The influence of MHC class II antigen blockade by perfusion with a monoclonal antibody on rat renal graft survival

Maciej Krzymański[1, 2], Ana Maria Waaga[1], Karin Ulrichs[1], Adam Deja[1, 2], Andrzej Oko[1, 2], Thomas Rommel[1], and Wolfgang Müller-Ruchholtz[1]

[1] Department of Immunology, Christian Albrechts University, Brunswikerstrasse 4, W-2300 Kiel 1, Federal Republic of Germany
[2] Department of Nephrology, Institute of Internal Medicine, Academy of Medicine, Al. Przybyszewskiego 49, Poznań 60–355, Poland

Abstract. To decrease immunogenicity of the rat kidney, grafts were perfused with an anti-MHC class II monoclonal antibody (mAb). How effectively this procedure blocked class II-positive cells, which were mainly dendritic in appearance, was checked by immunostaining renal sections after perfusion and comparing them with in vitro stained sections. Optimum conditions were applied for graft pretreatment before transplantation. This procedure prolonged graft survival, though not satisfactorily from the biological point of view (9.6 ± 0.8 versus 7.7 ± 0.5 days in the control group; $P < 0.02$). The dendritic cells were not killed but blocked. Several hours after transplantation, the mAb dissociated from these class II-positive cells. It was also shown that donor cells migrate into the recipient's spleen early after transplantation. The number of these cells was smaller when the transplanted organ was perfused with the mAb. Further studies are suggested to deplete the graft of donor dendritic cells more adequately. They should also combine graft perfusion with anti-class II mAb and recipient immunosuppression at reduced doses.

Key words: Class II antigen blockade, rat, renal transplantation – Monoclonal antibody, class II antigen blockade – Renal transplantation, rat, class II antigen blockade

The immunogenicity of the graft and the immunological reactivity of the recipient are the main factors that determine allogeneic graft rejection. Immunogenicity depends on the presence of minor and major histocompatibility complex (MHC) class I and II gene products on the cell surface. It has been shown that class II antigen expression is not stable, and various protocols may change the extent to which these antigens are expressed [1, 2, 16, 19, 22, 24, 25]. It has also been demonstrated in studies on the heart and pancreas [3, 21] that rat strains may be divided into two groups. One has a high density of interstitial dendritic cells (DC) that express class II antigens and the other a low density. For successful transplantation in different rat strain combinations, it may be advantageous to know the class II MHC antigen expression and to take it into account.

To decrease the immunogenicity of the graft, various procedures have been used [4, 9, 13, 14, 17, 18, 23]. Evidence is accumulating to support the hypothesis that graft immunogenicity may be altered by pretreatment with anti-class II monoclonal antibodies. However, evidence of the effectiveness of perfusion procedures, as presented in the literature, is quite fragmentary.

In the present study, MHC class II antigen expression was investigated in rat renal grafts to analyze the effectiveness of the perfusion procedure in pretreating the graft and to define the optimum conditions for blocking class II antigens expressed on DC. The efficacy of the method was assessed morphologically by the immunoperoxidase technique on renal tissue sections. Kidneys pretreated under optimum conditions were used for allogeneic rat transplantation.

Materials and methods

Animals

Inbred male LEW (RT1 l), DA (RT1 a), and CAP (RT1 c) rats, bred in our department, were used in the study. All rats were 3–4 months old. Male DA and LEW rats were used as transplant donors and recipients.

Monoclonal antibodies (mAb)

Organ perfusions were done with an anti-class II mAb (29A1) of the IgG1 type that reacts with the I-E subregion. This mAb was produced at the Department of Immunology in Kiel. For immunoperoxidase staining, the following mAbs were also used: MRC Ox6, which reacts with monomorphic determinant of rat class II antigen (I-A region), and MRC Ox3, directed against a polymorphic determinant of class II antigen present on cells from animals of the LEW strain but not of the DA or CAP strains (Serotec, Kidlington, Oxford, UK).

Organ perfusion

The left kidney was removed under ether anesthesia, perfused with 1.5 ml of cold phosphate-buffered saline (PBS) and then with mAb 29A1 (2.0 ml culture supernatant + 0.5 ml 20% glucose), and placed into an ice-cold bath for 20, 30, 60, or 120 min. The kidney fragments were snap-frozen and stored at −80°C.

Histology

Seven-micrometer cryostat sections were cut, air-dried, fixed in acetone, and stored at −20°C until use. MHC class II antigen expression in organs perfused with cold PBS was demonstrated using a three-stage immunoperoxidase technique. The first step was to incubate the organ sections with the mAb. In two further steps, peroxidase − conjugated antiglobulin antibodies were used: rabbit anti-mouse Ig (Dianova, Hamburg, FRG) and goat anti-rabbit Ig (Daco, Copenhagen, Denmark). After staining with diaminobenzidine, the slides were colored with hematoxylin. Sections of organs perfused with 29A1 were analyzed for the effectiveness of mAb binding to class II antigen-positive cells with the two-stage immunoperoxidase technique, the second and the third stages of the three-stage method.

Renal transplantation

Renal transplantations were performed according to the method employed by Lee [12] on rats weighing 250–300 g. In brief, the donor kidney was perfused with mAb 29A1 and incubated at 4°C for 60 min. Both of the recipient's own kidneys were removed. After incubation, the mAb was washed out of the donor kidney with PBS. The distal aorta and vena cava of the graft were connected to the re-
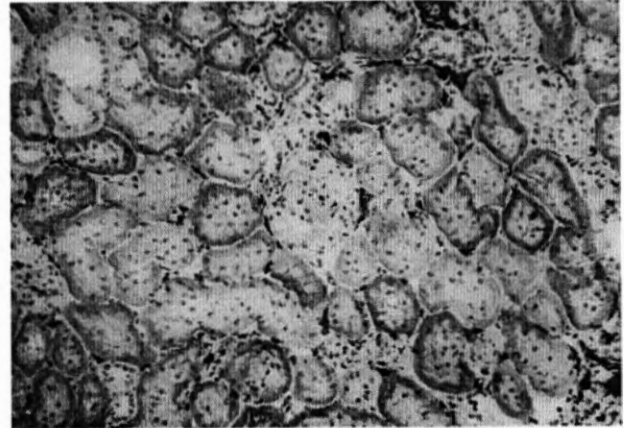

Fig. 1. Constitutive expression of MHC class II antigen in normal rat kidney. Sections of LEW rat kidney incubated with an anti-class II mAb (Ox6) and identified by the immunoperoxidase method. Interstitial dendritic cells and the cytoplasm of epithelial cells in proximal tubules are stained

spective structures of the recipient via end-to-side anastomoses. A ureter-ureter anastomosis was constructed over a fine polyethylene internal stent. The warm ischemia time did not exceed 20 min. If an obvious technical failure accounted for the death of the recipient, the animal was excluded from the study. Kidneys were transplanted in the following groups: (a) LEW to LEW for technical control after kidney perfusion; (b) group 1: DA to LEW, kidneys perfused with PBS; (c) group 2: DA to LEW, kidneys perfused with mAB 29A1; (d) group 3: LEW to DA, kidneys perfused with mAb 29A1; and (e) group 4: DA to LEW, kidneys perfused with mAb 29A1, anti-mouse IgG, and complement or mAb 29A1 and complement. Graft rejection was defined as death of the animal.

Results

Constitutive expression of MHC class II antigens in the rat kidney

Our first study dealt with the constitutive expression of class II antigens on rat kidney cells because we wanted to know how many of these cells bind the mAb when the graft has been perfused with an anti-class II mAb. Table 1 presents the results of the in vitro immunohistological analysis of the labelling of class II-positive cells with mAb 29A1. The localization of these cells was predominantly interstitial, especially between renal tubules. The glomeruli contained some densely stained cells, localized within the mesangium. The mean number of class II-positive cells per square millimeter varied significantly between rat strains ($P < 0.01$). Figure 1 shows the constitutive expression of class II antigen in the normal rat kidney.

Ontogenesis of class II expression in the rat kidney

Table 1 also presents results of the analysis of class II antigen expression by cells of the rat kidney in various age groups in three studied strains. In all of these strains, 3-week-old rats had a significantly lower number of posi-
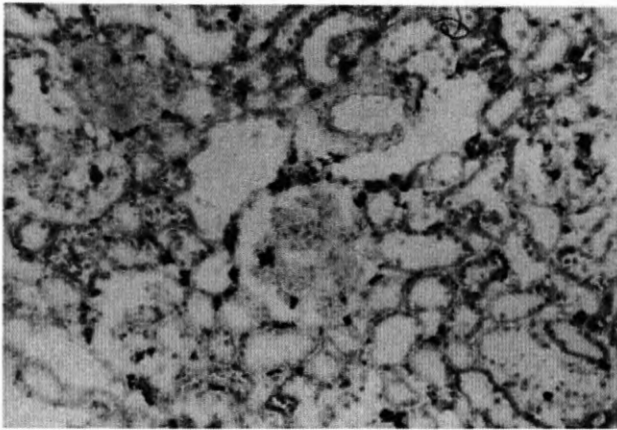
Table 1. Survey of MHC class II antigen expression on cells of the normal rat kidney in various inbred strains and an analysis of its ontogenesis

Rat strain	Age (weeks)	Class II-positive cells				Staining intensity of dendritic cells[a]
		Cells/glomerulus		Interstitial cells/mm²		
		n	$\bar{x} \pm SD$	n	$\bar{x} \pm SD$	
DA	3	18	0.1 ± 0.3	11	103 ± 30	2 +
(RT1 a)	6	21	0.8 ± 0.7	11	96 ± 25	4 +
	9	21	1.6 ± 1.4	11	113 ± 19	4 +
	12	36	0.9 ± 1.0	39	78 ± 24	4 +
	3– 6		$P < 0.05$		NS	
	3– 9		$P < 0.05$		NS	
	3–12		$P < 0.05$		NS	
LEW	3	19	0	23	19 ± 11	1 +
(RT1 l)	6	35	0.6 ± 0.7	19	23 ± 11	2 +
	9	24	1.5 ± 1.2	19	37 ± 16	3 +
	12	64	1.2 ± 1.4	38	38 ± 21	4 +
	3– 6		NS		NS	
	3– 9		NS		$P < 0.01$	
	3–12		NS		$P < 0.01$	
CAP	3	29	0.1 ± 0.3	15	78 ± 30	3 +
(RT1 c)	6	24	2.2 ± 1.5	14	80 ± 16	4 +
	9	20	3.4 ± 1.6	11	127 ± 26	4 +
	12	70	2.2 ± 1.5	27	78 ± 23	4 +
	3– 6		$P < 0.01$		NS	
	3– 9		$P < 0.01$		$P < 0.05$	
	3–12		$P < 0.01$		NS	
DA 12 – LEW 12			NS		$P < 0.01$	
CAP 12 – LEW 12			$P < 0.05$		$P < 0.01$	

[a] Grading of staining intensity: 1 +, weak; 2 +, medium; 3 +, strong; 4 +, very strong

Fig.2. Labelling of class II-positive cells in LEW rat kidney after perfusion with mAb 29A1 and 60 min of incubation. Sections of kidney incubated with anti-immunoglobulin conjugated with peroxidase. Staining of interstitial dendritic cells between tubules

tive cells per glomerulus than 6- and 12-week-old rats ($P < 0.05$–0.01). The number of class II-positive interstitial cells in 3-week-old DA and CAP rats was similar to that in 12-week-old rats. In kidneys from the LEW strain, however, the number of class II-positive interstitial cells was significantly smaller than in 9- and 12-week-old rats ($P < 0.01$), and the staining intensity was weaker.

Labelling of class II-positive cells by monoclonal antibody

Table 2 presents the results of the binding of class II-positive cells by ex vivo perfusion with mAb 29A1, followed by incubation for varying periods of time. These studies showed that perfusion with the mAb and a 60-min incubation procedure are sufficient to label all cells that express class II antigen. Therefore, this procedure was accepted for pretreatment of grafts prior to transplantation. An example of class II antigen-positive cells labelled by the mAb is presented in Fig. 2.

Rat kidney transplantation

Table 3 presents the results of kidney transplantation with and without graft pretreatment with mAb 29A1. Blockade of class II-positive cells with the mAb in a perfusion procedure was able to prolong graft survival significantly. To investigate how mAb pretreatment of grafts with varying densities of interstitial DC affects graft survival, transplantation was done in two different systems. In the first – DA-to-LEW – the graft had a high density of DC, and in the second – LEW-to-DA – the density was low. Yet, no difference in graft survival was found between these groups. IgG1 mouse antibody is known not to activate complement. Therefore, to induce cytolysis of DC within grafts, perfusion was followed by a second perfusion with anti-mouse immunoglobulin and complement or

complement alone. These procedures did not, however, prolong graft survival either.

Analysis of dissociation of the mAb from cells after transplantation

To answer the question how long the mAb is bound to class II-positive cells after transplantation, three kidneys in each group were explanted after 4, 8, 24, and 48 h. Sections of all kidneys were stained with Ox3, Ox6, or anti-immunoglobulin conjugated with peroxidase only (to detect mAb 29A1 on the cell surface). Four hours after transplantation, 37.5 ± 2.4 DC/mm^2 were labelled and

Table 2. Labelling of cells expressing class II MHC antigen by various perfusion-incubation procedures with the anti-class II monoclonal antibody 29A1

Rat strain	No. of organs	Incubation time (min)	Cells marked with mAb 29A1				Staining intensity[a]
			Cells/glomerulus		Interstitial DC/mm^2		
			n	$\bar{x} \pm SD$	n	$\bar{x} \pm SD$	
DA	2	30	41	1.8 ± 1.8	24	73 ± 19	2+
	2	60	21	1.7 ± 1.5	17	100 ± 32	3+
	2	120	40	1.5 ± 1.0	21	95 ± 23	3+
		30-Normal expression[b]		NS		NS	
		60-Normal expression[b]		NS		NS	
		120-Normal expression[b]		NS		NS	
LEW	2	30	36	0.8 ± 0.8	11	50 ± 18	2+
	1	60	40	0.6 ± 0.5	22	50 ± 17	2+
	2	120	35	1.5 ± 2.1	50	53 ± 17	3+
		30-Normal expression[b]		NS		NS	
		60-Normal expression[b]		NS		NS	
		120-Normal expression[b]		NS		$P < 0.05$	
CAP	1	20	38	1.1 ± 0.9	15	8 ± 10	1+
	2	30	30	2.6 ± 1.4	20	89 ± 18	2+
	2	60	43	2.0 ± 1.2	25	91 ± 28	3+
	2	120	47	1.6 ± 1.2	20	86 ± 24	3+
		20–30		$P < 0.02$		$P < 0.001$	
		20-Normal expression[b]		$P < 0.02$		$P < 0.001$	
		30-Normal expression[b]		NS		NS	
		60-Normal expression[b]		NS		NS	
		120-Normal expression[b]		NS		NS	

[a] Grading of staining intensity: 1+, weak; 2+, medium; 3+, strong; 4+, very strong
[b] Normal expression: number of class II-positive cells in in vitro staining of 12-week-old rat kidney

Table 3. Results of kidney transplantations in the rats

| Donor strain	Recipient strain	No. of animals	Pretreatment of the graft	Graft survival (days)	$\bar{x} \pm SD$
LEW	LEW	2	Perfusion mAb 29A1	> 150, > 150	> 150
DA	LEW	10	Perfusion PBS	7, 8, 7, 8, 8, 7, 8, 8, 8, 8	7.7 ± 0.5
DA	LEW	5	Perfusion mAb 29A1	10, 9, 11, 9, 9	9.6 ± 0.8 $P < 0.02$
LEW	DA	3	Perfusion PBS	10, 8, 8	8.6 ± 1.1
LEW	DA	5	Perfusion mAb 29A1	9, 8, 9, 10, 9	9.0 ± 0.6
DA	LEW	5	Perfusion mAb 29A1 anti mouse IgG rat complement	9, 10, 8, 9	9.0 ± 0.8 $P < 0.05$
DA	LEW	2	Perfusion mAb 29A1 rabbit complement	8, 9	8.5 ± 0.5 NS
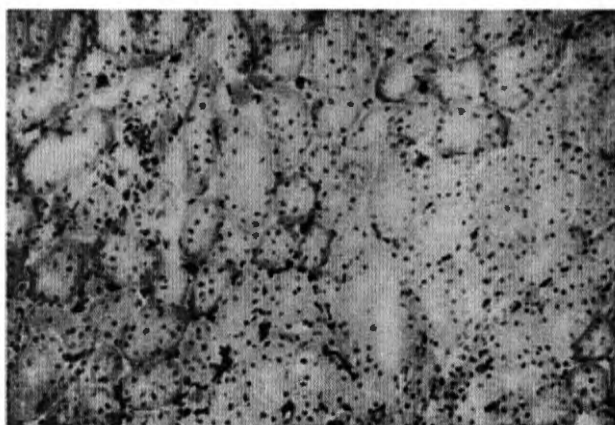
Fig. 3. Section of rat kidney perfused with mAb 29A1 and transplanted. Explantation done 4 h after grafting. Section incubated with anti-immunoglobulin conjugated with peroxidase. Interstitial dendritic cells are stained
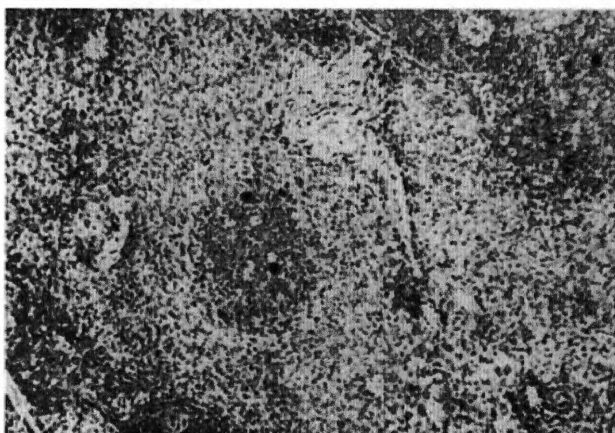

Fig. 4. Demonstration of donor-specific class II-positive cells in recipient's peripheral white pulp of spleen. Spleen of DA rat transplanted with LEW kidney. Section stained with anti-donor-specific class II mAb (Ox3)

proximal tubular cells were weakly stained (Fig. 3). Sections stained with Ox3 and Ox6 showed 42.9 ± 5.8 and 83.8 ± 1.5/mm² class II-positive cells. Eight hours postoperatively, the number of labelled interstitial DC was 29.3 ± 4.2/mm². Staining of additional sections with mAb Ox3 showed 33.8 ± 9.0/mm² donor-specific class II-positive cells. Twenty-four hours after transplantation, only sporadic cells were weakly stained (6.5 ± 5.3/mm²). At the same time, staining of kidney sections with anti-class II mAb Ox6 demonstrated the presence of 48.1 ± 8.0/mm² positive cells in interstitial tissue. Additional sections stained with anti-donor-specific class II antigen Ox3 (in the LEW-to-DA system) showed 28.6 ± 2.7 cells/mm² of donor origin. Thus, 24 h after transplantation, the number of donor DC was reduced and only 23% of them were labelled with mAb 29A1 given via perfusion. Forty-eight hours postoperatively, only a few cells was weakly stained, whereas 28.8 ± 7.1 cells/mm² reacted with Ox3. A similar number of Ox3-positive cells were observed on day 3 posttransplantation. These results showed that dissociation of the mAb begins earlier than emigration of DC and that

not all DC leave the graft during the first 3 days after transplantation.

Migration of cells between the host and the graft

On the basis of the observation that some regions of the kidney sections made 24–48 h after transplantation (LEW-DA) are depleted or even free of DC, recipient spleen and lymph nodes were analyzed for the presence of donor-specific class II cells. Donor-specific (LEW) class II-positive cells that reacted with mAb Ox3 were found in the peripheral white pulp of the recipient's (DA) spleen (Fig. 4). These cells were negative when stained with anti-mouse immunoglobulin to detect mAb 29A1 on their surfaces. There was a difference in the mean number of donor cells in the spleen between groups of rats pretreated with mAb 29A1 and those treated with PBS only (1.6 ± 0.6 versus 3.3 ± 1.7 cells per periarteriolar lymphoid sheath). In mesenteric lymph nodes of rats transplanted with organs perfused with PBS only, sporadic cells of donor class II antigen were found, whereas in those perfused with mAb 29A1, none were found.

Immunomorphological studies performed with MRC Ox3 in the DA-to-LEW system demonstrated that infiltration of the graft by recipient-specific class II cells begins 2 days after transplantation.

Discussion

Class II-positive interstitial DC are able to present alloantigen as well as to act as accessory cells [11, 15], and they constitute a major component of the renal cells that may induce graft rejection. Therefore, studies on blockade or elimination of these cells from the graft have been proposed in the hope of prolonging the survival of the graft. Several studies have been done with organs perfused with anti-MHC class II mAbs.

In 1983, Otsubo et al. [17] demonstrated that after perfusion of canine kidneys with anti-mouse class II antigens crossreacting with canine class II antigen, it is possible to prolong graft survival by a mean value of 15 days (7 days in the control group). On the other side, Pollak et al. [18], who used the anti-rat class II mAb Ox4, showed that this perfusion did not alter the immunogenicity of the graft, as there was no prolongation of renal graft survival. Jablonski et al. [9] obtained similar results.

Studies on pancreas transplantation have shown that a blockade of class II-positive cells in the graft did not change graft survival [4, 13, 14, 23]. In these studies, the effectiveness of the DC blockade was analyzed. They indicated that nearly all cells were labelled.

To better define the effectiveness of the procedure used for kidney graft pretreatment, we analyzed MHC class II antigen expression in the normal rat kidney. The influence of age on class II antigen expression was also studied. The results confirmed and extended earlier studies on class II antigen expression in the kidney, which were, however, not quantitative [5, 6]. Our studies showed that all class II-positive DC were labelled after a procedure that included perfusion and 60 min of incubation.

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Further studies have shown that this well-controlled procedure for graft pretreatment prolongs the graft's survival. The extent to which it does is not satisfactory, however, from the biological point of view. In light of these results, the question arose as to whether dissociation of an antibody is a factor responsible for this only short prolongation. Indeed, the studies done on sections of kidneys explanted at various time intervals after transplantation showed that the mAb became dissociated from the cell surface some hours after transplantation.

For several years the view has been accepted that sensitization of the host against kidney allograft occurs peripherally, within the graft. When we discovered in sections of our grafts explanted 24 and 48 h after transplantation that some regions of kidney tissue were free of DC, we considered this finding to be an effect of cytolysis induced by the mAb, allowing reduced graft immunogenicity. However, the brief survival of the perfused grafts led us to consider the alternative possibility that DC had migrated out of the graft and into the lymphatic tissue of the recipient and, thus, participated in the induction of graft rejection. For this reason we performed immunomorphological studies on the recipient's spleen and lymph nodes. As described above, it was found that as early as 1 day post-transplantation, donor-specific class II-positive DC begin to leave the graft and migrate into lymphatic tissues, preferentially the spleen. Similar studies on animals grafted with kidneys perfused with a mAb have shown that this procedure results in decreased migration of donor cells into the recipient's lymphatic tissues. Early migration of DC from a cardiac graft into the recipient's spleen and localization in peripheral white pulp was observed by Larsen et al. [10].

Taken together, this suggests that the reduction of DC within the graft explanted 24 and 48 h post-transplantation was the result of both depletion of their population and migration out of the graft.

We wish to point out that the number of class II-positive DC within the graft should be considered with caution, because some days after transplantation other cells, such as T lymphocytes or tubular epithelial cells, may be induced to express MHC class II antigen. In our perfusion experiments, however, induction of class II antigen on other cells may have been delayed, a suggestion that is supported by our earlier observation that only after mAb perfusion was class II antigen not expressed on distal tubular cells 4 days after transplantation.

Furthermore, considering that the mAb of IgG1 class, which we used in our study, does not activate complement, with the result that it becomes sufficiently cytolytic, the fact that some donor DC persisted within the graft is understandable. However, the combined perfusions with mAb, polyclonal anti-mouse immunoglobulin, and complement did not further prolong graft survival. Nor did destruction of DC within grafts by mAb targeted with A-chain ricin as immunotoxin [23] or by irradiation [9] inhibit graft rejection or prolong graft survival. Studies by Heart at al. [7] on retransplantation of rat kidney to another host showed only marginal prolongation of allogeneic graft survival. However, retransplantation of long-term surviving (DA x LEW) F1 grafts to fresh DA hosts,

resulted in very prolonged survival. The authors suggest that this result depends on the loss of donor DC from the graft.

On the second day after transplantation, we observed the beginning of infiltration of the graft by host cells. It may, therefore, be concluded that there is a bidirectional cell migration system between graft and host.

Our transplantation experiments showed that the survival of perfused grafts is only prolonged for about 3 days. This finding is in accordance with the observation that donor DC do not remain labelled with mAb for longer than 24 h. After that time, DC retrieved their alloantigen presentation ability and induced rejection.

Both migrating elements may participate in the induction of rejection by: (a) presentation of alloantigen by donor cells in the graft and later on in the recipient's spleen and (b) penetration of host cells into the graft and processing and presentation of alloantigen to T helper cells. The latter manner of alloantigen presentation has been suggested by Lechler and Batchelor [11], studied by Ishikura et al. [8], and confirmed by Sherwood et al. [20]. Participation of host cells in alloantigen presentation is good reason for combining mAb perfusion procedures with immunosuppression of the recipient. Thus, it may become possible (a) to apply appropriately reduced doses of immunosuppressive drugs and (b) to induce tolerance, allowing such treatment to be stopped several weeks or months after transplantation. Very promising studies along this line are in progress in our laboratory.

References

1. Autenried P, Halloran PF (1985) Cyclosporine blocks the induction of class I and class II MHC products in mouse kidney by graft-vs-host disease. J Immunol 135: 3922–3929
2. Bishop GA, Hall BM, Suranyi MG, Tiller DJ, Horvath JS, Duggin GG (1986) Expression of HLA antigens on renal tubular cells in culture. I. Evidence that mixed lymphocyte culture supernatants and gamma interferon increase both class I and class II HLA antigens. Transplantation 42: 671–678
3. Darden AG, Forbes RDC, Parfrey NA, Darden PM, Guttmann RD (1987) Genetics of Ia + interstitial cells in the heart and pancreas of inbred and congenic rats. Transplant Proc 19: 3047–3049
4. Dekker CC, Spillenaar EJ (1988) The effect of reduction of class II antigens in the graft on the survival of pancreatic allografts. Transplant Proc 21: 1770–1772
5. Halloran PF, Stylianos SK, Stubbs M (1981) Ia expression in mouse kidney. Transplant Proc 13: 1008–1010
6. Hart DNJ, Fabre JW (1981) Demonstration and characterization of Ia positive dendritic cells in the interstitial connective tissues of rat heart and other tissues, but not brain. J Exp Med 153: 347–361
7. Hart DNJ, Vinearls CG, Fabre JW (1980) Graft adaptation: studies on possible mechanisms in long-term surviving rat renal allografts. Transplantation 30: 73–80
8. Ishikura H, Natori T, Aizawa M (1985) Nonphagocytic, Ia-bearing cells of host origin in acutely rejected rat renal allografts. Transplantation 39: 561–563
9. Jablonski P, Kraft N, Howden B, Thomson NM, Atkins RC, Marshall VC (1989) Transplantation of rat kidneys following perfu-

sion with anti-Ia monoclonal antibody. Transplant Proc 21: 1123–1124

10. Larsen CP, Morris PJ, Austin JM (1990) Migration of dendritic leukocytes from cardiac allografts into host spleens. J Exp Med 171: 307–314

11. Lechler RI, Batchelor JR (1982) Restoration of immunogenicity to passenger cell-depleted kidney allografts by the addition of donor strain dendritic cells. J Exp Med 155: 31–41

12. Lee S (1967) An improved technique of the renal transplantation in the rat. Surgery 61: 771–773

13. Lloyd DM, Franklin W, Buckingham F, Buckingham M, Rizner JS, Stuart FP, Thistlethwaite JR (1987) Ex-vivo perfusion of the intact rat pancreas with anti-class II monoclonal antibody: labeling of dendritic cells. Transplant Proc 19: 620–624

14. Lloyd DM, Buckingham M, Stuart F, Thistlethwaite JR (1989) Does depletion of donor dendritic cells in an allograft lead to prolongation of graft survival on transplantation? Transplant Proc 21: 482–483

15. McKenzie JL, Beard MEJ, Hart DNJ (1984) The effect of donor pretreatment on interstitial cell content and rat cardiac allograft survival. Transplantation 38: 371–376

16. Milton AD, Spencer SC, Fabre JW (1986) Detailed analysis and demonstration of differences in the kinetics of induction of class I and class II major histocompatibility complex antigens in rejecting cardiac and kidney allografts in the rat. Transplantation 41: 499–508

17. Otsubo O, Sakai A, Watanabe T, Inou T, Takahashi I, Okumura K, Yamamoto K, Kitagawa H (1983) Effect of anti-mouse Ia

18. Pollak R, Blanchard JM, Mozes MF (1987) Strategies used to alter the immunogenicity of vascularized organ allografts. Transplant Proc 19: 258–261

19. Ruers TJM, Sproken EEM, Buurman WA (1987) The influence of prednisolone on the regulation of major histocompatibility complex class II expression. Transplant Proc 19: 4227–4229

20. Sherwood RA, Brent L, Rayfield LS (1986) Presentation of alloantigens by host cells. Eur J Immunol 16: 569–574

21. Ulrichs K, Nötling R, Keller R, Heusermann U, Müller-Ruchholtz W (1987) Genetically determined variation of constitutive major histocompatibility complex class II antigen expression in various rat strains and cell types. Transplant Proc 19: 181–186

22. Warren MK, Vogel SN (1985) Opposing effects of glucocorticoids on interferon gamma induced murine macrophage Fc receptor and Ia antigen expression. J Immunol 134: 2462–2469

23. Wiley KN, Powell CS, Henry L, Clark A, Fox M (1988) An A-chain ricin immunotoxin targeted against rat class II major histocompatibility complex molecules. In vitro and in vivo effects. Transplantation 45: 1113–1118

24. Willebrand E von, Pettersson E, Ahonen J, Häyry P (1986) CMV infection, class II antigen expression, and human allograft rejection. Transplantation 42: 364–367

25. Ziegler HK, Staffileno LK, Wentworth P (1984) Modulation of macrophage Ia-expression by lipopolisaccharide. I. Induction of Ia expression in vivo. J Immunol 133: 1825–1835

monoclonal antibody on canine renal allograft survival. Transplant Proc 15: 797–799