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Short Communication

LONG-STANDING RAT KIDNEY GRAFT SURVIVAL BY A COMBINATION OF ORGAN PERFUSION WITH MHC CLASS II MONOCLONAL ANTIBODY AND IMMUNOSUPPRESSION WITH REDUCED DOSES OF 15-DEOXYSPERGUALIN.

M. Krzymanski, A. M. Waaga, K. Ulrichs and W. Müller-Ruchholtz

Department of Nephrology, Medical Academy of Poznan, Poland,
and Department of Immunology University of Kiel, D-2300 Kiel 1, FRG.

To decrease the immunogenicity of rat kidneys, grafts were perfused with an anti-MHC class II (coded by the I-E subregion) mouse monoclonal antibody (MoAb 29A1, Dept. of Immunol. Kiel) as described previously (5). How effectively class II-positive cells, which were mainly dendritic in appearance, were surface labelled by this procedure was checked by staining MoAb-perfused kidney sections with peroxidase-conjugated rabbit anti-mouse antiglobulin and comparing them with in vitro MoAb-incubated sections. The results are presented in Table I.

It was found that after 60 min. of perfusion/incubation with MoAb almost all dendritic cells were labelled. The labelling was superior to that after 30 min. but not inferior to that after 120 min. Therefore 60 min. perfusion/incubation was chosen for graft pretreatment before transplantation, which was done in the rat inbred strain system DA (RT1 a) to LEW (RT1 1). This procedure prolonged graft survival significantly though not satisfactorily from the biological point of view (9.6±0.8 versus 7.7±0.5 days in the control group, $p<0.02$). It was shown that the dendritic cells were not killed but only blocked. Several hours after transplantation the MoAb dissociated from these cells. Similar observations were made by Jablonski et

Table I: Extent of labelling of MHC class II surface-positive kidney cells with MoAb.

Rat strain	Primary antibody (MoAb) incubation	No. of kidneys studied	Labelled interstitial dendritic cells/mm^2				Immunoperoxidase staining intensity
			n	$\bar{x}$	±	SD	
LEW	in vitro	2	38	38	±	21	4 +
LEW	perfusion	2	22	50	±	18	3 +
DA	in vitro	2	39	78	±	24	4 +
DA	perfusion	2	17	100	±	32	3 +

n total number of investigated sectors (mm^2) per kidney; $\bar{x}$ arithmetic mean value; SD standard deviation.

Table II: Duration of labelling of MHC class II surface-positive kidney cells with MoAb.

Hours after grafting	Cells labelled with MoAb	Staining intensity
4	100% dendritic cells	+++
8	30-40% dendritic cells	++
24	single cells positive	++
48	all cells negative	-

al. (4), though the time after which MoAb dissociated from dendritic cells was shorter than in our study. The results are presented in Table II.

Furthermore, it was shown that during the first day after transplantation donor cells migrated into the recipient's spleen and mesenteric lymph nodes. The number of these cells located within the spleen's lymphoid follicles was smaller when the transplanted organ was perfused with MoAb

Table III: Kidney allograft survival time after 15-DOS treatment.

Dose of 15-DOS	Control group	0.05 mg/kg	0.2 mg/kg	0.3 mg/kg	0.5 mg/kg
Graft survival days	7.7±0.5	8.3±0.5	16.5±0.5	>150	>150
n	10	3	3	3	3
p			p<0.01		

(1.6±0.6 versus 3.3±1.7 per lymphoid follicle section in the control group). On the second day after transplantation infiltration of the graft by host class II-positive cells began. This was delayed by one day when the graft was perfused with MoAb. Taken together, these results indicate that perfusion of the graft with MoAb reduced the number of donor cells, temporarily inhibited their function and delayed the migration of recipient cells into the graft.

Our findings suggest that a bidirectional system of cell migration between the graft and the host exists which may provide both "central" and "peripheral" alloantigen presentation. In "central" sensitization donor cells participate by migration into the recipient's spleen. The phenomenon of dendritic cell migration was recently reviewed by Austyn and Larsen (2). In "peripheral" sensitization host cells participate by penetration into the graft, and donor cells by local antigen presentation. The role of host cells in alloantigen presentation has been studied and confirmed by Ishikura at al. (3), and Sherwood et al. (7). Ruers at al. (6) concluded from their studies that the local events of allograft sensitization play a crucial role in the rejection response.

Taking into consideration that graft rejection may be induced in these two ways we found it reasonable to combine MoAb perfusion of rat kidney with a low dose of the new immunosuppressive agent 15-deoxyspergualin (15-DOS) (1)

which is known to interfere with antigen presentation. To determine the minimum effective dose of 15-DOS, doses of 0.05, 0.2, 0.3 and 0.5 mg/kg were given intraperitoneally for 14 days to LEW rats receiving DA kidney grafts. Graft survival in rats treated with various doses of 15-DOS is presented in Table III.

On the basis of these results a dose of 0.2 mg/kg was selected for further studies. In LEW rats receiving MoAb-perfused kidney grafts, mean graft survival was 146±16 days (n=3). These data suggest that the reduction of graft immunogenicity achieved by perfusion with MoAb became biologically relevant only in combination with a low dose of 15-DOS. This low-dose, short-term treatment initiates long-standing survival of an MHC fully allogeneic organ graft.

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