W. and J.N.M.I. contributed to interpretation of data and supervised manuscript; and D.K.C.C. supervised the experiments, in vivo management, interpretation of data, and writing of the manuscript. All authors reviewed the manuscript.

Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantjournal.com).

Received 9 November 2011. Revision requested 1 December 2011.
Accepted 22 December 2011.

ISSN 0041-1337/12/9308-769
DOI: 10.1097/TP.0b013e3182481168

Natural immunoglobulin (Ig)-M Abs develop during infancy, a process believed to be associated with colonization of the gastrointestinal tract with bacteria/viruses that express galactose-α1,3-galactose (Gal) antigens (*7*–*9*). This natural Ab production is considered to be T-cell independent (*10*), although there is some evidence that it may be T-cell dependent (*11*). The development of anti-Gal Abs is similar to that of other Abs directed to carbohydrate antigens, for example, the A and B blood group antigens (*12*). Natural anti-A/B Abs are usually absent during the first 3 months of life in humans and baboons but subsequently develop during the first year (*9*, *13*). The relative absence of Abs during the first few months has provided a "window of opportunity" during which AB-incompatible (AB-I) organ Tx can be carried out successfully (*14*). Infants that received an AB-I organ did not reject the graft, and subsequently developed donor-specific B-cell tolerance, defined by the absence of donor-specific Abs in the presence of a graft and normal development of nonspecific Abs, which was confirmed by a negative antidonor agglutination titer and enzyme-linked immunosorbent spot (ELISPOT) (*15*, *16*).

Anti-pig Abs are primarily directed against Gal antigens expressed on pig cells, which share their core structure with the ABO antigens (*12*). Similar to anti-A/B Abs, Abs to wild-type (WT) pig cells in human and baboon infants do not develop until approximately 3 months of age (*9*). Early studies demonstrated that hyperacute rejection does not occur after WT pig heart Tx into untreated newborn baboons (*17*).

We hypothesized that if an infant received a pig organ graft before the development of natural anti-pig Abs, these Abs might never develop, and B-cell tolerance to the pig graft would result. We have investigated this by carrying out WT pig or AB-I baboon artery patch Tx in baboons of 3 months of age. We could not confirm that this hypothesis is correct; however, all baboons that received anti-CD154mAb-based immunosuppressive therapy (IS), irrespective of the presence or source of a graft, showed inhibited development of both anti-pig and anti-A/B Abs compared with their age-matched controls, suggesting that natural Abs are T-cell dependent.

RESULTS

Group 1: Naïve Controls (n=6)

All naïve controls (Table 1) remained healthy and showed steady weight gain during the study.

All antiself-blood-group optical density values remained at less than 0.2, which we regarded as absent or undetectable Ab. All infants showed undetectable anti-AB-I (nonself) and anti-Gal IgM levels at 1 month of age, followed by a gradual increase of AB-I IgM and a more rapid increase of anti-Gal IgM; both could be detected by 4 months (Fig. 1A). Initial high anti-Gal IgG (but not anti-AB-I IgG) levels were detected in three infants (at 1–3 months), which was likely due to maternal IgG; at later ages, anti-Gal IgG steadily fell throughout the period of follow-up until undetectable after 15 months, whereas anti-AB-I IgG remained undetectable (Fig. 1B). No significant differences in Ab levels were noted between those housed in the specific pathogen-free facility at University of Oklahoma Health Sciences Center (UOHSC) (n=4) and those at the University of Pittsburgh (UPitt) (n=2) (not shown).

Flow cytometry confirmed the enzyme-linked immunosorbent assay (ELISA) data; all infants showed a gradual increase in IgM binding to porcine peripheral blood mononuclear cells (pPBMC) (Fig. 1C) and porcine aortic endothelial cells (pAEC) (not shown). IgG levels were high in some infants after birth but decreased over time (Fig. 1C).

Cellular responses of control and transplanted infants are discussed in the **Supplemental Results** (see **SDC,**

TABLE 1. Experimental groups

Groups	Baboon	Blood group	Graft type	IS (mo)	Tx at age (d)	Survival (age, mo)
1. Control (NO graft, NO IS)	4908	A	—	—	—	>22
	5108	A	—	—	—	>22
	12508	A	—	—	—	>16
	12708	B	—	—	—	>16
	12808	B	—	—	—	>16
	13408	B	—	—	—	>16
2. AB-I or WT (graft, NO IS)	7707	B	A	—	95	4[a]
	7607	B	Pig	—	102	4[a]
3A. AB-I (graft+IS)	7507[b]	B	A	3–12	98	13[a]
	5008[b]	A	B	3–8	107	>22
3B. Xeno (graft+IS)	5508[b]	B	Pig	3–8	98	8[a]
	5708[b]	B	Pig	3–8	87	>22
4. IS (NO graft)	209[c]	A	—	1–8	—	>14
	309[d]	B	—	1–8	—	>14

[a] Euthanized or died.
[b] Group 3 infants: immunosuppressive therapy: full regimen with ATG induction and maintenance anti-CD154 mAb+MMF.
[c] Group 4 infant B209: CTLA4-Ig only.
[d] Group 4 infant B309: anti-CD154 mAb only.
mAb, monoclonal antibody; Ig, immunoglobulin; Gal, galactose-α1,3-galactose; AB-I, A/B-incompatible; WT, wild type; IS, immunosuppressive therapy; Tx, transplantation; MMF, mycophenolate mofetil; Gp, group.

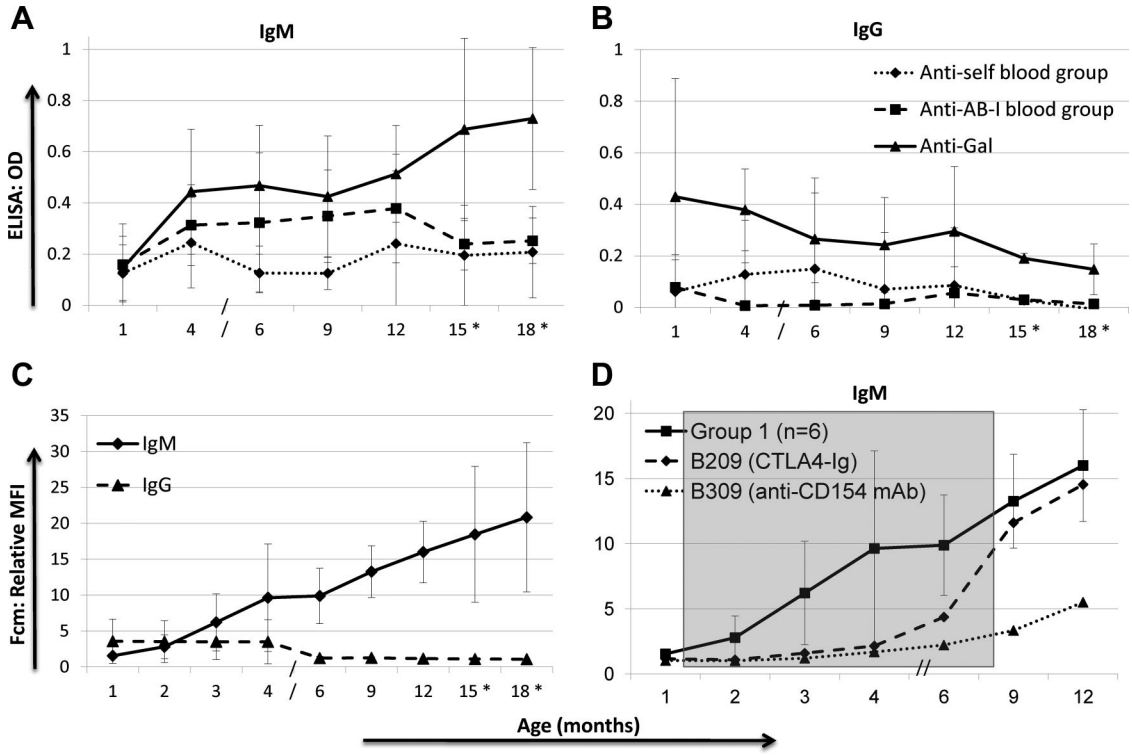

FIGURE 1. Anti-A, -B, -Gal, and -pig serum antibody levels in group 1 (n=6) and anti-pig levels in group 4 (n=2). (A) IgM levels (mean±SD) of blood group antiself, anti-AB-I, and anti-Gal, showing undetectable levels of anti-AB-I and anti-Gal at 1 month of age, comparable with antiself (optical density<0.2), and thereafter slowly increasing levels of anti-AB-I IgM and a more rapid increase in anti-Gal IgM with increasing age. (B) IgG levels (mean±SD) of blood group antiself, anti-AB-I, and anti-Gal showing a relatively high level of anti-Gal IgG at 1 month of age, with a slow decrease with advancing age, suggesting that these were maternal IgG antibodies. AB-I IgG were undetectable throughout the period of study. (C) The ELISA data were confirmed by flow cytometry analysis of infant baboon serum binding to WT pig PBMC. IgM levels were increasing by 3 months of age and continued to increase thereafter. Anti-WT pig IgG levels remained low (largely undetectable) for the entire period of follow-up (*15 and 18 months: n=2). (D) The single anti-CD154mAb-treated infant (Gp4, B309) showed low levels of binding to WT pig PBMC that remained low, but gradually rose after discontinuation of IS. In contrast, the single CTLA4-Ig-treated infant (Gp4, B209) showed levels that increased slightly before discontinuation of IS and rose more rapidly after discontinuation of IS. Colored area indicates time of IS in both baboons. Ig, immunoglobulin; Gal, galactose-α1,3-galactose; ELISA, enzyme-linked immunosorbent assay; SD, standard deviation; AB-I, A/B-incompatible; OD, optical density; PBMC, peripheral blood monunclear cells; WT, wild type; IS, immunosuppressive therapy.

http://links.lww.com/TP/A651) and are detailed elsewhere (van der Windt et al., *Transpl Int*, 2012, accepted for publication).

Group 2: Artery Patch Tx Without IS (n=2)

One infant received an AB-I (B7707) and one a WT pig (B7607) graft at 3 months, with no IS (Table 1).

In B7707 (AB-I graft, A→B), on ELISA there was a strong increase in anti-A IgM and IgG but not of anti-B or anti-Gal Abs (Fig. 2). Flow cytometry showed no significant increase in IgM or IgG binding to WT pPBMC or pAEC post-Tx. In B7607 (pig graft), a strong increase in anti-Gal IgM and IgG but not in anti-A or anti-B was detected by ELISA (Fig. 2). On flow cytometry, a strong increase in IgM and IgG binding to pPBMC and of IgG binding to pAEC after Tx was seen (not shown), indicating sensitization to the graft.

Histologic examination of the grafts showed extensive inflammatory cell infiltrates in both infants (not shown).

Altogether, these data confirmed that sensitization specific to the donor type (AB-I or WT pig) had occurred.

Group 3: Artery Patch Tx and IS (n=4)

Four baboons received an artery patch Tx and IS (Table 1). All baboons showed normal weight gain after Tx, and follow-up was uncomplicated for 4 to 9 months. At that time, within the same period of 4 weeks, all baboons developed features of a colitis (diarrhea and weight loss), which necessitated discontinuation of IS; one died and one was euthanized, and the other two recovered and remained in follow-up (Dons et al., *Inflamm Bowel Dis*, 2012, accepted for publication).

T-Cell Kinetics

After thymoglobulin induction, there was a marked reduction in T-cell ($CD4^+$ and $CD8^+$) numbers in all baboons. The number of $CD4^+$T cells remained low throughout the course of IS, after which slow recovery began (Fig. 3A). The immunosuppressive regimen or transplant did not have significant influence on B-cell ($CD20^+$) numbers (Fig. 3B). Cellular proliferative responses are discussed in the **Supplemental Results** (see **SDC,** http://links.lww.com/TP/A651)

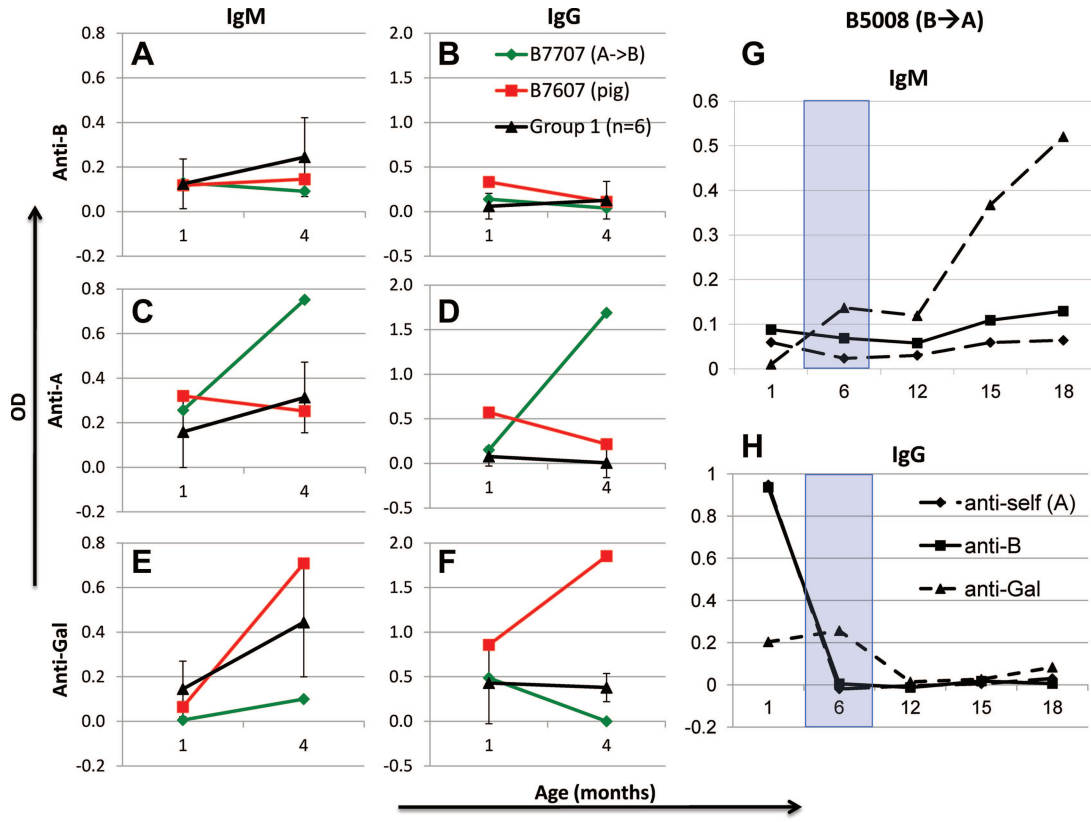

FIGURE 2. IgM and IgG anti-AB-I and anti-Gal antibodies measured by ELISA in group 2 baboons (n=2) and IgM levels in B5008 (group 3). In B7707 (AB-I graft, A→B), anti-A IgM (C), and IgG (D) increased rapidly post-Tx, whereas antiself-IgM (A) and IgG (B) and anti-Gal IgM (E) and IgG (F) remained low. In B7607 (blood group B, WT pig graft), antiself-IgM (A) and IgG (B), and anti-AB-I IgM (C) and IgG (D) remained low, whereas anti-Gal IgM (E) and IgG (F) strongly increased post-Tx. Donor-specific sensitization to the artery patch graft developed in these nonimmunosuppressed infant baboons. (G) In B5008, IS therapy was discontinued at 8 months. A delayed increase in anti-Gal IgM Ab was observed (beginning at 12 months, i.e., 4 months after discontinuation of IS), whereas there was an absence of AB-I (anti-B) IgM Abs for more than 10 months after discontinuation of IS, suggesting that a state of donor-specific B-cell tolerance may have been achieved. There was no increase in IgG levels (H). The colored area indicates the period of time during which B5008 received IS. Ig, immunoglobulin; Gal, galactose-α1,3-galactose; ELISA, enzyme-linked immunosorbent assay; AB-I, A/B-incompatible; WT, wild type; IS, immunosuppressive therapy; Tx, transplantation.

and are detailed elsewhere (van der Windt et al., accepted for publication).

Anti-A, -B, -Gal, and -Pig Abs

By ELISA and flow cytometry, all baboons showed no or minimal increase in anti-AB-I (Fig. 3C) or anti-Gal/pig (Fig. 3E) IgM Abs while IS was being administered, after which there was a slow increase in levels. In addition to the maternal IgG that was only detected pre-Tx, no anti-AB-I or anti-Gal/pig IgG Abs were detectable while IS was being administered. On ELISA, B5008 (AB-I graft, B→A) showed an absence of anti-B IgM Abs for more than 12 months after discontinuation of IS (Fig. 2G), whereas there was a delayed increase in anti-Gal IgM Ab (beginning 4 months after discontinuation of IS). Moreover, both anti-AB-I and anti-Gal IgG Abs remained undetectable long term after discontinuation of IS (Fig. 2H).

Histopathological Examination of the Aortic Patch Grafts

Microscopy of the Group 3 (Gp3) grafts showed that the two infants with AB-I grafts had developed some fibrosis in the connective tissue at the site of the graft, but there was no inflammatory cell infiltrate at the time of euthanasia (not shown). After pig xenografting, a minimal cell infiltrate was seen in B5508, which was euthanized shortly after discontinuation of IS, whereas the pig xenograft in B5708, which remained without IS for more than 13 months, contained numerous tortuous vascular channels suggesting previous thrombosis and recanalization, and one dense focus of inflammatory cells (not shown).

Staining of sections of native aorta and graft for anti-A, -B, and -Gal Abs showed that in all Gp3 infants, except for B5508 (pig graft), donor antigens could be identified on the vascular endothelium of the aorta and vasa vasorum (see **Figure S2, SDC,** http://links.lww.com/TP/A651), confirming that the transplanted tissue still presented antigen to the recipient at time of euthanasia in three of the four infants.

From these observations, we concluded that (i) donor-specific sensitization did not occur, (ii) natural Ab production to both A and pig antigens was inhibited, if not prevented, but (iii) B-cell tolerance to pig antigens was not achieved (evi-

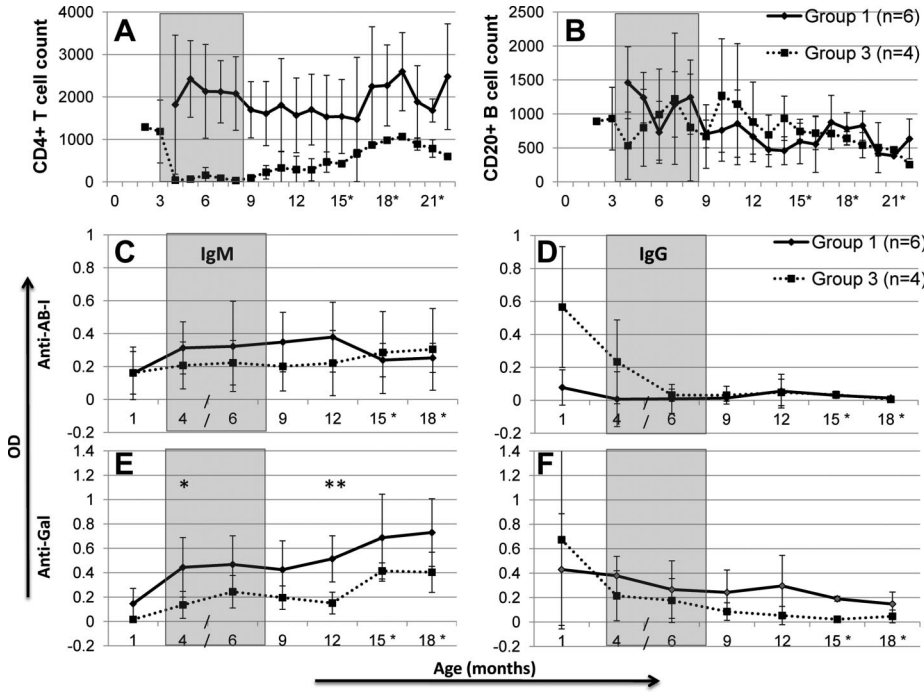

FIGURE 3. T-cell kinetics and antibody levels in group 3 baboons (n=4) compared with group 1 (n=6). (A) In the control Gp1, $CD4^+$ T-cell numbers remained steady. In Gp3, $CD4^+$ T-cell numbers were profoundly depleted by thymoglobulin induction therapy and maintained low throughout the course of IS. A slow recovery occurred after discontinuation of IS, but the number remained lower than in Gp1 for several months. (B) B cells were not affected by the immunosuppressive regimen: no difference was observed between Gp1 and Gp3. Mean anti-AB-I IgM (C) and anti-Gal IgM (E) levels in all Gp3 baboons remained lower than those in the Gp1 control baboons. The differences reached significance at two time points (4 months, $P<0.05$, and 12 months, $P<0.01$). Both anti-AB-I and anti-Gal IgG (D and F) remained low at all time points in Gp1 and Gp3, indicating that no sensitization occurred. Colored area indicates period of IS in three of the four Gp3 baboons (B7507 received IS for 12 months) (*15, 18, and 21 months: n=2 for both groups). Ig, immunoglobulin; AB-I, A/B-incompatible; IS, immunosuppressive therapy.

dence for B-cell tolerance to AB-I antigens was equivocal and is discussed later).

Group 4: IS Only (n=2)

As there was evidence that all Gp3 baboons showed an overall delayed production and lower level of Abs compared with their age-matched controls, which was not related to the type of graft, we added two infants to the study (Gp4). These received IS but no graft. One received only anti-CD154mAb and the other only cytotoxic T lymphocyte antigen (CTLA) u-Ig (Table 2); no induction or other maintenance therapy was administered. As they did not have to be weaned or undergo Tx, the IS was begun at 1 month of age and discontinued at 8 months.

Both ELISA and flow cytometry data indicated that in B309 (receiving anti-CD154mAb) both anti-A/B and anti-Gal/pig IgM remained undetectable at all time points (Fig. 1D). At 1 month of age, this infant had high maternal anti-A, -B, and -Gal IgG that fell to almost undetectable levels by 6 months of age and remained undetectable. B209 (receiving CTLA4-Ig) initially showed low levels of IgM, but when IS was discontinued at 8 months of age, the level was already comparable with that in the untreated Gp1 controls and rose thereafter (Fig. 1D).

These data indicate that, when compared with age-matched controls, anti-CD154mAb suppressed the development of Ab and the cellular response in an infant baboon even when given as monotherapy. In contrast, CTLA4-Ig as monotherapy did not have the same effect.

DISCUSSION

West et al. (*1*, *18*) and others demonstrated that the natural "plasticity" of the immune system in infants can provide a window of opportunity to induce B-cell tolerance to an ABO-I allograft. We investigated whether the encouraging results of ABO-I organ Tx in human infants in the absence of natural Abs to carbohydrate blood group antigens might be translated to pig tissue xenotransplantation.

A weakness of our study, as with many studies in nonhuman primates, is that it involved a limited number of experiments, and more data are required to confirm our initial tentative conclusions. The IS selected might also be criticized as not being clinically relevant, as anti-CD154mAb is unlikely to be approved for clinical application in view of its associated thrombotic complications (*19*, *20*). However, it was chosen as, in our experience, it is the only agent that effectively prevents a T-cell-dependent elicited Ab response in baboons to pig antigens (*21*, *22*). Nonetheless, our findings are of relevance to clinical ABO-I organ Tx and to future clinical xenotransplantation.

In this study, we provide further evidence of the development of natural anti-A/B and anti-Gal/pig Abs in infant

TABLE 2. Immunosuppressive and supportive therapy and monitoring

	Dose	Duration
Induction therapy		
Thymoglobulin	2.0–2.5 mg/kg IV	Days −3 and −1
Methylprednisolone	5 mg/kg IV	Before each dose of ATG and on day 0. The dose was then reduced by 1 mg/kg/d and discontinued on day 5
Maintenance therapy		
Anti-CD154 mAb[a]	20–25 mg/kg IV	Days −1, 0, 4, 7, 10, 14, then every 5–7 d
Mycophenolate mofetil (MMF)	20–150 mg/kg/d PO divided in two doses	Begun on day −2 (to maintain a blood through level of 3–6 μg/mL)
CTLA4-Ig (B209 only)	25 mg/kg IV×1/wk	Age 1–8 mo
Supportive therapy		
Cefazolin	25 mg/kg BID IV	For 3 d after surgery
Famotidine	0.25 mg/kg BID IV	From day −3 until 1 mo post-Tx
Ganciclovir	5 mg/kg IV	From day −4 until 1 mo post-Tx
Ketorolac	0.5 mg/kg IV	Before every dose of anti-CD154mAb
Buprenorphine	0.01 mg/kg BID IV	For 3 d after surgery

Monitoring	References	Frequency
Weight		Weekly. After discontinuation of IS: monthly
Blood cell count and chemistry		Weekly. After discontinuation of IS: monthly
Anti-CD154mAb levels	36	Weekly, until discontinuation of anti-CD154
MMF levels		Weekly, until discontinuation of MMF
T and B cell counts	37	Pre-Tx, before and after each dose of thymoglobulin, weekly for 1 month, then monthly
Anti-A/-B/-Gal Abs (ELISA)	38	Pre-Tx, and at age 4, 6, 9, 12, 15, and 18 mo
IgM and IgG binding to anti-WT pig PBMC and pAEC (FCM)	39	Pre-Tx, and at age 4, 6, 9, 12, 15 and 18 mo (at 15 and 18 mo, only PBMC were tested)
IgM binding to anti-GTKO pig PBMC (FCM)	39	Pre-Tx, and at age 6, 12, 15, and 18 mo
Complement-dependent cytotoxicity assay	39 and 40	At age 1, 6, 12, and 18 mo
Mixed leukocyte reaction	41	Pre-Tx, and at age 4, 6, 9, 12, 15, and 18 mo
Histology (H&E staining)		Transplanted artery patch and native aorta harvested at time of necropsy
Immunofluorescence (anti-A, -B, and -Gal staining)	42	Transplanted artery patch and native aorta harvested at time of necropsy

[a] Selected as, in our experience, it is the only immunosuppressive agent that effectively prevents a T-cell-dependent elicited Ab response in baboons to pig antigens (*22*). Group 3 infants (n=4) received the full regimen, including all the afore-listed drugs, except for CTLA4-Ig. Group 4 infants (n=2) received weekly CTLA4-Ig (B209) or weekly anti-CD154 mAb (B309) only, plus weekly ketorolac to prevent thrombus formation.

IV, intravenously; PO, orally; Ig, immunoglobulin; Gal, galactose-α1,3-galactose; ELISA, enzyme-linked immunosorbent assay; PBMC, peripheral blood mononuclear cells; WT, wild type; IS, immunosuppressive therapy; Tx, transplantation; BID, two times per day; H&E, hematoxylin-eosin; pAEC, porcine aortic endothelial cells.

baboons (Gp1). Anti-A Ab developed more quickly (in type B baboons) than anti-B Ab (in type A baboons). We additionally confirm our previous observation (*9*) that anti-non-Gal Abs remain minimal throughout the first year of life (see **Results, SDC,** http://links.lww.com/TP/A651). We also demonstrate that a small artery patch graft from an AB-I or a pig donor is sufficient to elicit donor-specific sensitization in infant baboons not receiving IS (Gp2).

Infant baboons that received IS in the presence of a graft did not become sensitized to their graft while IS was continuing. There was no or minimal increase in IgM and IgG levels, and the cellular responses remained weak. After discontinuation of IS, there was a delayed increase in Ab production and in the cellular response on mixed leukocyte reaction. B-cell tolerance to AB-I blood group antigens may possibly have developed in B5008, as it failed to develop anti-B IgM Abs after discontinuation of IS, while development of anti-Gal IgM Abs occurred. However, as this was a B-group graft into an A-group baboon, this observation needs to be placed in the perspective of the slow development of anti-B Abs in the control baboons of Gp1. Unfortunately, it was not possible to investigate this possibility by ELISPOT, as this would have required an excessively large blood draw from the small baboon to obtain sufficient B-cell numbers. In the other Gp3 baboon that was followed up for 21 months (B5708), anti-Gal Ab production did not increase until 4 months after discontinuation of IS, but B-cell tolerance was clearly not achieved.

A striking observation was the finding that all infants receiving anti-CD154mAb (n=5) showed a marked inhibi-

tion and delay in development of both anti-AB-I IgM and anti-pig/anti-Gal IgM Abs, which was irrespective of the presence or type of graft. This was not only seen in the infants receiving thymoglobulin induction and maintenance therapy (Gp3) but also in the single infant that received only anti-CD154mAb (with no graft or adjunctive IS) (Gp4). Despite the small number of animals, when comparing Gp3 (±B309 from Gp4) (total n=5) and Gp1 (n=6), there was a significant difference in anti-Gal IgM levels at 4 months and 12 months.

These observations question whether natural Ab production is T-cell independent. Ohdan et al. (*10*) showed that, after depletion of $CD4^+$ and $CD8^+$ T cells in α1,3-galactosyltransferase gene-knockout mice, anti-Gal IgM increased significantly, indicating that the natural development of Abs was not inhibited by the absence of T cells, suggesting that the production of anti-Gal Ab is T-cell independent. In contrast, Cretin et al. (*11*) presented evidence indicating that natural anti-Gal Ab production is T-cell dependent. Our limited data, which to our knowledge, are the first in nonhuman primates suggest that the development of natural Abs to carbohydrate antigens is inhibited by the administration of T-cell-directed IS and, therefore, may not be entirely T-cell independent. In baboons, anti-CD154mAb, when combined with mycophenolate mofetil, is associated with a profound $CD4^+$ T-cell depletion but not in B-cell depletion (*20*, *21*, *23–25*). In contrast, costimulatory blockade with CTLA4-Ig did not seem to inhibit natural Ab production so effectively, although this was tested in only one baboon.

Our finding that anti-CD154mAb-based IS can delay natural Ab production in infants has implications for clinical organ Tx. An infant needing an organ transplant at birth could possibly be maintained on anti-CD154mAb-based IS to prevent natural Ab production while waiting for a donor organ. Although in our study in baboons this effect was only seen with anti-CD154mAb, further studies involving combinations of IS agents might possibly achieve the same result. The complete inhibition of the production of natural Ab, however, might be associated with a risk of infection. In clinical studies of ABO-I heart Tx in infants, B-cell tolerance to donor-specific antigens was achieved, but no generalized reduction in Abs to nonspecific antigens was observed (*1*, *15*, *16*). Of possible relevance is that three of the four Gp3 grafts (including B5008, to which no anti-B Abs developed) still expressed AB or Gal antigens some months after Tx; expression of antigen is important in the development of specific tolerance. Of possible relevance, there is evidence from rodent studies that both anti-CD154mAb (*26*) and CTLA4-Ig (*27*) may be associated with decreased auto-Ab production. Variations in the effect of IS on Ab production may be related to differences between animals and humans or to the IS administered, which in clinical cases has not included costimulatory blockade.

It is encouraging to believe that the absence of natural anti-pig Abs might allow the possibility of pig organ Tx, at least as a bridge to allogeneic Tx (*28*), avoiding Ab-mediated rejection or endothelial cell activation, which may be associated with the development of thrombotic microangiopathy and consumptive coagulopathy. If a xenograft was to be transplanted into a human, the immature immune system of infants would be advantageous, as it offers the possibility of modulating its immune response. Theoretically, if a pig organ was transplanted early in infancy, the child might never develop Abs to the graft, and conventional T-cell-directed IS might be sufficient to maintain the graft.

This might be a particularly successful approach if the heart is taken from a genetically modified pig (*22*, *29*, *30*), thus reducing the humoral and cellular immunologic responses further (*31–33*). Pig hearts of suitable size for infants could readily be obtained. However, our experience is that the response to a pig organ in nonhuman primates is complex. In addition to natural Abs and the adaptive cellular response, it involves an innate cellular response, coagulation dysregulation, and a marked inflammatory response (*32*). Nevertheless, particularly with respect to genetically modified pig heart Tx, it might provide a bridge to allotransplantation.

MATERIALS AND METHODS

Animals

Infant baboons (*Papio anubis*, UOHSC, Oklahoma City, OK) were housed at the UPitt or in the specific pathogen-free facility at UOHSC (*34*). Donor pigs (White/Landrace, Country View Farms, Schellsburg, PA) and donor baboons (UOHSC) were housed at UPitt. Baboon aorta or pig carotid artery was harvested on the day of Tx.

Selection of Baboons

AB blood type was determined in the first month after birth by staining of buccal smears from 43 colony-raised infant baboons (*35*) and was confirmed at least once after 3 months of age. To allow monitoring of development of Abs to nonself (AB-I) -blood group antigens over time, only baboons of A or B blood group were selected. Five A and nine B baboons were selected for the study (Table 1).

Experimental Groups

Four groups of infants were studied (Table 1). Control baboons (Gp1: n=6) received no IS and no graft. Gp2 (n=2) received an AB-I or WT pig graft but no IS; both baboons were followed up until 1 month after Tx. Gp3 received AB-I grafts+IS (3A: n=2) or WT pig grafts+IS (3B: n=2). Gp4 (n=2) received IS only (either anti-CD154mAb or CTLA4-Ig only) but no graft.

Artery Patch Tx

In Gps2+3 infants, a length of donor carotid artery (pig) or aorta (baboon) was transplanted as a full-thickness onlay graft (approximately 1.0×0.5 cm, see **SDC, Figure S1,** http://links.lww.com/TP/A651) into the wall of the abdominal aorta at 3 months of age (Table 1). Donor grafts were obtained from adult AB-I baboons or from WT pigs of blood type O.

Immunosuppressive and Supportive Therapy

Immunosuppressive and supportive therapy is described in Table 2. Gp3 (n=4) received therapy from 3 months of age (Table 1). Maintenance IS was discontinued at 8 months, except in one baboon in Gp3A (B7507, discontinued at 12 months). Gp4 received anti-CD154mAb or CTLA4-Ig alone, which was discontinued at 8 months.

Monitoring of Recipient Baboon

Follow-up in Gp1 was for 12 to 21 months, Gp2 for 1 month post-Tx, Gp3 for 5 to 18 months post-Tx, and Gp4 for 14 months. Monitoring is detailed in Table 2.

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