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Received 9 February 2000.
Revision 21 March 2001.
Accepted 1 April 2001.

0041-1337/01/7209-1541/0
TRANSPLANTATION

Vol. 72, 1541–1548, No. 9, November 15, 2001
Printed in U.S.A.

IN VITRO RECOGNITION AND IMPAIRMENT OF PIG ISLET CELLS BY BABOON IMMUNE CELLS

SIMILARITY TO HUMAN CELLULAR REACTIONS

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Background. **Grafting pig islets into patients with type 1 diabetes requires control of the strong cellular xenogeneic rejection. This in vitro study compared the cellular reaction of baboons and humans to pig islet cells (PICs) to confirm the validity of using these animals for further in vivo preclinical trials.**

Methods. **Baboon or human peripheral blood mononuclear cells (PBMCs) or subsets were co-incubated with PICs from specific pathogen-free adult pigs for 7 days to determine the mechanisms and intensity of PBMC proliferation. Interleukin (IL) 10 and interferon (IFN) γ secretion were assessed by enzyme-linked immunosorbent assay. Because proliferation was not indicative of aggression, a test based on perifusion analysis of the alteration of basal and stimulated insulin releases from PIC incubated with different baboon and human cells was developed.**

Results. **Baboon PBMCs strongly proliferated in response to PICs (stimulation index [SI]=24.8±6.9 [n=8] vs. 23.9±3.4 [n=34] for human PBMCs), showing considerable variation in intensity among animals (2.3<SI<63) and humans (1.8<SI<97). PBMC proliferation was inhibited in baboons and humans by anti-CD4 (% inhibition of SI: 71±10% and 75±7%, respectively) and anti-DR (75±35% and 80±6%) monoclonal antibodies (MoAbs) or by depletion of MHC class II+ cells (99±1% and 90±6%). Blocking by anti-CD8 or anti-CD16 MoAbs was weaker and variable among both animals and humans. IL-10 production by baboon and human PBMCs in response to PICs increased more than IFN-γ production after 2 days of coculture, but the IL-10/IFN-γ ratio was inverted after 5 days of coculture. After 7 days (and even after only 2 days) of coculture with baboon (n=8) or human (n=18) PBMCs, basal and glucose-stimulated insulin secretions**

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from PICs were almost completely abolished ($P<0.0001$). The drop in insulin release could have mainly resulted from lysis of PICs, because the number of PICs decreased by 78% after 7 days of co-incubation with PBMCs. A decrease of insulin release by PBMCs was reproduced with plastic-adherent cells and was abolished by depletion of MHC class II+ cells or by addition of 100 μg/ml gadolinium (which inhibits macrophages), but not by cyclosporine. In baboons, as in humans, insulin release was also decreased after coculture of PICs with enriched T lymphocytes remixed with antigen-presenting cells (APCs).

Conclusions. **This study provides the first data on in vitro comparison of baboon and human cell-mediated recognition and impairment of PICs. Proliferation of PBMCs against PICs involves mainly CD4 T cells, with indirect recognition mediated by baboon or human MHC class II+ APCs. The Th2/Th1 profile of cytokines secreted in response to PICs was similar in baboon and human PBMCs. The model based on alteration of insulin secretion indicates that PIC impairment by whole mononuclear cells was strong and rapid and that a crucial role was played by MHC class II+ and plastic-adherent cells. Two mechanisms appear to be responsible for the role of these cells: (1) early and strong direct effect, which is potentially involved in vivo in primary nonfunction of islets aggressed by monocytes and macrophages; and (2) presentation of PIC xenoantigens, which leads to impairment by T lymphocytes possibly involved in in vivo–specific cellular rejection. The mechanisms and intensity of baboon cellular reactions to PICs in vitro were similar to those observed in humans, which suggests that the baboon is a suitable model for the study of cellular mechanisms during preclinical trials of pig islet xenografts.**

Adult pig islet grafts may be effective in the treatment of human type 1 diabetes (*1*) and do not appear to be susceptible to hyperacute rejection (*2*). However, before such xenografts are attempted in humans, preclinical studies are required in large animal models, particularly non-human primates, to evaluate their efficacy for glycemia control, graft safety, and xenogeneic cell-mediated rejection. As a first step, immune cellular reaction of non-human primates to pig islets needs to be compared with that of humans to determine whether the process is similar in both species.

The present report applied methods previously used in humans (*3*) and rodents (*4*) to in vitro study of the different cellular components of baboon reaction against pig islet cells (PICs). These findings were compared with human data to determine whether the baboon is a suitable model for further in vivo preclinical trials. Few studies have concerned the rejection of pig islets in non-human primates in vivo (*5*) or in vitro (*6*), whereas the pig-to-primate combination has been better evaluated for the grafting of other tissues (*7–9*). However, these studies investigated humoral immunity and not cell-mediated rejection of islets.

The present report studied the mechanisms and intensity of baboon and human lymphocyte proliferation and cytokine secretion to PICs. Additionally, because lymphocyte proliferation was not indicative of PIC impairment, a test was developed to assess consequences of the cell-mediated immune process. Perifusion was used to analyze basal and stimulated insulin secretion of PICs incubated with baboon or human peripheral blood mononuclear cells (PBMCs), i.e., a complex mixture of cells. Two components were then analyzed separately: (1) impairment mediated by non-T cells potentially involved in vivo in the early event known as islet primary nonfunction (PNF) (*10*) and (2) alteration by T lymphocytes potentially involved in vivo in specific cellular rejection of pig islets (*11–13*).

MATERIALS AND METHODS

PBMCs and subset preparations from baboon and human subjects. PBMCs from 8 adult baboons (7 females and 1 male) and 34 human adult volunteers were obtained from venous blood by lymphocyte separation medium (Eurobio, Les Ulis, France) centrifugation and suspended in RPMI 1640 (Eurobio). In some experiments, PBMCs were depleted of MHC class II–positive antigen-presenting cells (APCs) by negative selection using incubation with magnetic microspheres (Dynabeads, Dynal, Oslo, Norway) coated with anti-MHC class II monoclonal antibodies (MoAbs). The effectiveness of APC depletion was confirmed by the abolition of proliferation during 3-day co-incubation of PBMCs with 5 μg/ml phytohemagglutinin. Enriched APC preparations were recovered after plastic adherence of PBMCs by incubations on plastic Petri dishes (Becton Dickinson, San Diego, CA) for 3 hr at 37°C. Enriched T cells were obtained by negative selection using microspheres (Dynabeads) and a cocktail of MoAbs directed against CD14/CD16 (a and b)/CD56/MHC II DR-DP. Enriched $CD4^+$ and $CD8^+$ T-cell fractions were obtained by positive selections using microspheres (Dynabeads) coated with anti-CD4 or anti-CD8 MoAbs. The efficiency of depletions or enrichments was controlled by flow cytometry analysis (FACScan analyser, Becton Dickinson, San Jose, CA).

Specific pathogen-free (SPF) pigs: isolation of PICs. Young (20 week old) large, white SPF pigs (80–120 kg) from our stock in the west of France (*14*) were used. Pancreata were removed under sterile conditions in an operating room with a protected environment so as not to lose the benefit of using SPF pigs raised in sanitary conditions. They were soaked in Betadine solution, immediately inflated with 100 ml of ice-cold sterile modified University of Wisconsin (mUW) solution, and transported in a sterile receptacle containing 250 ml of mUW. Inflated pancreata were then inflated again with 0.5 ml/g of mUW solution containing 2 mg/ml Liberase (Roche Diagnostics, Meylan, France) and placed in a digestion chamber filled with mUW solution kept at 36°C. The flow rate of the medium circulating through the chamber was 40 ml/min. Enzymatic digestion was controlled by dithizone staining. Crude preparations were pelleted by centrifugation, suspended in mUW, and purified on continuous Optiprep gradient (Life International Technology, Cergy-Pontoise, France) with a COBE 2991 processor. Islet cells were directly obtained and purity, as assessed by dithizone staining, exceeded 90%. Insulin release from SPF PICs in response to different stimuli was highly reproducible. Islet cells were numerated and cultured in Ham's F10 medium (5.5 mmol/L glucose) (Biomédia, Boussens, France) supplemented with 2% Ultroser (BioSepra SA, Villeneuve la Garenne, France), antibiotics (penicillin and streptomycin) (Seromed, Berlin, Germany), and Fungizone (Bristol, Paris, France) for a period of 8 days before the experiments.

Proliferation and cytokine production of baboon and human PBMCs in response to pig cells. Baboon and human PBMCs were aliquoted into flat-bottomed 96-well microtiter plates (Costar, Cambridge, MA) at 5×10^5/well with 60000 PICs in 0.3 ml of culture medium that contained 10% autologous decomplemented baboon or human plasma. Plates were incubated at 37°C in 5% CO_2 for 7 days and pulsed by adding 1 μCi [^{3}H] thymidine (specific activity 20 Ci/mmol; Amersham, Orsay, France) for the final 18 hr. Cells were then harvested, and the filters were counted. Data were obtained from the mean values of triplicate wells, and the results were also expressed by a stimulation index (SI), i.e., the ratio between the mean count with PICs and the mean basal count.

Similar proliferation assays in response to PICs were performed while PBMC subsets were blocked, and different MoAbs were introduced (10 μg/ml) for the entire co-incubation period. Rat immunoglobulin G MoAbs directed against baboon CD4 (13B.8.2), human CD4 (RPA-T4) or CD8 (OKT8), or against CD16 from natural killer (NK) cells (B73–1) were used. To determine whether the recognition involved MHC class II–restricted mechanisms, similar proliferation assays in response to PICs were performed while PBMCs were blocked with MoAbs directed against HLA class II DR (L243) or DQ (SPVL3) and that did not cross-react with pig cells, as determined by flow cytometry analysis. MoAbs directed against human CD8, CD16, and HLA DQ and DR showed 100% cross-reactivity with baboon, as determined by flow cytometry analysis. To determine whether baboon or human T cells interact directly or indirectly with PICs, T cells were depleted of APCs by negative selection, as described above, before being introduced into the proliferation assay. Cocultures of whole PBMCs with PICs were also performed in the presence of different doses of cyclosporine (CsA) (Sandoz, Rueil-Malmaison, France) or gadolinium chloride (Sigma, St. Louis, MO).

Under conditions similar to those described for proliferation, 100 μl of supernatant were removed from each well after 2 or 5 days of co-incubation for baboon or human interferon (IFN) γ and interleukin (IL) 10 assays (rhesus monkey INF-γ and human IL-10 Cytoscreen immunoassay kit, Biosource International, Camarillo, CA) using a solid phase that involved a bound capture antibody and a horseradish peroxidase–conjugated antibody for detection. Standard curves were generated using recombinant interleukins.

Perifusion analysis of basal and stimulated insulin releases after co-incubation of PICs with baboon and human PBMCs or subsets. Islet β-cell function was assessed after incubations of 3×10^5 PICs alone or in the presence of different types of baboon or human cells in flat-bottomed 24-well microtiter plates (Nunc, Rostilde, Denmark) incubated at 37°C in 5% CO_2 in a final volume of 2 ml for 2, 4, or 7 days. For the study of whole PBMC effect, 3×10^6 baboon or human PBMCs were co-incubated with PICs. In some experiments, 3×10^6 PBMCs were introduced in the assay after removal of MHC class II+ cells. On the contrary, 600000 plastic-adherent cells (PACs) were directly incubated with PICs in other experiments. Cocultures of PICs with whole PBMCs were also performed in the presence of different doses of gadolinium chloride (Sigma) or CsA (Sandoz). Finally, insulin release was measured after co-incubation of PICs with 1.5×10^6 T cells, remixed or not with 100000 plastic-adherent APCs.

In all cases, insulin release was then determined by perifusion. Cocultures were placed in a perifusion chamber (Swinnex 13, Millipore Corp, Bedford, MA) supplied with Krebs-Ringer bicarbonate buffer that contained 0.5% bovine serum albumin; 5 mmol/L pyruvate, fumarate, and glutamate (Sigma); and 2.8 mmol/L glucose. The flow rate was 1 ml/min. Chambers and perifusion media were kept at 37°C in a water bath, and the perfusates were gassed with a 95% O_2-5% CO_2 mixture. PICs were first perifused for 30 min in 2.8 mmol/L glucose, then for 20 min in 11 mmol/L glucose, and finally in 2.8 mmol/L glucose. Effluents were collected and stored at −20°C for insulin radioimmunoassay (CIS Bio International, Gif-sur-Yvette, France) using porcine insulin as standard (Endopancrine, Organon, Serifontaine, France).

Statistical analysis. Data are presented as mean±SEM. Statistical significances of differences were calculated in each point of time during perifusions and evaluated using the Student's t test or the Mann-Whitney test. $P<0.05$ was considered significant.

RESULTS

Proliferation of baboon and human PBMCs in response to PICs. Baboon PBMCs strongly proliferated in response to PICs after 7 days of co-incubation (Fig. 1) and showing a mean SI (n=8, SI=24.8±6.9, $P<0.01$) similar to that of human PBMCs (n=34, SI=23.9±3.4, $P<0.001$). The intensity of proliferation varied greatly among animal and human subjects (SI ranged from 2.3 to 63 in baboons and from 1.8 to 97 in humans).

In baboons, as in humans, CD4+ T cells were mainly involved in proliferation against PICs (Fig. 2A). The SI decreased sharply (% inhibition of SI: 71±10%, $P<0.04$, in baboons vs. 75±7%, $P<0.02$, in humans) when CD4+ were blocked by MoAbs. In both species, the involvement of CD8 T cells was much lower (the SI decreased less when CD8+ were blocked by MoAbs), and NK cells were only marginally involved (the SI was only very slightly reduced after blocking by anti-CD16 MoAbs).

MHC class II–restricted mechanisms were involved in both baboon and human cellular responses to PICs, i.e., proliferation was blocked by anti-DR (75±35%, $P<0.04$, and 80±6%, $P<0.01$, respectively) but not anti-DQ MoAbs. Moreover, the removal of MHC class II–positive APCs from baboon PBMCs led to strong inhibition of proliferation (99±1%, $P<0.01$), which indicated that the dominant pathway for recognition of PICs was indirect, which was similar to that for human subjects (90±6%, $P<0.03$).

Proliferation of baboon PBMCs in response to PICs was inhibited in a dose-dependent manner by CsA, which prevents lymphocyte division, and by gadolinium, which inhibits macrophages (Fig. 2B). Immunosuppressive drugs were efficient in inhibiting proliferation of baboon PBMCs at the same levels as for human PBMCs.

T helper cell (Th) 1/Th2 cytokine secretion from baboon and human PBMCs in response to PICs. After 2 days of coculture, production of IL-10 by baboon or human PBMCs in response to PICs (compared to basal production) increased more than production of IFN-γ (Fig. 3), suggesting that Th2 involvement was greater than that of Th1 at that time. However, after 5 days of coculture, the increase of IFN-γ production in response to PICs became higher than that of IL-10 in both species (Table 1).

Loss of basal and stimulated insulin releases from perifused PICs incubated with baboon and human PBMCs or subsets. In comparison with control PICs cultured alone or co-incubated with autologous pig splenocytes, co-incubation of PICs with baboon PBMCs for 7 days led to loss of insulin release ($P<0.0001$, Fig. 4A) similar to that induced by human PBMCs ($P<0.0001$). Basal and glucose-stimulated insulin releases were abolished ($P<0.0001$ for both). The drop in insulin release could have mainly resulted from lysis of PICs, because the number of PICs counted in light microscopy after 7 days of co-incubation with human PBMCs decreased by 78±11% in comparison with PICs alone. In baboons, as in humans, a loss of insulin secretion was detected even after 2 or 4 days of co-incubation (S.L., P.G., E.G., and P.S., unpublished data, 2000). A set of complementary depletion or enrichment experiments was then designed to determine the cell subsets involved in alteration of PICs by baboon PBMCs and to compare the mechanisms with those of humans.

The decrease of both basal and stimulated insulin releases was reproduced when baboon PACs were co-incubated alone with PICs for 7 days ($P<0.0001$; Fig. 4B), an effect also observed with human PACs ($P<0.001$). The magnitude of this alteration depended on the number of PACs because the deleterious effect disappeared when PICs were co-incubated with 100000 or fewer PACs (Fig. 6). Conversely, the effect of whole baboon PBMCs on PIC insulin secretion disappeared

($P<0.0001$; Fig. 4C) after 7 days of co-incubation when MHC class II+ cells were removed from PBMCs. An identical depletion effect was obtained with human PBMCs ($P<0.0001$).

Gadolinium chloride abolished the loss of insulin release from PICs cultured with baboon or human whole PBMCs ($P<0.0001$ for both; Fig. 5A), which is consistent with the involvement of MHC class II+ and PACs in PIC impairment. On the contrary, the loss of insulin release from PICs co-incubated with baboon or human PBMCs was not modified by CsA (Fig. 5B). The doses of CsA and gadolinium themselves did not alter insulin release from PICs (S.L., P.G., E.G., and P.S., unpublished data, 2000), but they blocked proliferation of the same PBMCs incubated with PICs (Fig. 2B).

A second alteration mechanism was also detected, i.e., 7-day co-incubation of PICs with baboon enriched T cells remixed with 100000 plastic-adherent APCs led to a decrease ($P<0.0001$) of insulin release (Fig. 6). This effect was also observed with human-enriched T cells remixed with APCs ($P<0.0001$).

DISCUSSION

To validate the use of baboons as preclinical models for in vivo grafts of pig islets, different cellular components of baboon reactions to PICs were studied in vitro and compared with those of humans. This in vitro study provides the first detailed information about several mechanisms of baboon cell-mediated impairment of PICs.

Lymphocyte proliferation in response to PICs was as intense in baboons as in humans. Although this does not imply that the mechanisms are identical in both species, it tends to validate the baboon for preclinical trials. However, lymphocyte proliferation in response to PICs was much less intense in the mouse model than that observed in humans and baboons (*4*). The intensity of proliferation varied considerably among baboons and humans, which suggests that in vivo cell-mediated rejection of PICs would not be strong in all monkeys or human subjects. The cause of this variability was not specifically studied here but may depend, at least in part, on the matching of donor and recipient MHC antigens (*15*). This varying intensity may also reflect differences in the ability of individual APCs to process and present xenoanti-

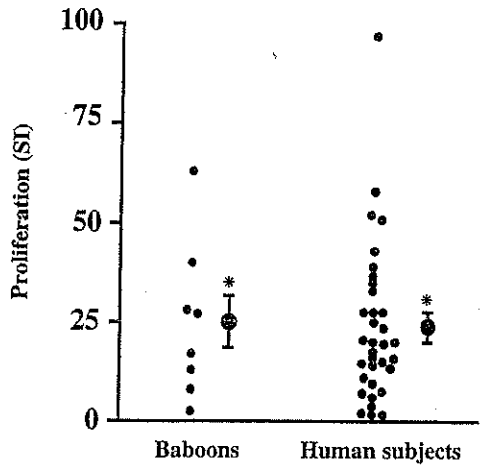

FIGURE 1. Proliferation of baboon and human PBMCs in response to PICs. In vitro PBMC (5×10^5) responsiveness of baboon (n=8) or human subjects (n=34) to 60000 PICs. Each individual point represents the SI of PBMCs from a given animal or subject co-incubated for 7 days with PICs. The mean SI±SEM is also represented. The SI was calculated as the ratios of the mean ^{3}H-thymidine uptakes with PICs to the mean basal counts. Absolute values of basal ^{3}H-thymidine uptake by PBMCs in the absence of PICs were not different between baboons and humans (1313±402 cpm vs. 8822±1947 cpm, respectively). * ($P<0.01$) indicates significant differences between basal and ^{3}H-thymidine uptakes stimulated by PICs. There was no significant difference between baboon and human subjects.

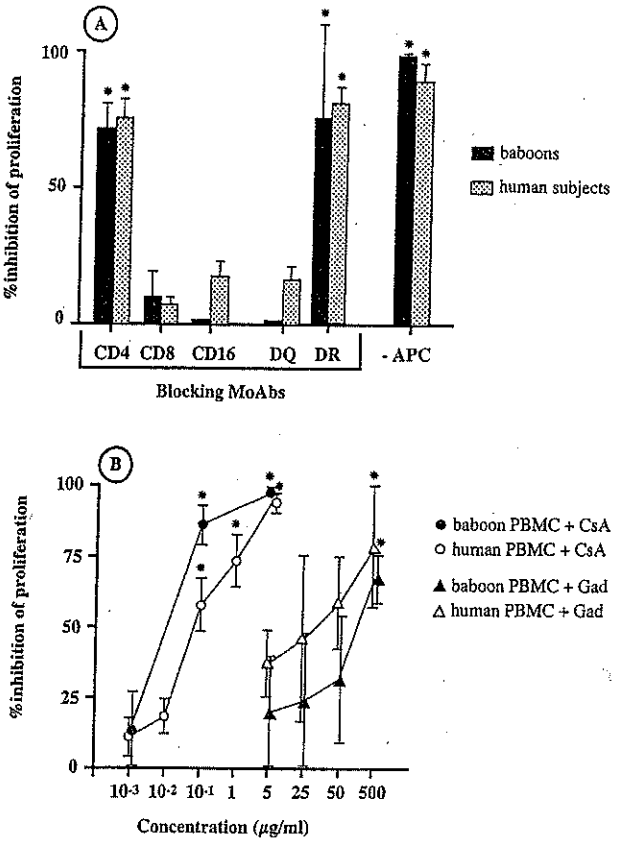

FIGURE 2. Effects of MoAbs and immunosuppressive drugs on PBMC proliferation in response to PICs. A, Proliferation of PBMCs from baboon (n=4) or human subjects (n=18) cocultured with 60000 PICs for 7 days in the presence of blocking MoAbs directed against CD4, CD8, CD16, MHC class II DQ, or MHC class II DR. The proliferative responses of PBMCs from baboon (n=5) and human subjects (n=7) to PICs after removal of APCs are also represented (−APC). Absolute values of ^{3}H-thymidine incorporation by baboon PBMCs cocultured with PICs were 49750±9562 cpm (vs. 161233±16962 cpm for human PBMCs), whereas incorporation after removal of baboon APCs was 5014±1036 cpm (vs. 14583±3941 cpm after removal of human APCs). B, Effects of different doses of CsA (circles) or gadolinium chloride (Gad) (triangles) on the proliferation of PBMCs from baboon (full symbols; n=5 and n=3, respectively) or human subjects (open symbols; n=9 and n=4, respectively) cocultured with PICs. Results are expressed as the mean percentage of inhibition±SEM of the SI. * ($P<0.05$) indicates significant differences from control SI with PICs, but in the absence of MoAb or drugs and without removal of APCs.

gens or individual variations in the T-cell receptor for antigen repertory.

Blocking experiments with MoAbs showed that the proliferation of baboon PBMCs in response to PICs involved mainly CD4+ T lymphocytes, which is consistent with studies indicating that CD4+ cells are the main cell type infiltrating xenografts (*16*). Blocking experiments also showed that MHC class II–restricted mechanisms were involved. Thus, the response of CD4+ cells could have required indirect presentation of PIC antigens by class II molecules borne by baboon APCs, which was confirmed by the considerable decrease in PBMC proliferation after APC depletion. These results in baboons were similar to those obtained in humans, which suggests that the dominant pathway for recognition of PIC antigens is indirect in both species, a mechanism reported for other pig targets (*15*, *17–19*). Despite dominant indirect CD4+ recognition, the blocking experiments suggested that CD8+ might also be implicated in the proliferation of baboon or human PBMCs in response to PICs. However, they were involved much less than CD4+ cells and showed variations among individuals. The blocking experiments suggest that NK cells were only marginally involved (if at all) in the proliferation of baboon or human PBMCs in response to PICs.

Assays of IL-10 (Th2 cytokine) and IFN-γ (Th1 cytokine) produced by baboon or human PBMCs in response to PICs indicated that Th2 activation was greater than that of Th1 in both species after 2 days of co-incubation, whereas the Th1 activation was greater in mouse splenocytes confronted to the same PIC preparations during the same period (*4*). However, after 5 days of coculture, the increase of IFN-γ production in response to PICs became higher than that of IL-10 in baboons and humans, and the Th1/Th2 ratio was, thus, inverted as compared to 2-day co-incubation. Absolute cytokine levels were higher in humans than baboons, which may have been partly due to the incomplete cross-reactivity of the reagents used in the assay.

Finally, lymphocyte proliferation in response to PICs showed sensitivity to similar doses of immunosuppressive drugs in baboons and humans, which may also be of interest for further in vivo trials. Proliferation was sensitive to CsA, which prevents T-cell activation. Gadolinium chloride, which blocks monocytes and macrophages (*20*), also decreased proliferation, probably through interference with the indirect pathway of PIC xenorecognition.

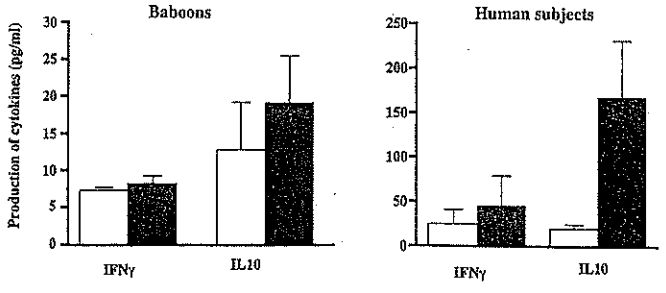

FIGURE 3. Th1/Th2 cytokine secretion from baboon or human PBMCs in response to PICs after 2 days of coculture. In vitro production of cytokines by 5×10^5 PBMCs from 6 baboon and 4 human subjects cultured for 2 days with 60000 PICs. Black columns represent the mean production of IFN-γ and IL-10 in the presence of PICs. Basal cytokine production in the absence of stimulator cells is also indicated (open columns).

Thus, the mechanisms and intensity of baboon and human cell-mediated proliferation in response to PICs, as well as sensitivity to immunosuppressive drugs, were similar in both species. This suggests that the baboon could be a particularly relevant model for the study of cell-mediated rejection of PIC xenografts in humans. The response of rodents to PICs was notably different, in particular showing much less intensity (*4*).

Because lymphocyte proliferation is not necessarily indicative of impairment of target cells, the effects of baboon immune cell activation on the in vitro function of PICs were also studied to compare the mechanisms and intensity of this aggression in baboons and humans. Dynamic perifusion was used to analyze the alteration of insulin release from PICs incubated with different baboon or human cells potentially involved in successive rejection stages. To the best of our knowledge, xenogeneic cellular aggressiveness against pig islets has not yet been reported in the pig-to-baboon model, and the few studies performed in the pig-to-human combination have only concerned the release of tracers from radiolabeled islet cells (*21–25*).

The perifusion test showed that baboon PBMCs abolished basal and glucose-stimulated insulin releases from PICs almost completely and very rapidly in a manner identical to that of human PBMCs. Insulin release from the same PIC preparations co-incubated with mouse splenocytes was also reduced, but not so markedly (*26*). The number of PICs used in these experiments was determined on the basis of preliminary experiments (S.L., P.G., E.G., and P.S., unpublished data, 2000) and optimal conditions for proliferation. Although the number of xenogeneic PBMCs was relatively high, hypothesis of overcrowding can be ruled out. For example, the co-incubation of PICs with the same number of baboon or human PBMCs depleted of MHC class II–positive cells did not lead to decreased insulin secretion. Moreover, co-incubation of PICs with the same number of autologous pig splenocytes did not alter insulin release. This suggests that the decreased insulin secretion observed with whole xenogeneic PBMCs was actually indicative of xenogeneic mechanisms. The drop in insulin release from PICs induced by human PBMCs could have been due to lysis or functional inhibition of PICs, but a decrease in PIC number in the presence of PBMCs, as revealed by microscopy, was consistent with the notion of PIC lysis. This cell-mediated impairment of PICs was apparent from the first days of PBMC islet confrontation, i.e., well before lymphocyte proliferation, which peaked after 7 days.

Both baboon and human PBMCs are complex mixtures. However, depletion, enrichment, and pharmacological experiments have identified two cellular mechanisms involved in baboon or human cell-mediated impairment of PICs. Both mechanisms depend on MHC class II–positive cells and PACs, i.e., those belonging to the category of APCs.

In baboons, as in humans, a first mechanism involved in the rapid loss of insulin secretion is related to direct effect of MHC class II–positive cells and PACs of the recipient. Depletion reduced impairment, whereas enrichment reproduced the alteration of insulin release. Their effect in vitro is comparable to in vivo early islet PNF, a non–T-cell macrophage-mediated phenomenon not yet revealed in primates but

TABLE 1. In vitro Th1/Th2 cytokine secretion from baboon or human PBMCs in response to PICs after 5 days of coculture

	Baboon			Human		
	PBMC	PBMC+PIC	SI	PBMC	PBMC+PIC	SI
IFN-γ	8.3 pg/ml	16.9 pg/ml	2.0	9.3 pg/ml	63.3 pg/ml	6.8
IL-10	14.0 pg/ml	24.5 pg/ml	1.7	49.4 pg/ml	81.3 pg/ml	1.6
IL-10/IFN-γ	1.7	1.4	0.9	5.3	1.3	0.2

Production of cytokines (IFN-γ and IL-10) by 5×10^5 PBMCs from one baboon or human subject cultured for 5 days alone or in the presence of 60,000 PICs. Stimulation Indexes were calculated as ratios between cytokine production in the presence of PICs and basal cytokine production.

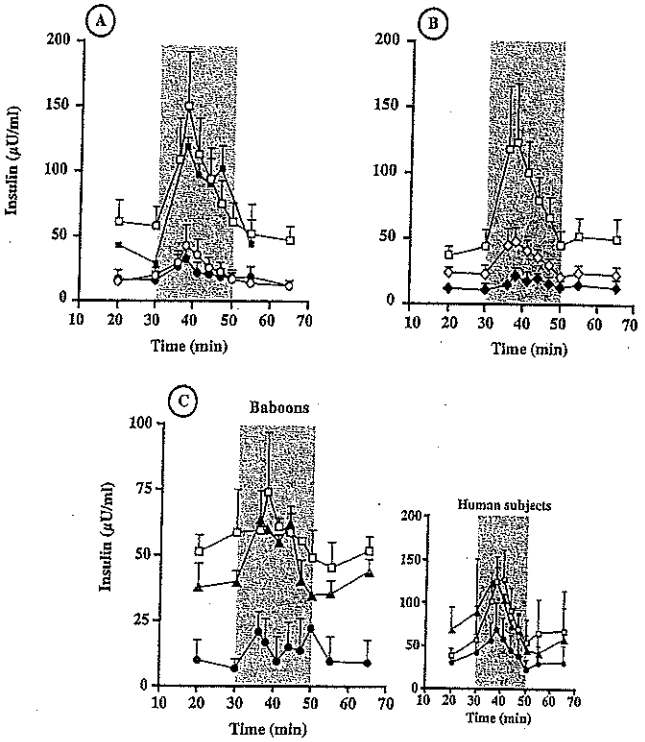

FIGURE 4. Inhibition of dynamic insulin release from perifused PICs incubated with baboon or human PBMCs or cell subsets. A, Insulin secretion of 300000 PICs cultured for 7 days alone (open squares, n=20), with 3×10^6 autologous pig splenocytes (full squares, n=2), or with 3×10^6 PBMCs from baboon (full circles, n=8) or human subjects (open circles, n=18). $P<0.0001$ between insulin secretion from PICs cultured alone or with baboon and human PBMCs. B, Insulin secretion of 300000 PICs cultured for 7 days alone (open squares, n=11) or with 600000 PACs from baboons (full lozenges, n=3) or human subjects (open lozenges, n=11). $P<0.0001$ between insulin secretion from PICs cultured alone or with baboon PACs (vs. $P<0.001$ for human PACs). C, Insulin secretion of 300000 PICs cultured alone (open squares, n=2), with 3×10^6 whole baboon PBMCs (full circles, n=2), or 3×10^6 PBMCs depleted of MHC class II–positive cells (full triangles, n=2). A similar experiment was performed with human cells (same symbols). $P<0.0001$ between insulin secretion from PICs cultured with whole baboon or human PBMCs or PBMCs depleted of class II–positive cells. The glucose stimulation period is indicated by the grey area. Each point indicates the mean±SEM.

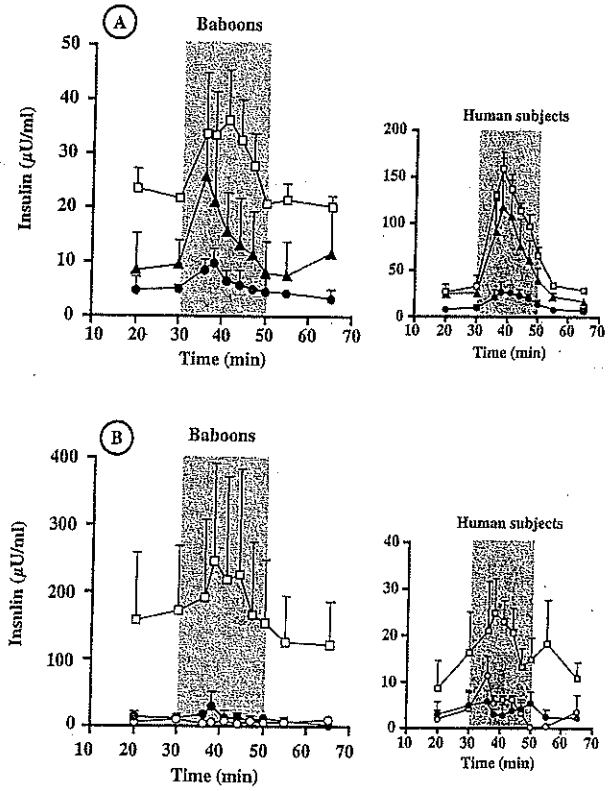

FIGURE 5. Effects of gadolinium chloride (Gad) and CsA on impairment of insulin release from PICs induced by baboon and human PBMCs. Effects of 100 μg/ml Gad (full triangles, panel A, n=3) and 1 μg/ml CsA (open circles, panel B, n=2) on insulin secretion of 300000 PICs cocultured with 3×10^6 baboon whole PBMCs. Comparisons were performed with PICs alone (open squares) or with cocultures of PICs and PBMCs without immunosuppressive drugs (full circles). Similar experiments were performed with human cells (same symbols). The glucose stimulation period is indicated by the grey area. Each point indicates the mean±SEM. $P<0.0001$ between insulin secretion from PICs cultured with baboon or human PBMCs in the absence and presence of Gad.

observed in rodents receiving xenogeneic islets (*27, 28*). The role of macrophages in PNF of islets grafted into xenogeneic rodents has also been suggested by the improvement of early graft function after gadolinium chloride–induced macrophage inhibition (*29*). In this respect, our study also found that the use of gadolinium in the in vitro assay suppressed the early PIC impairment by baboon or human PBMCs without causing toxicity to PICs. Conversely, CsA was unable to abolish this impairment, confirming the involvement of a non–T-cell phenomenon in both baboons and humans. This direct effect of macrophages on PICs could have been due in our study to direct cell-mediated toxicity or to production of soluble factors such as cytokines or oxygen radicals. Addi-

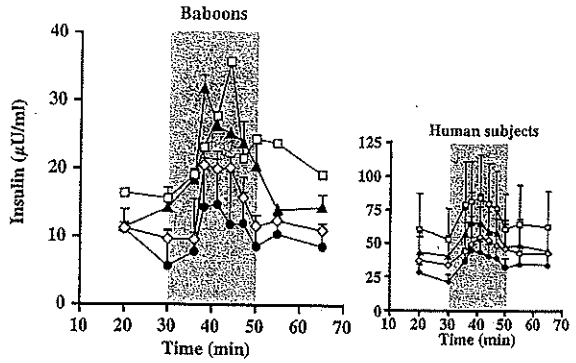

FIGURE 6. Alteration of insulin release from PICs by baboon and human T cells. Insulin release of 300000 PICs cultured alone (open squares) or with baboon cells. PICs were co-incubated for 7 days with 100000 baboon plastic-adherent APCs (full triangles, n=2), 1.5×10^6 baboon-enriched T cells (open lozenges, n=2), or T cells remixed with APCs (full circles, n=2). A similar experiment was performed with human cells (same symbols). The glucose stimulation period is indicated by the grey area. Each point indicates the mean±SEM. $P<0.0001$ between insulin secretion from PICs alone and PICs cultured with T cells remixed with APCs.

tional experiments are required to investigate these two hypotheses.

In vitro analysis of insulin secretion from perifused PICs enabled us to detect a second mechanism mediated by baboon or human T lymphocytes. In baboons, as in humans, the impairment of PICs in vitro was marked in the presence of T cells remixed with autologous APCs. This may correspond to indirect activation of T lymphocytes mediated by APCs, which is consistent with the dominant indirect recognition pathway for PBMC proliferation (*3*). However, the presence of porcine MHC class II molecules shared by non-endocrine cells (dendritic cells, endothelial cells), which could contaminate the islet cell preparations, cannot be excluded because immunofluorescence with anti-MHC class II antibodies revealed approximately 1% of class II–positive cells in one of our islet cell preparations.

The loss of insulin secretion and lysis from PICs exposed to baboon or human PBMCs for 7 days in our study is consistent with the findings that adult PICs grafted into immunodeficient mice reconstituted with human peripheral blood lymphocytes (*22, 23*) were rejected after 14 days and with recent report of in vitro killing of porcine islets by human cells (*25*). However, it is not consistent with other reports that indicate that PICs are not susceptible to lysis by human NK cells or cytotoxic T lymphocytes (*21, 24*). However, the latter studies were performed with neonatal or fetal PICs that may be less susceptible than adult PICs to cytotoxicity. Moreover, PICs were cocultured in these studies with human cells depleted by plastic adherence from monocytes and macrophages, i.e., from cells that appear to be crucial for direct cytotoxicity and for the presentation of xenoantigens that induce aggression by T lymphocytes. Furthermore, these studies used tracer release from radiolabeled islet cells to assess xenogeneic aggressiveness after coculture of only 4 or 6 hr, whereas in our study, the insulin secretion of PICs was evaluated after at least 24 hr of co-incubation with human cells. Finally, the resistance of neonatal PICs to cell-mediated injury could result from a lack of adhesion or costimulatory molecules on neonatal PICs, which may limit interactions with human effector cells. The expression of these molecules is not fully described in the pig, but they may depend on the differentiation state of PICs, thus contributing to differential sensitivity to lysis of adult, neonatal, or fetal PICs. It is also noteworthy that lysis of PICs was obtained in these studies by addition of exogenous IL-2 or lectin.

In conclusion, the baboon appears to be a suitable model for the study of cellular rejection mechanisms during preclinical trials of pig islet xenografts. Strategies for combating cellular rejection of PICs in baboons and humans should include action against recipient APCs. These cells play a major role in the strong, direct early effect potentially involved in vivo in the PNF of islets as well as in the presentation of PIC xenoantigens responsible for recognition and impairment by T lymphocytes that could be implicated in in vivo–specific cellular rejection.

Acknowledgments. We are grateful to Mrs. M. Allard, Mrs. M. Ouary, and Mrs. S. Pogu for their excellent collaboration. This work was supported by grants from the Pays de la Loire and Brittany regions. Pig islets were isolated from SPF pigs obtained by a collaboration with AFSSA (Ploufragan, France, Dr. Ph. Vannier).

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Received 17 October 2000.
Accepted 3 April 2001.

0041-1337/01/7209-1548/0
TRANSPLANTATION

Vol. 72, 1548–1555, No. 9, November 15, 2001
Printed in U.S.A.

CD103+ CTL ACCUMULATE WITHIN THE GRAFT EPITHELIUM DURING CLINICAL RENAL ALLOGRAFT REJECTION[1]

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Background. **We have previously reported that activated CD8+TCR$\alpha\beta$+ cells that express high levels of the β7 integrin CD103 (formerly α_E, MLA) are present at the graft site during clinical renal allograft rejection. This observation potentially provides new insight into the mechanisms underlying renal allograft destruction because the ligand of CD103 is the epithelial cell-specific molecule E-cadherin, which is known to be expressed by critical graft functional elements such as the renal tubular epithelium. We herein used combined fluorescence-activated cell sorter (FACS) and immunohistochemical (IHC) analyses of transplant nephrectomy (TN) specimens to demonstrate that CD103+ cytolytic T lymphocytes (CTLs) specifically home to the graft epithelium during rejection episodes.**

Methods. **Serial sections of TN specimens undergoing histologically confirmed cellular rejection (n=7) were stained with anti-CD8 or anti-CD103 and were scored for the presence of positively stained cells within the tubular basement membrane. Freshly isolated graft-infiltrating lymphocytes were subjected to three-color FACS analyses to define the extended phenotypic characteristics of CD103+ cells detected by IHC.**

Results. **CD103+ cells in all specimens were biased towards an intratubular localization. On average, the percentage of CD103+ cells with an intraepithelial localization was 52.2±13.1 compared to 12.0±3.5 for pan CD8+ cells (mean±SE, n=5). FACS analyses confirmed that CD103+ cells detected by IHC exhibited the salient characteristics of CD8+ CTLs (large CD8+TCR$\alpha\beta$+ CD62L$^-$CD11a^{hi}perforin+). The CD103− subset of graft-infiltrating CD8 cells also exhibited a CTL phenotype, but these were predominantly restricted to the graft interstitium.**

[1] This work was supported by a grant from the National Institutes of Health (AI36532) (to G.A.H.) and a University of Maryland Special Research Initiative Support Award (to G.A.H.).

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