

Human and non-human primate anti-galactosyl response after injection of rat monoclonal antibody bearing galactosyl epitopes

Dehoux JP, de la Parra B, Latinne D, Bazin H, Squifflet J-P, Gianello P. Human and non-human primate anti-galactosyl response after injection of rat monoclonal antibody bearing galactosyl epitopes. *Xenotransplantation* 2000; 7: 109–117. © Munksgaard, Copenhagen

Abstract: In the case of clinical use of pig-to-human xenografting, any exogenous source of α -galactosyl epitopes will elicit an anti-galactosyl immune response, which could be deleterious for the xenograft. The presence of Gal α (1–3)Gal residues was thus examined by western blotting on various rat monoclonal antibodies (mAb), which are used in clinical trials. In parallel, the anti-galactosyl humoral response was assessed in the serum of kidney allograft recipients and experimental baboons, which received these mAbs. Galactosyl residues were evidenced on all rat monoclonal antibody tested. The anti-galactosyl response was weak in kidney allograft recipients receiving a basic immunosuppression (Cyclosporine, Azathioprine, Prednisolone) and iterative injections of rat mAbs. In contrast, untreated or immunosuppressed baboons that received rat mAbs developed a major anti-galactosyl humoral response. These results suggest that anti-galactosyl sensitization produced by therapeutic agents will have to be considered in the case of clinical xenotransplantation.

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Key words: anti-Gal sensitization –
rat monoclonal antibody – xenograft

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Received 21 February 2000;
Accepted 9 May 2000

Introduction

The major epitope recognized by natural xenotransplantation antibodies is Gal α (1–3)Gal saccharide, a sugar commonly found on all tissues of mammals and New World monkeys. In contrast, humans, apes, or Old World monkeys do not express galactosyl epitopes on tissue, but produce anti-Gal α (1–3)-Gal antibodies in response to colonization of the gut by galactosyl-bearing bacteria [1,2]. Thus, in the clinical situation of pig-to-human xenotransplantation, any exogenous source of galactosyl epitopes would elicit a strong anti-galactosyl response, which could damage the xenotissue, except if knock-out pigs for galactosyl-transferase are generated. Because galactosyl residues have previously been identified on mouse mAb and human cell lines [3], the presence of galactosyl residues has been assessed on several rat monoclonal antibodies, Anti-Thymocyte Globulins

(ATG) and one humanized monoclonal antibody, which are used in clinical trials [4,5]. The anti-galactosyl response was studied in immunosuppressed kidney allograft recipients, and compared with the anti-Gal response obtained in experimental baboons.

Materials and methods

Monoclonal antibodies and immunoglobulins

LO-BM2 (Experimental Immunology Unit, Université catholique de Louvain, Brussels, Belgium) is a rat IgG2a mAb that binds to baboon IgM and does not cross-react with baboon or human IgA, IgD or IgG isotypes. LO-CD2b (Experimental Immunology Unit) is a rat IgG2b mAb that binds both human and baboon CD2 molecules. LO-CD2a/BTI322 (Biotransplant Inc, Boston, USA) is a rat IgG2b mAb that binds to human and

chimpanzee CD2+ cells. MEDI 507 (MedImmune, Gaithersburg, USA) is the humanized form of BTI322 (linked to human IgG1). LO-CD2a, LO-CD2b and LO-BM2 have been produced in vivo in rat ascitis (Experimental Immunology Unit) by a rat hybridoma, which is the result of the fusion of the rat cell line IR 983F and rat splenocytes. BTI322 is similar to LO-CD2a, but has been produced in vitro by the same rat hybridoma for clinical use (Sorebio, Bordeaux, France). MEDI507 (MedImmune, Gaithersburg, USA) is the humanized form of BTI322 and has been produced in vitro by the expression vector SP2O (which is a murine myeloma cell line). ATG are rabbit anti-human T-cell globulins purchased from Pharmacia and Upjohn, USA.

Western blotting

Twenty-five microliters of each mAb [1 mg/ml of LO-BM2, LO-CD2b, BTI 322, MEDI 507 and mouse anti-rat kappa-MARK1 (IgG1)] and human or baboon IgG (1 mg/ml) were electrophoresed on a Bis-Tris-HCL buffered (pH 6.4) polyacrylamide gel (NuPAGE, NOVEX, San Diego, USA) under reducing conditions and transferred to nitro-cellulose membrane

(NOVEX, San Diego, USA). After blocking with 1% skimmed milk (MERCK, Darmstadt, Germany), the blot was coated overnight with 1/20 000 biotinylated lectin. After washing three times in PBS Tween Buffer, the blot was stained with I^{125} -labeled streptavidine (50 μ l/5 ml) (Amersham Int., UK, 20 μ Ci/0.5 ml). After incubation for 1 h at 4 °C, the blot was washed with PBS Tween buffer and autoradiographed.

Glycosidase digestion and ELISA

Nitro-cellulose (0.4 μ)-coated 96 microwell plates (Maxisorp, Nunc Products, Denmark) were incubated in borate buffer (pH 9.5) overnight at 4 °C with (Gal α (1-3)Gal-O-spacer)n-BSA (250 μ g/microwell) (Glycorex AB Lun, Sweden) or with the previously described mAb and immunoglobulins: LO-BM2 (250 μ g), LO-CD2b (250 μ g), LO-CD2a/BTI 322 (187.5 μ g); MEDI 507 (187.5 μ g), ATG (200 μ g), human IgM (200 μ g) and baboon IgM (200 μ g) in borate buffer (pH 9.5). The plates were washed three times in PBS 0.1% Tween and submitted to glycosidase digestion. A serial dilution of alpha- and beta-galactosidase enzyme was carried out on (Gal α (1-3)Gal-O-spacer)n-BSA in order to determine the most effective dilution. Alpha- and

Table 1. Kidney transplant recipients. At days 1,3,10 and 21, the anti-galactosyl immunization is expressed in ng/ml when assessed by ELISA and in Mean Fluorescence Intensity (MFI), when flow cytometry on PAECs was used. The day of appearance of the anti-murine sensitization is shown as well as the day of the disappearance of free Ig in the serum of the patients

Group and identification	Treatment	Frequency and dosage	Anti-galactosyl sensitization				Anti-murine response appearance (day)	Day of end of circulating free Ig
			Days	D1	D3	D10		
Human BZ	BTI 322	10 $\times$ 0.15 mg/kg	ng/ml	162	143	151	10	11
			MFI	81.9	65.5	66.7		
Human LJ	BTI 322	10 $\times$ 0.15 mg/kg	ng/ml	85	87	90	11	10
			MFI	183.9	203.3	211.2		
Human LE	BTI 322	10 $\times$ 0.15 mg/kg	ng/ml	380	290	285	10	12
			MFI	110.2	58.6	63.0		
Human KO	BTI 322	3 $\times$ 0.4 and 2 $\times$ 0.07 mg/kg	ng/ml	62	ND	57	9	9
			MFI	47.8	ND	50.0		
Human TS	BTI 322	3 $\times$ 0.4 and 2 $\times$ 0.07 mg/ml	ng/ml	112	ND	90	11	9
			MFI	34.9	ND	31.7		
Human VG	BTI 322	3 $\times$ 0.4 and 2 $\times$ 0.07 mg/kg	ng/ml	291	ND	274	12	11
			MFI	54.6	ND	55.3		
Human MR	ATG	10 $\times$ 4 mg/kg	ng/ml	145	97	118	12	36
			MFI	72.7	65.4	70.9		
Human DL	ATG	10 $\times$ 4 mg/kg	ng/ml	168	160	170	12	51
			MFI	68.3	64	56.6		
Human TM	ATG	10 $\times$ 4 mg/kg	ng/ml	330	317	326	9	42
			MFI	131.4	108.6	109.6		
Human OM	ATG	10 $\times$ 4 mg/ml	ng/ml	18	21	33	10	56
			MFI	64	66.6	69.4		
Human HY	MEDI 507	1 $\times$ 0.015 and 3 $\times$ 0.4 mg/kg	ng/ml	143	79	210	Neg	18
			MFI	82.4	ND	54.9		
Human HP	MEDI 507	1 $\times$ 0.015 and 3 $\times$ 0.4 mg/kg	ng/ml	1320	1010	1340	Neg	27
			MFI	177.1	ND	125.5		
Human BJ	MEDI 507	1 $\times$ 0.015 and 3 $\times$ 0.4 mg/kg	ng/ml	280	340	107	Neg	34
			MFI	39.7	ND	33.9		

Rat monoclonal antibody and anti-galactosyl response

Table 2. Baboons after treatment with several doses of rat mAb. At days 1,3,10 and 21, the anti-galactosyl immunization is expressed in ng/ml when assessed by ELISA and in MFI, when flow cytometry on PAECs was used. The day of appearance of the anti-murine sensitization is shown as well as the day of the disappearance of free Ig in the serum of the untreated and treated baboons

Group and identification	Treatment	Frequency and dosage	Anti-galatosyl sensitization				Anti-murine response appearance (day)	Day of end of circulating free Ig
			Days	D1	D3	D10	D21	
Baboon 04	PLP*	—	ng/ml	59	49	506	ND	—
			MFI	68.5	81	239	ND	
Baboon 25	LO-CD2b	12×0.25 mg/kg	ng/ml	131	194	320	592	8
	allograft		MFI	41.5	44.5	64.9	99.5	10
Baboon 35	LO-CD2b	3×0.5 mg/kg	ng/ml	52	61	102	204	8
			MFI	52.5	51.6	81	343.6	5
Baboon 40	LO-CD2b	3×2 and	ng/ml	17	59	124	134	10
	and FK 506	14×0.1 mg/kg	MFI	60.2	81.6	92.6	133.7	10
Baboon 43	LO-CD2b	3×2 mg/kg	ng/ml	71	110	270	283	8
	allograft		MFI	109.9	150	167.6	215.0	8
Baboon 32	LO-CD2b	1×2,5 mg/kg	ng/ml	20	29	18	13	7
			MFI	68.4	ND	92.2	101.3	6
Baboon 42	LO-BM2	1×2 mg/kg	ng/ml	52	134	251	1205	9
			MFI	160.7	351.2	846.6	1004	3
Baboon 15	LO-BM2	10×20 mg/kg	ng/ml	48	78	2300	2600	7
			MFI	71.9	72.7	638.7	788.7	12
Baboon 22	LO-BM2	7×20 and	ng/ml	18	32	340	790	7
	and FK506	3×2 mg/kg	MFI	65	64.6	412.1	827.7	10
Baboon 06	LO-BM2	8×20 mg/kg	ng/ml	67	84	2450	2740	8
	Xenograft		MFI	62.8	85.4	757.9	874.2	10
Baboon 09	LO-BM2	7×20 mg/kg	ng/ml	52	75	1980	2480	7
	Xenograft		MFI	65.4	81.2	745.2	985.4	11
Baboon 39	ATG	3×50 mg/kg	ng/ml	302	310	240	382	ND
			MFI	63.9	66	60	64.9	ND

*PLP: pig liver perfusion.

beta-galactosidase (Sigma Chemical Company, St Louis, MO, USA) digestion were performed under optimal conditions for each enzyme: *alpha-galactosidase digestion* in buffer-morpholinoethanesulfonic acid (MES, Sigma) 20 mm/l, pH 6.0; 3 U per microtiter plate well; enzymatic conditions (21 h at 37°C); *beta-galactosidase digestion* in buffer-PBS, pH 7.2; 0.03 U per

microtiter plate well; enzymatic conditions (21 h at 37°C). Similar digestion was carried out using buffer alone as the control. After 21 h of incubation at 37°C, the plates were washed and incubated (1 h, 37°C) with gelatin 0.5% (Merck) in PBS and washed three times in PBS 0.1% Tween. The plates were then incubated for 1 h with 5 µg biotinylated *Bandeiraea simplicifolia* (BS-I) (Sigma) followed by peroxidased streptavidine (1/2000, Sigma). After washing, the revelation was done by Ortho-phenyldiamin (OPD, Sigma), 0.03% H₂O₂ (MERCK) in citrate buffer (pH 5.5) solution. Optical density was measured at 492 nm using an optical reader (Labsystems multiskan RC).

97 -

52 -

21 -

MW A B C D E F G H I

Fig. 1. Lectin binding to different immunoglobulins. Column A: LO-BM2; column B: MARK1 (mouse IgG1 mAb); column C: LO-CD2a/BTI 322; column D: no Ig; column E: MEDI 507; column F: no Ig; column G: human IgG; column H: baboon IgG and column I: LO-CD2b. Molecular weight is shown on the left. As evidenced, galactosyl residues are observed on all rat and mouse Igs, but not on baboon and human Igs.

Human kidney allograft recipients

Thirteen kidney allograft recipients were studied. In addition to the basic immunosuppression (Cyclosporine, Azathioprine, Prednisolone), these patients received one of the following antibody/globulins during several days (see Table 1): BTI 322 (at high or low doses), MEDI 507 and rabbit ATG. Sera from these patients were obtained at D1, D3, D10 and D21 after the first mAb injection and studied by ELISA or flow

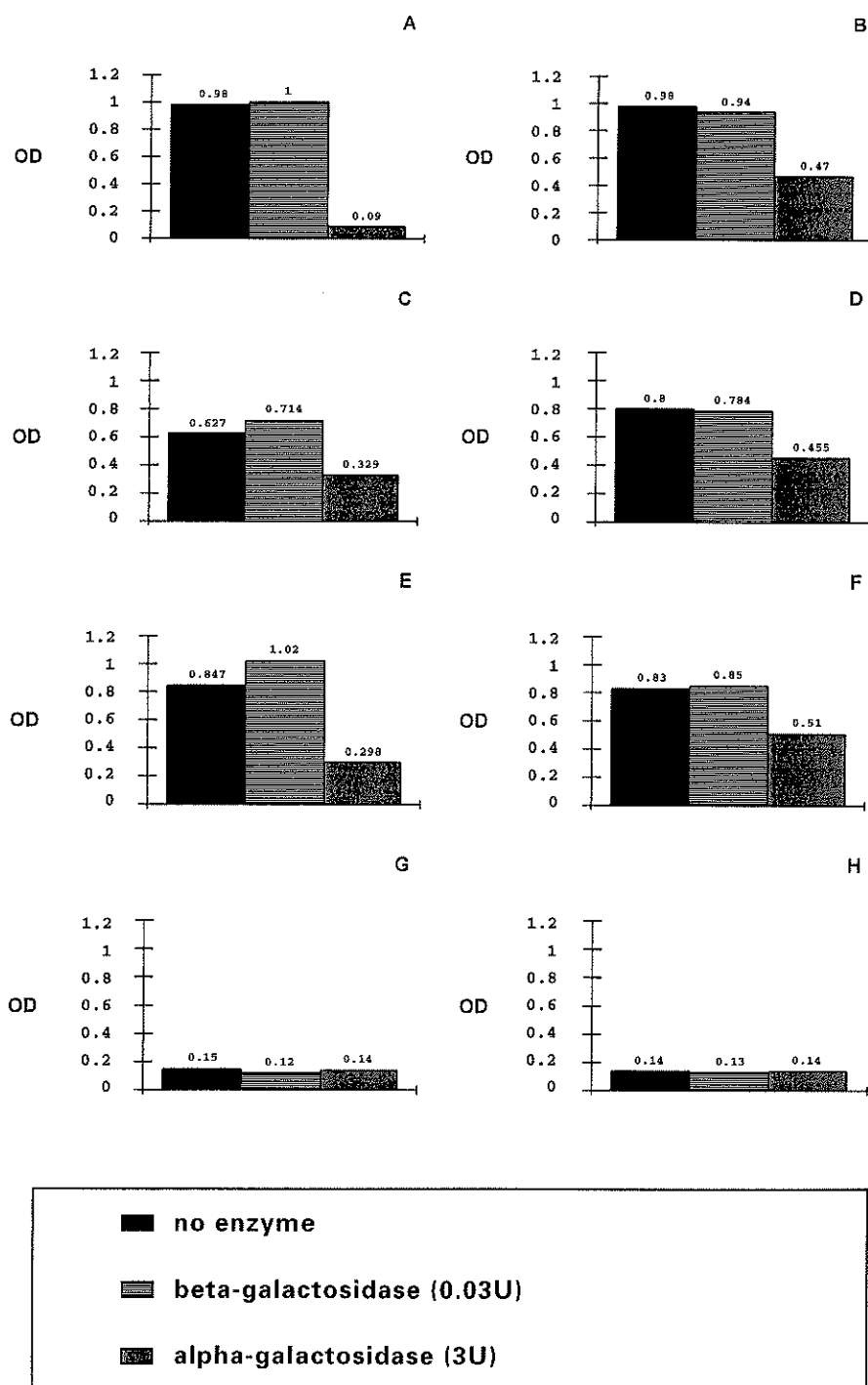


Fig. 2. Optical density of lectin binding with no enzyme, after α -galactosidase and β -galactosidase digestion on microwells coated with (A) Gal α (1-3)Gal-bovine serum albumin (BSA), (B) LO-CD2b, (C) LO-BM2, (D) LO-CD2a/BTI 322, (E) MEDI 507, (F) ATG, (G) human IgM and (H) baboon IgM.

cytometry (FC) for the presence of antigalactosyl antibodies (Table 1).

Non-human primate experiments

Ten baboons (*Papio hamadryas*) received LO-BM2 or LO-CD2b mAb at different doses and

iterative injections with or without additional immunosuppressive drug (see Table 2). Among these animals, three underwent a kidney allograft (Bab25, 40 and 43) and two baboons that were injected with LO-BM2 underwent a pig kidney xenograft (Bab06 and 09). One animal (Bab39) received three doses of ATG at 50 mg/kg and one

underwent a pig liver perfusion (Bab04) for 1 h (PLP). Baboon sera were obtained at D1, D3, D10 and D21 after the first mAb injection or the beginning of the procedure and assessed for the presence of anti-Gal antibodies by ELISA and FC.

Serum anti-galactosyl sensitization

The production of anti-galactosyl IgG antibodies in humans and baboons was assessed by ELISA. Microplates were first coated with (Gal α (1-3)-Gal-O-spacer)n-BSA (0.25 mg/well), and human or baboon sera were serially diluted from 1/50 and revealed by peroxidized rat anti-baboon IgG (LO-BG1) and by rat anti-human IgG (LO-HG22).

Anti-rat and rabbit sensitization

The production of antibody against rat and rabbit determinants was assessed by ELISA: the plates were either coated with 0.5 μ g/well of rat LO-CD2a, rabbit ATG, MEDI507, rat LO-CD2b or rat LO-BM2 and serial dilutions of human serum (from 1/10 for BTI322, from 1/1000 for ATG and from 1/100 for MEDI 507) or baboon serum (from 1/5 for LO-CD2b and 1/00 for LO-BM2) were then added. The human and baboon serum IgG concentration was then revealed by peroxidized rat anti-human mAb (LO-HG-6 and 22) to determine the anti-mAb sensitization.

Serum concentration of mAb and ATG

To assess the serum BTI 322 concentration, the plates were first coated with mouse anti-rat IgG2b (MARG2b-3), and serial dilutions of human sera (starting dilution: 1/10) were added. Anti-rat antibodies were detected by peroxidase-labeled mouse anti-rat mAb (MARK-1). Purified rat IgG2b (BTI 322) was used as the standard. For rabbit ATG concentration, the plates were first coated with rat anti-rabbit IgG1, and serial dilutions of human sera (from 1/1000) were added. Pig anti-rabbit Ig peroxidase (DAKO, Denmark) was used to determine the level of ATG concentration. For MEDI 507 concentration, the plates were coated with rat anti-human IgG (LO-HG20), serial dilutions of serum added (starting dilution: 1/10) and the peroxidized rat anti-human kappa chain (LO-H kappa) was used for the revelation. For LO-CD2b concentration, the plates were coated with mouse anti-rat IgG2b, and serial dilutions of baboon serum (1/5). Peroxidized mouse anti-rat (MARK-1) mAb was used for the revelation. For LO-BM2 concentration, the plates were coated with purified

baboon IgM, and then baboon sera serially diluted from 1/100. Peroxidized mouse anti-rat IgG2a (MARG2a-1 and 8) was used to reveal the concentration of serum LO-BM2 level.

Flow cytometry on porcine aortic endothelial cells

Freshly harvested pig aortic segments were stored in a sterile tube in HBSS (Biowhittaker, Petit Rechain, Belgium) until isolation of the endothelial cells. The porcine aortic endothelial cells (PAECs) were obtained by scraping the intimal surface of the aortic segments. The cells were washed once in HBSS salt solution, resuspended in 5 ml of Medium 199 (Biowhittaker) supplemented with 20% fetal calf serum, 2 mm l-glutamine, 16.5 UI/ml heparin, 100 U/ml penicillin and 100 μ g/ml streptomycin. The cells were seeded on a gelatin-coated 25 cm²-culture flask (Cornil, International Medical, Belgium). After 24 h, the medium was renewed and 50 μ g/ml of Endothelial Cell Growth Supplement (from bovine pituitary, Sigma, Bornem, Belgium) was added. Thereafter, the medium was changed twice a week. When the cells were confluent, subcultures were obtained by trypsinization. Specific anti-porcine IgG antibodies were assessed by FC on PAECs. 1×10^6 endothelial cells per tube were incubated for 30 min with 20 μ l of each sera. After incubation, three washings were performed with HBSS. Fluoresceinated rat anti-baboon IgG (LO-BG3) or rat anti-human IgG (LO-HG22) were incubated for 30 min. The mean fluorescence intensity (MFI) was measured and used as a reference.

Statistics

In order to compare the anti-galactosyl response obtained by ELISA and FC in either all patients or experimental baboons, an Analysis of Variance (anova) for repeated measure was used. A *P*-value under 0.05 was considered as significant.

Results

Identification of Gal α (1-3)Gal epitopes on immunoglobulins and mAbs

The western blot evidenced that the *Bandeiraea simplicifolia* (BS-I) lectin binds molecules that have an approximate molecular weight of 52 and 20 Kda (which corresponds to the MW of the heavy and light chain of immunoglobulin) on rat immunoglobulins (LO-BM2, LO-CD2a/BTI322, LO-CD2b), mouse Igs (MARK1/mouse IgG1)

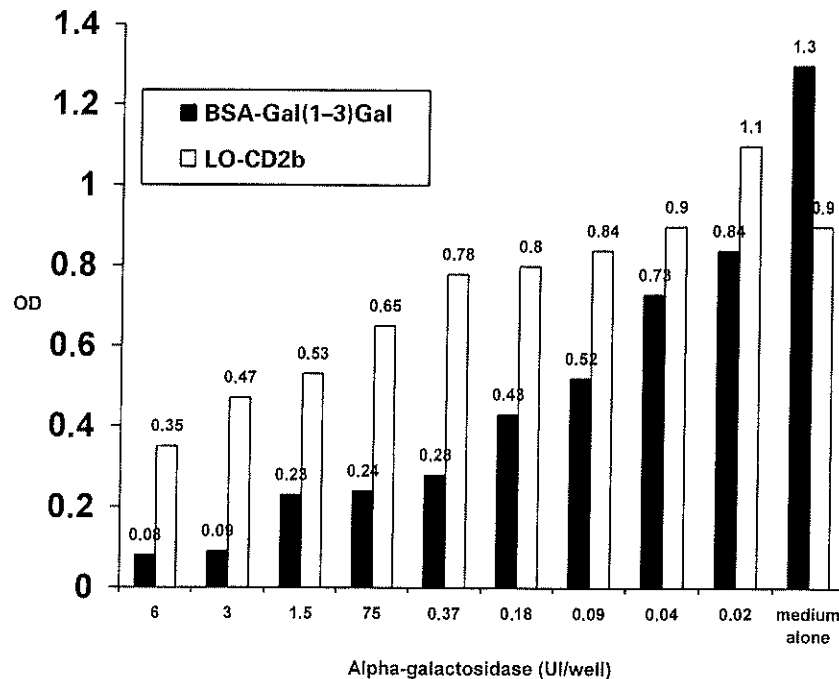


Fig. 3. Effect of serial dilutions of α -galactosidase concentration (from 6 to 0.02 UI/well) on lectin binding (optical density) after coating with either Gal α (1-3)Gal-BSA or LO-CD2b.

and humanized MEDI 507, but not on human and baboon IgM antibodies (Fig. 1). This blot, therefore, confirmed the presence of galactosyl determinants on rat and mouse immunoglobulins as well as on the humanized mAb, but not on baboon or human Igs.

As shown in Fig. 2, digestion with α -galactosidase resulted in a 90.6% decrease of the lectin binding for Gal α (1-3)Gal-BSA, and a decrease of 52%, 48%, 43%, 65% for LO-CD2b, LO-BM2, BTI322 and MEDI 507, respectively. The reduction of the lectin binding was only 38% for ATG. This experiment was designed to assess the presence of galactosyl residues on the monoclonal antibodies, but not to completely eliminate these residues. A previous dilution curve (Fig. 3) showed that a higher concentration (6 UI/well) could provide a stronger digestion on LO-CD2b, but for practical reasons (commercial concentration) the dose of 3 UI/well was chosen. In contrast, α -galactosidase had no or little effect on the binding. This ELISA, therefore, confirmed that Gal α (1-3)Gal epitopes are detectable on all the mouse and rat Igs, but not on baboon or human Igs, as observed by the western blot.

Anti-galactosyl and murine sensitization in kidney allograft recipients

The results are presented in Table 1. The anti-galactosyl response was assessed by ELISA and FC on PAECs. No significant immunization against Gal α (1-3)Gal or PAECs was evidenced

in patients treated with low doses of BTI 322 (10×0.15 mg/kg) or with ATG. Patients treated with higher doses of BTI 322 (3×0.4 mg/kg), demonstrated a slight but not significant increase of the anti-Gal α (1-3)Gal IgG response (1.6 ± 0.4 fold the pre-treatment level), while the level of anti-porcine IgG antibodies remained unchanged. Among the three patients who received the humanized MEDI 507 mAb, two developed a mild increase of anti-galactosyl antibodies (1.2 ± 0.2 fold the pre-treatment level), but no increase of anti-porcine response was observed. Overall and according to these results, immunosuppressed patients did not elicit a significant anti-galactosyl response after iterative injections of mAb bearing galactosyl residues. The anova test for repeated measures was used to analyze all the patients treated and was under significance at FC ($F=2.284$; $P=0.1236$) or ELISA ($F=0.8353$; $P=0.4460$).

In the same patients, however, an anti-murine response was detected in all but the patients treated with the humanized mAb (MEDI507). As expected, the half-life of MEDI507 mAb was significantly longer than the half-life of rat mAbs. The half-life of anti-human rabbit globulins was also prolonged (Table 1).

Anti-galactosyl and murine sensitization in experimental baboons

The results are summarized in Table 2 and show that, except for the animal that received

ATG, all the baboons developed a strong and significant anti-Gal and anti-PAECs immunization. The animal that underwent a pig liver perfusion for 1 h (4), developed rapidly a strong anti-galactosyl response as evidenced by both ELISA and FC. Baboons treated with different LO-CD2b doses and regimen [12×0.5 mg/kg (25) or 3×0.5 mg/kg (35)], comparable to the doses used in humans, developed a strong anti-Gal immune response. At equal dose and frequency (3×2 mg/kg), the injection of concomitant FK 506 (0.5 mg/kg during 10 d) to LO-CD2b-treated animal did not protect against the anti-Gal immunization [3.9-fold the pre-treatment level (43) vs. 7.8-fold for FK 506 treated baboon (40)]. High doses of LO-BM2 (20 mg/kg) produced an anti-galactosyl response that reached 54.1-fold the pre-treatment level. A concomitant treatment with FK506, only slightly decreased the xenogeneic response after injection of a similar dose of LO-CD2b (43.8-fold the pre-treatment level). A low dose (2 mg/kg) of LO-BM2 elicited an 23.1-fold increase of the anti-galactosyl response in comparison with the pre-treatment value. There was a dose-response effect, but LO-BM2 clearly elicited a significantly increased response in non-immunosuppressed baboons. In contrast, the baboon that received ATG demonstrated a low level of anti-galactosyl immunization, but by ELISA and glycosidase digestion, ATG has been shown to bear less galactosyl determinants. Overall (i.e. all the baboons together) the immune response against galactosyl determinants was significantly increased in baboons after injection of various rat mAbs as assessed by the anova test for repeated measures [at FC ($F=7.236$; $P=0.0049$) and ELISA ($F=4.884$; $P=0.0229$)].

In all these baboons, there was a clear correlation between the period of disappearance of the mAb from the serum and the first signs of anti-rat immunization (Table 2).

Discussion

The presence of Gal α (1-3)Gal residues was demonstrated on both the heavy and light chains of rat monoclonal and rabbit polyclonal antibodies by western blot, whereas galactosyl residues were not detectable on human and baboon immunoglobulins. These results were confirmed by α -galactosidase digestion, an enzyme that releases terminal α -galactosyl residues [6]. The presence of galactosyl residues on rat and rabbit immunoglobulins was expected, and

the detection of similar determinants on humanized MEDI 507 mAb is explained by the fact that the expression system used was a murine SPO2 cell line, able to process a murine glycosylation [7-10]. As found in this study, there was no expression of galactosyl residues on primate and human immunoglobulins, but the presence of galactosyl residues has been anecdotally shown on humanized Ig, human Ig [3,11], old red blood cells and human cells lines [12-14]. In these observations, however, the Gal α (1-3)galactosyltransferase gene has probably undergone a dysregulation, thereby resulting in a synthesis of Gal α (1-3)Gal residues on human glycoconjugates.

Despite the presence of galactosyl epitopes on the mAbs injected to the kidney allograft recipients, these patients did not elicit a significant anti-galactosyl humoral response. The lack of galactosyl sensitization in these recipients could be the consequence of: (i) the dose of mAb used; (ii) the quantity of galactosyl residues expressed on the mAb; (iii) the immunosuppressive regimen; or (iiii) the biological way used to produce the mAb. In fact, the patients treated with a 10-d regimen of *low dose BTI322* (10×0.15 mg/kg) did not show any anti-galactosyl response, whereas patients receiving a *higher dose regimen of BTI322* (3×0.4 mg/kg plus 2×0.07 mg/kg) or *MEDI507* (3×0.4 mg/kg and 1×0.015 mg/kg) evidenced a slight but not significant increase of anti-galactosyl response. This slight difference could reflect a dose effect. The quantification of the galactosyl residues on the various mAbs has not been assessed in this study, and it is therefore not possible to analyze the effect of the galactosyl residue quantity on the intensity of the anti-galactosyl sensitization. The immunosuppressive regimen, however, could have an impact on the anti-galactosyl immune response, although such a humoral immune response is known to be T-independent and no drug given in the basic immunosuppression (cyclosporine A, azathioprine, prednisone) to the kidney allograft recipients has such characteristics. Another possible explanation for the lack of anti-galactosyl sensitization in kidney allograft recipients is the biological way of production of the injected mAbs. In fact, BTI322 and MEDI507 have been produced in vitro for safety reasons, and the glycosylation could be less important in vitro than the level obtained in vivo in rat ascitis.

In contrast to the lack of galactosyl sensitization after rat mAbs injection in humans, most of the patients that had been exposed in vivo to porcine tissues or organs produced a significant anti-galactosyl humoral response. Cotterell et al.

[15], in fact, showed that after cross-perfusion of porcine organs by human patients receiving immunosuppression therapy, there is a seric increase of antibodies directed against porcine antigens and against Gal α (1-3)Gal. This sensitization could be the consequence of the dose of antigens and memory B cells, already committed by cross-reactive epitopes on intestinal bacteria, which are stimulated to produce increasing amounts of Gal α (1-3)Gal antibodies. The increased anti-Gal activity in patients receiving xenografts occurred in spite of massive immunosuppressive regimens and was likely to be the result of selective proliferation of lymphocytes clones producing high-affinity anti-Gal antibody [7,16]. In patients exposed to pig hepatocytes after bioartificial liver treatment, Baquerizo et al. [17] also concluded that a T-cell independent response was suggested by the inability of conventional immunosuppression to decrease the xeno-antibodies. Although inhibition of xeno-antibodies has been reported in rodent models [18,19], there is no immunosuppressive regimen that produced a similar effect in large animal models or in humans [20]. Overall, these results suggest that the absence in kidney recipients of anti-galactosyl sensitization is the consequence of an exposition to a low dose of galactosyl antigens as compared with the amount of xeno-antigens presented by an porcine tissue or organ.

Although the presence of galactosyl residues on rat mAbs and rabbit ATG does not seem to provide a significant and potentially deleterious anti-galactosyl sensitization, the clonage and the expression of the rat genes for the heavy and light chains in a host cell that does not have an active α (1-3)galactosyltransferase could overcome the presence of galactosyl residues on monoclonal antibodies [2]. The absence of this enzyme would prevent the addition of Gal α (1-3)Gal residues to all proteins produced. The choice of the host cell is very important and the best choice would eventually be a human monoclonal antibody, because human molecules are devoid of any Gal α (1-3)^oresidues [3]. As recently reported, amino-acid substitution represents another biotechnological technique able to prevent the glycosilation of humanized mAb [21].

In contrary to the lack of anti-galactosyl immune response in immunosuppressed patients, the anti-galactosyl response was strong in untreated or immunosuppressed baboons. At comparable low doses in humans, two animals receiving LOCD2b (25 and 35) developed a strong and significant anti-

galactosyl response 10 or 21 d after the beginning of infusion. All other animals receiving a higher dose of LO-CD2b mounted an anti-galactosyl response that reached almost 50-fold the pre-treatment value, whatever FK506 was given concomitantly. This strong anti-galactosyl response emphasized the importance of the dose of mAb injected, the presence or not of a simultaneous xenograft or the in vivo production of the LO-CD2b.

In conclusion, the presence of galactosyl residues was evidenced on rat mAbs and ATG by lectin binding. Although the rat mAbs and rabbit globulins injected in the patients bore galactosyl residues, the kidney allograft recipients did not elicit a significant anti-galactosyl response. In these conditions, the use of such mAbs in recipients of pig xenotransplantation seems to be safe, but this study also suggests that the use of biological therapeutic agents in the context of clinical xenotransplantation should be carefully evaluated prior to clinical use.

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