Virchows Archiv A Pathol Anat (1991) 418:105–110

Virchows Archiv A
Pathological Anatomy and Histopathology
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Interdigitating reticulum cells in human renal grafts

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Received May 8, 1990 / Received after revision Oktober 25, 1990 / Accepted October 26, 1990

Summary. Seventeen human renal graft biopsies taken 1 h to 50 days after transplantation and 3 human renal non-graft biopsies (2 minimal change and 1 non-tumour portion of angiomyolipoma) were investigated with immunoelectron microscopy in order to identify interdigitating reticulum cells (IDC) or dendritic cells (DC) in renal tissues. The antibodies used consisted of a rabbit polyclonal antibody of antihuman S100β protein, mouse monoclonal antibodies of antihuman HLA-DR, anti-CD3, and anti-CD1a. IDC or DC were identified in 11 renal grafts. They were found both in the glomerular and interstitial (peritubular) capillary lumens but not in the interstitium of 1 case; both were present in the interstitial capillary lumens and interstitium of another case, and in the interstitium only of 9 cases. In the remaining 6 grafts and 3 non-grafts they were not detected. These 6 grafts and 3 non-grafts did not show any pathological change except for foot process fusion of the glomerular epithelia in 2 cases of minimal change. These findings suggest that IDC or DC are not normally present in human renal tissues. The presence of the cell in the glomerular and peritubular capillary lumens of a biopsy taken after 1 h and their presence in the interstitial capillary lumens of another graft biopsy, suggest that the IDC or DC in human renal grafts are derived from recipients, not donors, and that they migrate from the circulating blood toward the interstitium.

Key words: Human renal graft – Interdigitating reticulum cell – Dendritic cell – S100β protein – Immunoelectron microscopy

Introduction

The interdigitating reticulum cell (IDC) was first found in the thymus-dependent area of the rabbit lymph node by Veldman (1970) and later in the similar area of the rat spleen by Veerman (1974). The dendritic cell (DC) was found in cultured cells of the mouse spleen by Steinman and Cohn (1973). Later Steinman et al. (1975) identified it in situ in the mouse spleen.

Nowadays the IDC and DC are thought to be the same type of cell with different designations. The DC has been regarded as a potent stimulator of the mixed lymphocyte reaction and as an antigen-presenting cell to T lymphocytes not only in mice (Nussenzweig et al. 1980; Sunshine et al. 1980; Austyn et al. 1983) and rats (Phillips et al. 1980; Klinkert et al. 1980) but also in humans (Van Voorhis et al. 1982; Kuntz Crow and Kunkel 1982; Van Voorhis 1983). It was Lechler and Batchelor (1982) who first focused on the DC in renal transplantation. Later, several papers (Raftery et al. 1983; Ishikura et al. 1985; Milton et al. 1986) appeared which studied the role of the DC in renal acute rejection. To date it is controversial as to whether, donors or recipient DC are involved in acute rejection.

However the distribution of the IDC or DC in a normal kidney (Raftery et al. 1983; Alpers and Beckstead 1985) and other non-lymphoid tissues (Hart and Fabre 1981; Daar et al. 1983) has been extensively investigated. All these reports except for one (Alpers and Beckstead 1985) concluded that IDC or DC were present in various normal tissues. The investigators arrived at this conclusion by using the techniques of immunofluorescence, immunoperoxidase or histochemistry; none of them used immunoelectron microscopy. We have identified IDC in biopsied human renal grafts by immunoelectron microscopy but conclude that they are not normally present in renal tissues. IDC in human renal grafts are thought to be derived from recipients. We emphasize the reliability of immunoelectron microscopy for identification of IDC or DC.

Patients and methods

The patients were 14 males and 3 females, from 17 to 37 years in age. The donors consisted of 2 unrelated cadavers, a brother,

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an identical sibling, an identical twin, 6 mothers and 6 fathers. The age range of the donors was 25–71 years. All patients except for 2 received random blood transfusions before transplantation. One of the 2 patients received donor-specific blood transfusions only and the other did not receive any blood transfusions prior to transplantation. Biopsy was done 1 h to 50 days after transplantation. One-hour biopsy was performed in 5 patients. At the time of biopsy clinical rejection was absent in all the patients except for 1. All patients were administered daily dosages of cyclosporine A, azathioprine and prednisolone. The biopsied specimens of 16 renal allografts, an isograft, and two non-grafts of minimal change, and the non-tumour portion of a non-graft carrying angiomyolipoma were examined by light and immunoelectron microscopy. For light microscopic observation a large portion of each biopsy was processed routinely and stained with haematoxylin and eosin, periodic acid-Schiff reaction, Masson-Goldner method, elastic-van-Giesen, periodic acid-methenamine silver, and phosphotungstic acid-haematoxylin. For electron microscopic observation several pieces of each biopsy were processed. A small cortical portion of each biopsy was prepared for immunoelectron microscopy. The size of the latter portion was as small as 1 mm in diameter × 4–5 mm in length. These small specimens were fixed in 2% periodate-lysine paraformaldehyde solution (PLP) (McLean and Nakane 1974) in 0.05 M phosphate buffer adjusted at pH 7.4 at 4° C for 4 h, rinsed in 10, 15 and 20% sucrose solution in 0.05 M phosphate buffer pH 7.4 at 4° C for 6 h, respectively, and finally in 20% sucrose solution to which was added 0.05% glycerin in 0.05 M phosphate buffer at pH 7.4 at 4° C for 1 h. These specimens were then mounted in an embedding medium OCT compound (Miles Laboratories, USA) and snap frozen in dry ice-aceton, cut into 6-μm thickness with a cryostat and placed on slides coated with poly-L-lysine. For these sections immunostaining was performed by the ABC method (Hsu et al. 1981). First, endogenous biotin and avidin were blocked with 0.02% avidin D (Vector Laboratories, USA), phosphate buffer saline at pH 7.4 (PBS) and 0.002% *d*-biotin (Vector) PBS at room temperature for 20 min, respectively and rinsed in PBS for 5 min, respectively. The sections were incubated with 1% normal horse serum at room temperature for 1 h and subsequently incubated with the first antibodies at 4° C overnight. The first antibodies and dilutions were as follows: anti-human HLA-DR mouse monoclonal antibody (Becton-Dickinson, USA), Leu 4 mouse monoclonal antibody (Becton-Dickinson) for CD3, OKT 6 mouse monoclonal antibody (Orthomune, USA) for CD1a, and rabbit antihuman S 100*β* antibody (donated by Dr. K. Kato of the Institute for Developmental Research, Aichi Prefectural Colony, Aichi, Japan) (Haimoto et al. 1987) were used at 1 in 100 dilution. For control sections, normal mouse serum or normal rabbit serum were substituted for the primary antibodies.

After incubation with the first antibodies, the sections were rinsed in PBS for 5 min 3 times. Then biotinylated anti-mouse or anti-rabbit IgG antibodies (Vector) added with 5% normal human serum were reacted at room temperature for 2 h. After rinsing in PBS, the sections were reacted with avidin-biotin-peroxidase complex (ABC kit, Vector) at room temperature for 30 min. After rinsing in PBS, the sections were fixed in 2.5% glutaraldehyde in phosphate buffer at pH 7.4 at 4° C for 10 min. After rinsing in PBS, the sections were reacted with 0.6 mg/ml diaminobenzidine tetrahydrochloride (DAB) (Sigma, USA) at room temperature for 20 min, rinsed in PBS, and again reacted with DAB containing 0.01% hydrogen peroxide at room temperature for 5 min. After rinsing in phosphate buffer the sections were postfixed with 1% osmium tetroxide in phosphate buffer pH 7.4 at room temperature for 20 min. After rinsing in phosphate buffer the sections were dehydrated in graded concentrations of alcohol and overlayed with Epon-812 filled capsules, and polymerized at 60° C for 2 days. The Epon-812 filled capsules were detached from the slides by heating and ultrathin sections were prepared with a Reichert-Jung ultramicrotome. Ultrathin sections were stained with 4% uranyl acetate solution for 10 min and some sections were unstained for each block. Both stained and unstained sections were observed under an Akashi LEM 2000 electron microscope. In addition the formalin-fixed paraffin sections of all specimens were immunostained with anti-human S100*β* antibody after deparaffinization using the same method as above. Following light microscopic observation all the S100*β*-positive cells or fragments on the paraffin sections were observed by electron microscopy to detect IDC not found in the corresponding PLP-fixed sections.

Results

On light microscopic in 3 cases (cases 7, 11 and 14) relatively large foci of mononuclear cells were observed. In the remaining cases mononuclear cell infiltration was relatively mild or scanty. Otherwise no remarkable changes were found. On immunoelectron microscopic the localization and number of the IDC determined in the PLP-fixed sections are summarized in Table 1. In all cases most of the infiltrating cells were positive for CD3. About one-half of the lymphocytes were HLA-DR positive and the rest negative. A small number of macrophages were observed and were also HLA-DR positive. In addition to the lymphocytes and macrophages a few larger cells than lymphocytes showed HLA-DR positivity on their cytoplasmic membrane and rarely on the endoplasmic reticulum (Fig. 1). These cells had long, relatively thick processes and an oval or irregularly shaped nucleus with euchromatin. They were rich in vesicles and endoplasmic reticulum. Mitochondria were relatively large. Ribosomes were few. Lysosomes and phagosomes were absent. In 1 case with relatively large foci of mononuclear cells, many cellular processes bearing HLA-DR antigen on their membranes were observed. Such cells were present only in the interstitium or were

Table 1. The localization and number of interdigitating reticulum cells detected by immunoelectron microscopy in the PLP-fixed frozen sections of the human renal grafts

Case no	Post-transplant time	S100*β*-positive IDC + HLA-DR-positive IDC		
		Glomerular capillary	Interstitial capillary	Interstitium
1	1 hour	–	–	–
2	1 hour	–	–	–
3	1 hour	–	–	–
4	1 hour	–	–	–
5	1 hour	+ (1)	+ (1)	–
6	15 days	–	–	+ (3)
7	15 days	–	+ (2)	+ (3)
8	18 days	–	–	+ (2)
9	23 days	–	–	–
10	29 days	–	–	+ (1)
11	29 days	–	–	+ (3)
12	31 days	–	–	–
13	36 days	–	–	+ (1)
14	37 days	–	–	+ (4)
15	38 days	–	–	+ (1)
16	48 days	–	–	+ (1)
17	50 days	–	–	+ (1)

The numerals within parentheses indicate the number of IDC

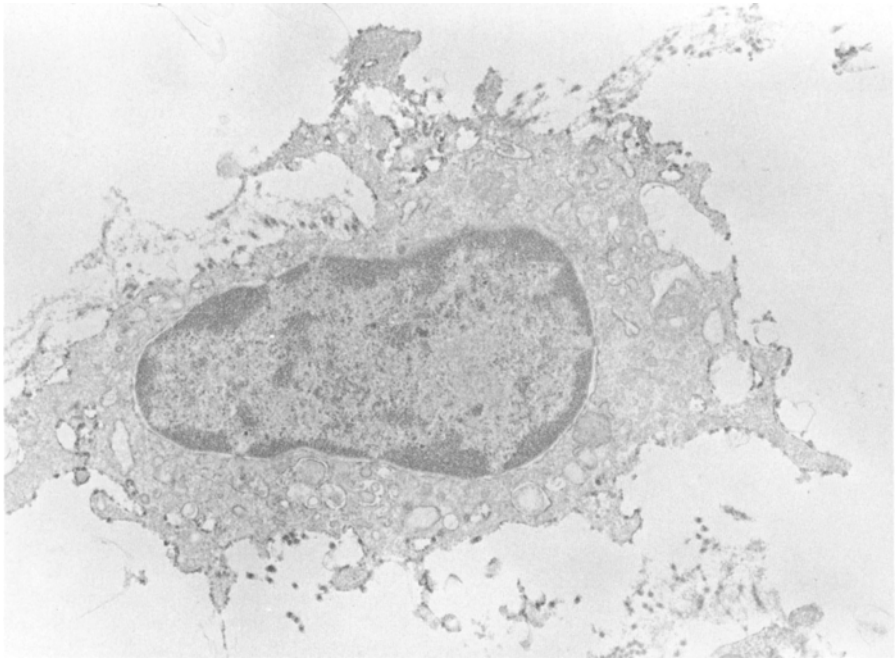

Fig. 1. This cell larger than a lymphocyte carries HLA-DR antigen on the cytoplasmic membrane, has many vesicles, and relatively long processes. Ribosomes are few. Lysosomes and phagosomes are lacking. It was identified as an interdigitating reticulum cell (IDC). ×10216

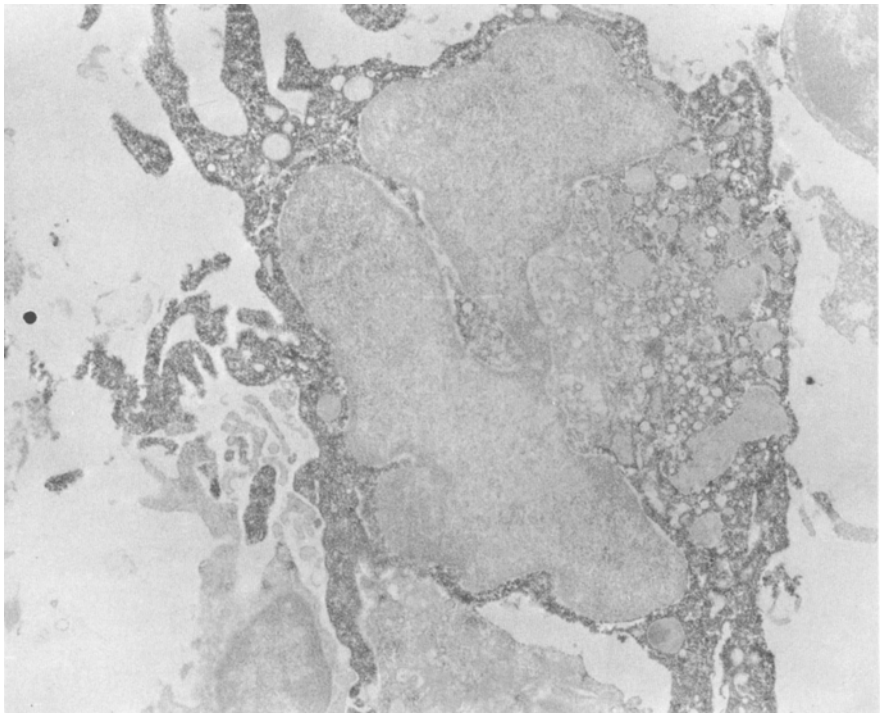

Fig. 2. In the cytoplasm S100β is densely distributed and vesicles are very rich. The nucleus is irregularly shaped. The cytoplasmic processes are long and thick. It is larger than a lymphocyte and was identified as an IDC. ×8916

present both there and in the interstitial blood capillaries (case 7).

S100β-positive cells were also few and composed of small- and large-sized cells. S100β was densely distributed in the cytoplasm of these cells. The characteristics of the morphological features of large S100β-positive cells (Takahashi et al. 1984) were almost the same as those of large HLA-DR-positive cells just described. They were present in the interstitium (Fig. 2) or in the interstitial (peritubular) and glomerular capillary lumens. In case 5 (Fig. 3) S100β-positive cells were observed in the glomerular and peritubular capillary lumens but not in the interstitium. This case was a transplant between identical twins and the specimen examined was a 1-h biopsy. These IDC were smaller than those described above and had fewer organelles, but showed an irregularly shaped nucleus and relatively long cellular processes. All these S100β- and HLA-DR-positive cells were thought to belong to the same lineage and were identified as IDC. The IDC were found in 11 of the total of 17 grafts. Among those 11 grafts, apart from the previously described 1-h biopsy IDC were observed

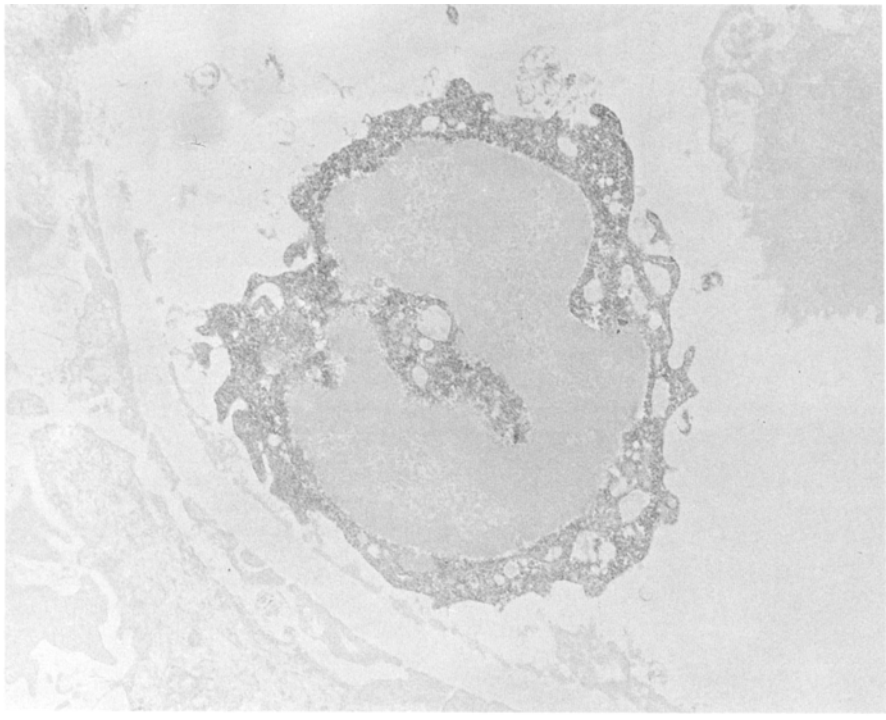

Fig. 3. An S100β-positive cell is noticed in the glomerular capillary lumen. It has less organelles than those shown in Figs. 1 and 2 but was supposed to be an immature IDC from its nuclear features and relatively long cytoplasmic processes. $\times$10780

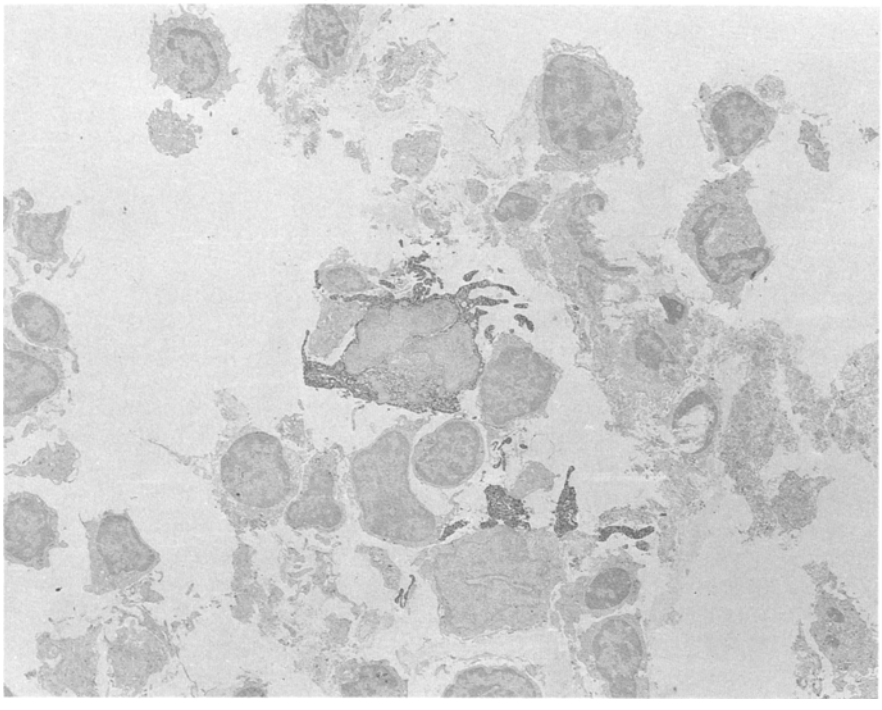

Fig. 4. The same IDC as demonstrated in the Fig. 2 is seen accompanied by lymphocytes. $\times$2280

in the capillary lumens in the interstitium of an allograft (case 7). In the remaining 9 allograft biopsies they were found only in the interstitium. Six allograft biopsies showed no IDC. All these 6, including 4 1-h biopsies, were almost normal histologically.

In most cases the IDC were observed to be accompanied by lymphocytes to a greater or lesser extent (Fig. 4).

Small S100β-positive cells (Takahashi et al. 1985) were regarded as lymphocytes from their morphological features which showed a chromatin-rich nucleus, rather scanty organelles and short or absent cellular processes.

No CD1a-positive cells were found.

In 3 non-graft biopsies no IDCs were detected. These 3 biopsies were almost normal histologically except for foot process fusion of the glomerular epithelium of minimal change biopsies on electron microscopic observation. The light microscopic findings of paraffin sections immunostained with antihuman S100β antibody in-

cluded large S100β-positive cells, generally observed in the aggregate of lymphocytes. Their processes were seen or not seen depending on the case. In the cases where IDC were not found in the PLP-fixed sections large S100β-positive cells like IDC were not detected on the corresponding paraffin sections. Small spindle-shaped S100β-positive cells or fragments were occasionally observed in perivascular areas. In addition S100β-positive structures were rarely observed in the interstitium. All these S100β-positive cells or structures on the paraffin sections were sought electron microscopically in the cases in which the IDC were not detected in their PLP-fixed sections, but were not found. In case 5 (1-h biopsy) the IDC were found in the PLP-fixed frozen sections as already mentioned. The corresponding paraffin section was also observed by electron microscopy in order not to overlook the IDC in the interstitium. However, none were found anywhere in the paraffin section.

Discussion

Reports focusing on the IDC in human renal transplantation are few (Wakabayashi et al. 1988). In our paper these cells were clearly demonstrated in 10 human renal allografts and an isograft, using immunoelectron microscopy. The cells were not recognized in 6 renal allografts or 3 renal non-grafts (2 minimal change and 1 angiomyolipoma). It may be that slight or no rejection was occurring at the time of biopsy in these cases that we found so few IDC. It is noteworthy that IDC were not only observed in the interstitium but also in the capillary lumens of a renal allograft and in the glomerular and peritubular capillary lumens of an isograft. The latter specimen was a 1-h biopsy of an isograft in which the blood of the recipient had just begun to flow and where the IDC were recognized only in the vessel lumens but not in the interstitium. In the remaining 4 1-h biopsies and 2 other graft biopsies they were not found. These 6 graft biopsies were histologically normal. In addition, 3 non-graft biopsies were normal except for foot process fusion of the glomerular epithelia in minimal change cases. These observations strongly suggest that the IDC are originally absent in normal renal tissues and that those noticed in renal grafts are derived from recipients rather than donors. Since IDC in human peripheral blood are very few it is unlikely for donor IDC to remain in the blood vessels of renal grafts perfused prior to transplantation. In the strict sense, it cannot be concluded whether the IDC in the interstitium originated from the donor or recipient. However, they were not found in each 1-h biopsy of 4 renal allografts which were almost normal (without rejection) and 3 renal non-graft biopsies which did not show any pathological change apart from foot process fusion of the glomerular epithelium (minimal change). One-hour renal graft biopsies are not supposed to differ in appearance from the renal tissues of donors before transplantation, if hyperacute rejection does not occur. Therefore, the IDC are not normally thought to be present in the interstitium but must migrate from the circulating peripheral blood toward the interstitium when needed. This speculation is not in accord with several investigators (Hart and Fabre 1981; Daar et al. 1983; Raftery et al. 1983) who reported that the IDC were present ubiquitously in the interstitium of various organs. They derived their conclusion from light microscopic observation using immuno- and histochemical staining. They stained HLA-DR antigen and ATPase, but with these stains it was very difficult to distinguish IDC from endothelium (Hinglais et al. 1984); both cell types are positive for HLA-DR antigen and ATPase. In contrast an S100β-positive cell is much easier to find by both light and immunoelectron microscopy because endothelium is negative for S100β. Even if S100β immunostaining is performed, light microscopic observation alone is thought to be insufficient to identify the IDC accurately. Immunoelectron microscopy is required. Small numbers of T lymphocytes are also S100β positive, but they differ in size and morphological appearance from IDC which are larger and richer in organelles than S100β-positive T lymphocytes and possess long cytoplasmic processes. Their nuclei are clear and irregular, and the cells can generally be readily differentiated from S100β-positive T lymphocytes. The Langerhans cell in the skin is related to the IDC and is OKT6 positive (Murphy et al. 1981) but the IDC examined in this study were all negative. One IDC was observed in the glomerular capillary lumen. This cell was smaller, lymphocyte-sized and had fewer organelles than the other ones observed in this study. However, irregularly shaped nuclei and longer processes were seen than those of lymphocytes. Takahashi (1989) recently found that S100-positive T lymphocytes were transformed into IDC-like cells by TPA stimulation and suggested that S100-positive T lymphocytes could be precursors of a part of IDC. This seems to explain the appearance of such a cell as observed in a glomerular capillary very well. Since this IDC was recognized in the 1-h biopsy it may be thought to be an immature IDC.

We conclude that IDC are not normally present in the interstitium in human renal tissues but migrate from the circulating blood to the interstitium when needed. The IDC recognized in renal grafts are assumed to originate from recipients principally.

Acknowledgements. The authors thank Prof. M. Shamoto of Fujita Gakuen Health University School of Medicine for the valuable discussions on the morphology of the IDC. The authors wish to express their thanks to Dr. K. Kato of the Institute for Developmental Research, Aichi Prefectural Colony for generously providing the rabbit antibody of anti-human S100β protein. We thank Prof. S. Mori of the Institute of Medical Science, the University of Tokyo for generosity in using commercial antibodies and laboratory facilities in a part of this work. We are grateful to Prof. N. Akiyama and all members of the Department of Organ Transplantation and Physiology of the same institute for providing us with materials and the clinical data of the patients. We also thank Mr. Y. Morishita of the same institute for excellent technical assistance.

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