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Class II antigen expression of epidermal Langerhans cells in renal allograft recipients

Mats Jontell[1], Håkan Gäbel[2], Sven-Christer Öhman[1], and Hans Brynger[2]

[1] Department of Oral Diagnosis, Faculty of Odontology, University of Gothenburg and Public Dental Service, S-433 00 Gothenburg, Sweden
[2] Department of Surgery, Sahlgrens Hospital, S-413 45 Gothenburg, Sweden

Abstract. This study was designed to investigate the influence of systemic immunosuppressive therapy on the HLA-DR expression of epidermal Langerhans cells. Fifteen renal allograft recipients immunosuppressed with cyclosporin A and steroids were studied. Skin biopsies were taken from the upper arm prior to transplantation and at different intervals during the post-transplantation period. The epidermis was separated from the dermis, and the epidermal sheet was subjected to immunohistochemistry in order to make the HLA-DR antigens on the Langerhans cells visible. Following 2 days of immunosuppression, the number of Langerhans cells expressing HLA-DR antigens started to decrease and after 1 week, only 60% of the initial number of positive cells were detected. The number of positive cells remained low throughout the experimental period. It is suggested that systemic immunosuppressive therapy will suppress the expression of HLA-DR antigens on epidermal Langerhans cells, something which may mirror a systemic effect on other antigen-presenting cells.

Key words: HLA-DR - Immunosuppressive therapy - Cyclosporin A - Immunohistochemistry.

Epidermal Langerhans cells have been designated an antigen-presenting role in the immunosurveillance system of the skin [9, 19]. These suprabasilar dendritic cells are of mesodermal origin and express class II transplantation antigens encoded in the major histocompatibility complex. These cell surface antigens have to be presented together with the foreign antigen in order to initiate a proliferative response of T-lymphocytes (for review see [13]). This proliferation of T-lymphocytes is believed to be essential in order to elicit the immune response.

Offprint requests to: H. Brynger

Administration of immunosuppressive drugs has been shown to decrease the number of epidermal Langerhans cells demonstrated by cell surface ATPase activity [2, 6, 15, 17]. In animal experiments, both topical and systemic administration reportedly have a similar effect on the number of class II antigen-expressing Langerhans cells [1, 15] and endothelial cells [11]. However, information is still lacking about the influence of systemic immunosuppressive therapy on human class II antigen-expressing Langerhans cells. Therefore, the present investigation was designed to answer two questions. First, does systemic immunosuppression affect the expression of HLA-DR antigens on human epidermal Langerhans cells? Second, if an effect is observed, at what time following the administration of the immunosuppressive drugs does this effect occur?

Material and methods

Patients

Fifteen consecutive renal allograft recipients were included in the study. The demographic and clinical data are given in Table 1. The immunosuppression was achieved by administration of cyclosporin A and steroids (Table 2). A nonimmunosuppressed recipient of an isograft from his twin donor (one-egg twin) served as the control.[1]

Biopsies

All 15 patients were biopsied preoperatively. Ten of these patients had biopsies once a week for 10 weeks while five were subjected to a biopsy daily during the first week. The biopsies were taken under local anesthesia (Carbocain, Astra, Södertälje, Sweden) from the inside of the upper arm with a 3-mm punch.

[1] This study was approved by the Ethics Committee of the Medical Faculty, University of Gothenburg

Table 1. Demographic features of patients studied. CGN, Chronic glomerulonephritis; CPN, chronic pyelonephritis; PCK, polycystic kidney disease; SLE, systemic lupus; CD, cadaver donor; LRD, living related donor

	Sex	Age (years)	Renal disease	Dialysis/ predialytic	Source of renal allograft	Immuno-suppression
Group A (patients having biopsies once a week for 10 weeks)						
1	M	67	CGN	Dialysis	CD	CyA + steroids
2	M	56	Nephrosclerosis	Predialytic	LRD (1 shared haplotype)	CyA + steroids
3	F	57	SLE	Dialysis	CD	CyA + steroids
4	M	53	CGN	Dialysis	CD	CyA + steroids
5	F	46	Diabetes	Dialysis	LRD	CyA + steroids
6	F	59	CPN	Dialysis	CD	CyA + steroids
7	F	47	PCK	Predialytic	LRD (HLA-identical)	Azathioprine + steroids
8	M	38	CGN	Dialysis	LRD (1 shared haplotype)	CyA + steroids
9	M	14	PCK	Dialysis	LRD (1 shared haplotype)	CyA + steroids
10	F	53	CGN	Dialysis	CD	CyA + steroids
Group B (patients having biopsies once a day for 7 days)						
11	M	63	CGN	Dialysis	CD	CyA + steroids
12	F	66	CGN	Dialysis	CD	CyA + steroids
13	M	40	CGN	Predialytic	LRD (1 shared haplotype)	CyA + steroids
14	M	34	Diabetes	Dialysis	CD	CyA + steroids
15	M	28	CGN	Predialytic	LRD (1 shared haplotype)	CyA + steroids
Control						
16	M	21	CGN	Dialysis	LRD (isograft)	None

Table 2. Immunosuppressive therapy

	Cyclosporin A (mg/kg)	Steroids (mg)
Preoperatively	10 (i.v.)	Prednisolone: 50
Peroperatively	-	Hydrocortisone: 500
Postoperatively	-	Prednisolone: 50
Days 1-13	15 (orally)	Day 1: 90
		Tapered to:
14	13	Day 8: 20
30	11	Day 30: 15
60	9	Day 60: 12.5

The biopsies were kept in Histocon (Histolab, Bethlehem Tradings, Gothenburg, Sweden) at +4°C until they were placed on a filter paper strip, frozen to −70°C, and stored at the same temperature until further use.

Immunohistochemical staining

The biopsies were floated, dermis side down, on an EDTA solution for 60 min at +37°C [14]. The epidermis was easily separated from the dermis with forceps. The epidermal sheets were fixed for 10 min at +4°C in an acetone/water solution (1:1), and additional fixation was achieved with 100% acetone. The sheets were washed in phosphate-buffered saline (PBS) for three 20-min cycles. This washing procedure was repeated between each of the subsequent three incubations. Monoclonal mouse antihuman HLA-DR (clone L243, Becton Dickinson, Sunnyvale, Calif.) was used as the primary antibody and diluted 1:80 in PBS. This and the subsequent incubations were performed at +4°C for 8 h. The sheets were further incubated in biotinylated horse-anti-mouse IgG (1:350, Vector Laboratories, Burlingham, Calif.). The final incubation was performed in an FITC-avidin conjugate (1:200, Vector Laboratories, Burlingham, Calif.). The specimens were mounted in a gelatin/glycerin solution with the dermis side up. Specificity of the staining reaction was tested by incubation without primary antibody. The sheets were examined on the day of incubation in a Leitz Orthoplan microscope with an Osram HBO 200 W lamp equipped for incident light excitation with a Ploemopak 2.1 fluorescent illuminator. The filter blocks used were specifically for FITC-illumination.

Photomicrographs were taken with an Orthomat camera using Ectachrome 400 film. Five photomicrographs were taken of each sheet of epidermis, representing 8.2% of the total epidermal surface. A cell was required to have a bright fluorescence with at least one dendritic structure to be deemed an HLA-DR-expressing Langerhans cell. The numbers of cells were counted on projected slides and read by three independent examiners without their knowledge of the treatment.

Statistical analysis

The mean variance was calculated to estimate the measurement error. The significance of the change in number of Langerhans cells was determined by Student's *t*-test for paired samples [10].

Results

Prior to immunosuppression, the HLA-DR-expressing Langerhans cells were found to be evenly distributed in the epidermal sheets (Fig. 1). The highly dendritic appearance and the expression of HLA-DR antigens made identification as Langerhans cells rather easy. The mean number of HLA-DR-expressing cells per mm^2 was calculated to be 377 (Fig. 2).

Following the administration of immunosuppressive drugs, the mean number of HLA-DR antigen-bearing cells was calculated to be

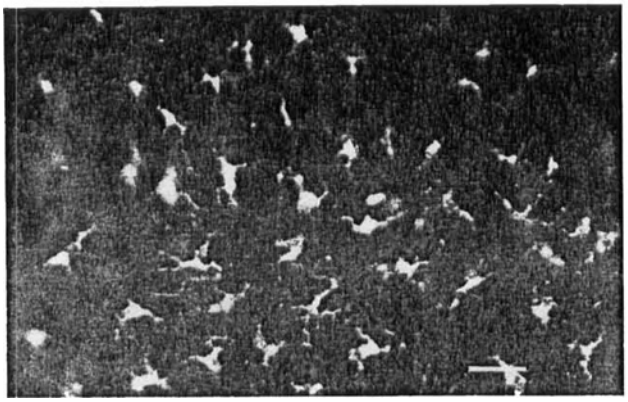

Fig. 1. Immunofluorescent HLA-DR expressing Langerhans cells in an epidermal sheet one day prior to administration of immunosuppressive therapy (×365). *Bar* represents 20 μm

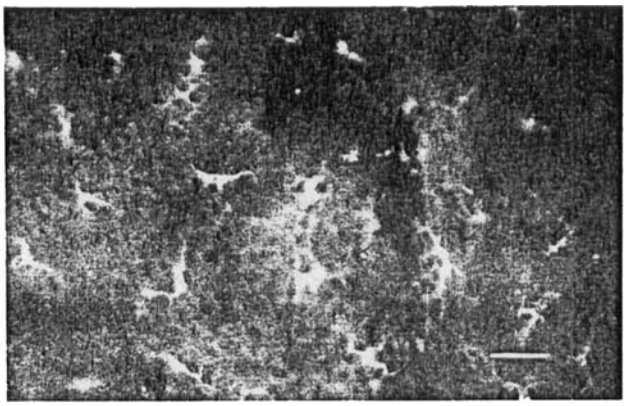

Fig. 3. Immunohistochemistry of an epidermal sheet 15 days after immunosuppression had been instituted. The number of HLA-expressing Langerhans cells are fewer compared to Fig. 1 (×365). *Bar* represents 20 μm

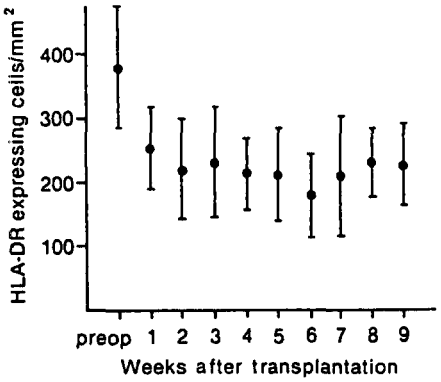

Fig. 2. Number of HLA-DR positive Langerhans cells/mm² of epidermal tissue examined preoperatively and weekly for 9 weeks following transplantation in 10 renal allograft recipients

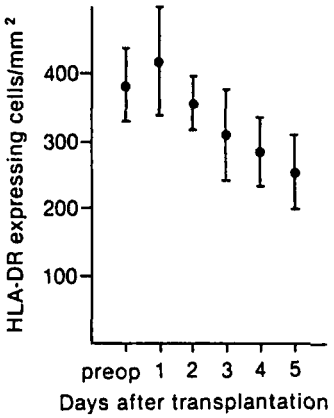

Fig. 4. Number of Langerhans cells expressing HLA-DR antigens per mm² of epidermal tissue examined preoperatively and daily for 5 days following transplantation in 5 renal allograft recipients

226 cells/mm², a decrease of 40% ($P<0.05$; Figs. 2, 3). The number of cells remained low throughout the experimental period and did not increase in concert with the decrease in dosage of the immunosuppressive drugs. The major reduction in the number of HLA-DR-expressing cells was seen during the first 5 days after the introduction of immunosuppression (Fig. 4). The recipient of the isograft and his donor had the same number of HLA-DR-expressing epidermal cells before and after transplantation.

Discussion

The present data show that systemic immunosuppressive therapy results in a rapid decrease in the number of HLA-DR-expressing Langerhans cells. It is unlikely that this decrease is the result of the allografting procedure itself as the effect did not appear in the epidermis of the isograft recipient. Furthermore, the number of cells prior to immunosuppressive therapy was found to be in the same range as that reported for healthy individuals [5]. Thus, compromised renal function did not seem to have a negative influence on the frequency of HLA-DR expression.

The present observations may have implications beyond the effect on Langerhans cells since other HLA-DR-expressing dendritic cells and macrophages, which also have an antigen-presenting capacity [16, 18], might be affected as well. However, mixed-lymphocyte reactions (MLR) with epidermal cells, serving as antigen-presenting cells, seem to be more sensitive to immunosuppressive therapy than MLR with peripheral mononuclear blood cells acting as accessory cells [4]. This indicates that immunosuppressive therapy more effectively suppresses the HLA-DR antigens on epidermal cells than on mononuclear blood cells.

In a recent report, the observation was made that topical but not systemic administration of immunosuppressive drugs diminishes the number of ATPase-positive Langerhans cells [12]. It was suggested that only long-term systemic drug treatment would cause a substantial reduction in the

number of Langerhans cells. Such a long-term effect has been demonstrated by Sontheimer et al. [17]. This suggestion is not in agreement with the findings of the present study, where an effect was demonstrated after a few days, using HLA-DR antigens as a marker for Langerhans cells. However, controversy exists over whether ATPase activity and the expression of HLA-DR antigens should be considered equivalent for the enumeration of Langerhans cells [2, 3]. A decrease in HLA-DR-expressing cells does not necessarily imply a decrease in ATPase-positive Langerhans cells [3]. It may be better to use ATPase activity as a numerical marker and the expression of HLA-DR antigens as a functional marker of the Langerhans cells' antigen-presenting capacity. To evaluate the effect of immunosuppressive therapy on the functional state of Langerhans cells, the HLA-DR marker is probably more relevant than the ATPase activity.

It is uncertain whether the decrease in HLA-DR-bearing Langerhans cells is the result of a decreased number of cells or whether the synthesis of the HLA-DR antigens is suppressed. The rapid down-regulation of HLA-DR-expressing cells may suggest that the synthesis of HLA-DR antigens is affected. A similar decrease over time has been observed after intraperitoneal injections of steroids in guinea pigs [2]. A decrease in the expression of class II antigens, rather than a decreased number of cells, might thus be responsible for the diminished function of the Langerhans cells following immunosuppression [8]. A deficient function of these cells may interfere with the presentation of tumor antigens. In addition, tolerance of these antigens may explain the increased incidence of cutaneous tumors in patients subjected to immunosuppressive therapy [7]. However, further studies are needed to determine whether patients with contracted skin tumors have a decreased frequency of HLA-DR-expressing Langerhans cells compared to other immunosuppressed patients.

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