Identification of T-Lymphocytes in Human Renal Allografts and Urine by the Fluorescent Antibody Technique Using Anti-Human T-Lymphocyte Serum (AHTS)

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Immunopathological investigations of the small round cells infiltrating into the tissues of human renal allografts and also those in urinary sediments were performed, using anti-human T-lymphocyte serum (AHTS). AHTS made from ALS is highly specific for T cells, but not for B cells. Tissues obtained by needle biopsy were examined by immunofluorescent staining and conventional histopathological methods. Urinary sediments were obtained from centrifugated fresh urine. Sediments were checked by immunofluorescent and Giemsa staining. Fluorescent positive cells were seen in the interstitial tissues and occasionally in renal glomeruli. Clustered cell-debris in the lumen of renal tubules was also stained by AHTS. In the urinary sediments, about a half of the lymphoid cells were stained by AHTS. The relationship between lymphocyturia and numerous infiltrating cells in the grafts during the rejection crisis has not been clarified. In this experiments, we tried to identify the detailed process of excretion of T cells to urine from renal tissues, using immunopathological methods.

renal transplantation; T-cells; transplantation immunology; anti-T-cell serum; lymphoid cells in urine

T-lymphocytes are considered to play an important role in transplantation immunity. However, immunopathological investigations on the small round cells, especially on the lymphocyte subpopulations in human renal allografts, have not been heretofore reported. Several methods to identify T-lymphocytes are currently developed. These are rosette formation with sheep erythrocytes (Fröland and Natvig 1973), selective reactivity to lectins (Greaves and Janossy 1972) and characteristic morphological differences of cell surface structures by scanning electron microscopy (Polliack et al. 1973). In the present study we...
tried to identify T-lymphocytes in human renal allografts and urinary sediments using anti-human T-lymphocyte serum (AHTS).

**Materials and Methods**

*Anti-human T-lymphocyte serum (AHTS).* The properties of AHTS and its specificity for T-lymphocytes have been described elsewhere (Nishihira et al. 1977). Six rabbits were immunized with $5 \times 10^7$ of fresh human thymocytes on their foot pads with Freund’s complete adjuvants. 7 weeks after this immunization $4 \times 10^8$ of fresh human thymocytes were injected intravenously and antiserum was obtained 2 weeks after boosting. The antisera were absorbed subsequently with fresh human erythrocytes, polymorphonuclear cells, liver and kidney homogenates, and Daudi cells of Burkitt lymphoma as a source of B cells. The absorbed serum was cytotoxic against human thymocytes at a titer of 1:64, and inhibited rosette formation with sheep erythrocytes at a titer of 1:1024. Specificity of AHTS was examined in peripheral lymphocytes and renal tissues as follows: E-rosette forming cells (RFC), surface immunoglobulin bearing cells and EAC rosette forming cells in peripheral blood of 8 healthy volunteers were examined by AHTS. Numbers of RFC were proportional to the numbers of cells which reacted with AHTS. Specificity of AHTS on renal tissues was examined using tissues from several patients with some renal diseases suspected. These experiments by immunofluorescence proved the specificity of AHTS against T-lymphocytes.

*Renal biopsies.* 21 allograft tissues were obtained by needle biopsy from 16 recipients (Table 1). All the recipients had transplants from living related donors. 6 biopsies in 6 recipients were performed within 3 months after transplantation (from 24 to 74 postoperative days). 9 biopsies were performed in 8 patients whose kidney functioned well over 2 years after transplantation.

*Tissue immunofluorescence.* Biopsy specimens were fixed in 95% ethanol overnight at 4°C and embedded in paraffin (at 54°C to 56°C). Tissue sections were cut at 4 μm in thickness and placed on non-fluorescent glass slides. All procedures were carried out at

<table>
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**Table 1. Summary of 16 recipients**

4°C except for the embedding. After the removal of paraffin by xylene, the sections were washed in phosphate buffer (PBS) at pH 7.2 and incubated with AHTS for 1 hr at room temperature. Fluorescein conjugated swine anti-rabbit IgG serum (Dakopatts, Denmark) was placed on the tissue sections after washing out AHTS with PBS, and incubated for 2–3 hr at room temperature. After the fluoresceinated reagents were washed out with PBS, the tissue sections were examined with a fluorescent microscope (Olympus, Japan).

Urinary sediments. Urinary sediments were obtained by centrifugation (1000 rpm, 5 min) of urine, and spread over the slide glasses. The dried sediments were fixed with 95% ethanol. The indirect fluorescent antibody technique, using AHTS and fluorescein conjugated swine anti-rabbit IgG serum, was carried out following the procedures as described above. At the same time the urinary sediments were stained with Giemsa.

Results

In early renal allografts (within 3 months), numerous cells accumulated around Bowman’s capsules (Fig. 1) and a few small vessels (Fig. 2) and were distributed in

![Fig. 1 (upper). Numerous small round cells are seen around the capsule of Bowman. 37 days after transplantation (Case 15). × 320, H & E stain.](image)

![Fig. 2 (lower). Small round cells are seen to accumulate around the small vessels. 37 days after transplantation (case 15). × 80, H & E stain.](image)
the interstitial tissues among renal tubules. These cells seemed to be composed of a large number of small round cells and a few plasma cells. These histological changes were similar to the results reported by Foker and Najarian (1972). In one allograft, focal cell infiltration was seen about 2 weeks before the onset of rapid deterioration of renal function which was caused by acute rejection. And some tubules were surrounded with the infiltrating lymphoid cells and lost their epithelia. Clustered cell-debris was seen in the lumen of renal tubules (Fig. 3). Urinary examinations of such cases revealed massive excretion of lymphoid cells in urine at the onset of rejection crisis (Fig. 4). In the allografts which functioned over 2 years after transplantation, apparent cell infiltrations were seen in 4 cases (5 biopsies), including 2 cases of acute rejection in late period. The other cases
showed no remarkable cell infiltration.

Fluorescent positive cells were seen in accumulations of infiltrating cells in the interstitial tissues (Fig. 5) and occasionally in renal glomeruli (Fig. 6). Clustered cell-debris in the lumen of renal tubules was also stained by AHTS (Fig. 7). In the urinary sediments, about a half of the lymphoid cells were stained by AHTS (Fig. 8).

**DISCUSSION**

From our previous studies (Nishihira et al. 1977), we concluded that the fluorescein-stained cells present in renal allograft are T-lymphocytes. The evidence for this conclusion is as follows: (a) AHTS stains approximately 60% of normal lymphocytes in peripheral blood and about 95% of thymocytes, but B-cell origin lymphoma cells are not stained. (b) AHTS stains only E-rosette forming cells in peripheral blood. (c) ATHS does not stain any other tissues or cells except...
the mononuclear cells in tissue sections. In addition to these findings of immuno-fluorescence, in vitro studies revealed that prior treatment of AHTS and complements to normal lymphocyte completely inhibit the response against T-cell mitogen (phytohemagglutinin: PHA), and that AHTS (diluted to 1:64) lysed 80% of thymocytes, 40% of peripheral lymphocytes, 10% of chronic lymphocytic leukemia cells, and 4% of Raji cells (B-cell origin). We concluded, therefore, that the stained cells are T-lymphocytes.

Massive cell infiltration was seen in renal allografts obtained shortly after the transplantation. This finding is known as one of the most important sings of acute rejection crisis. Our immunopathological investigations suggest that large numbers of these infiltrating cells belong to AHTS reacting cells, and those cells may be excreted in large quantities into urine during the rejection crisis.

Several investigators have systematized these infiltrating cells in relation to cellular immunity by the experiments of canine renal transplantation. Häyry and his coworkers (1972) reported interesting findings that these infiltrating cells in renal parenchyma had high activities of DNA synthesis, especially during the rejection crisis. Morphologically these cells were divided into three types by electron microscopy (Lindquist et al. 1971). Moreover, these infiltrating cells harvested from the rejected cardiac allografts of rats consisted of 35 to 45% of B-lymphocytes, which had no Fc-receptors and were highly cytotoxic against the donor thymocytes (Tilney et al. 1975). These active cells have often been detected in lymph flow from renal allografts or in peripheral blood of acute rejection (Pedersen and Morris 1970; Parker and Mowbray 1971).

T-lymphocytes in tissue sections were reported by Balch et al. (1973). They
showed T-lymphocytes experimentally in rat renal allografts by immunofluorescent staining using rabbit anti-rat thymocyte sera. Moreover, they observed that the infiltrating T-lymphocytes reached the maximum number 3 days after grafting and the cells diminished at 6 days. In our experiment, the exact percentage of infiltrating cells which reacted with AHTS could not be determined. But it was clarified that number of AHTS reacting cells was more than that of immunoglobulin bearing cells, which were investigated in the same tissue section by various kinds of anti-immunoglobulin sera. Our data also suggest that infiltrating cells which reacted with AHTS continued to appear in the graft at any days as long as grafts are functioning.

Lymphocyturia during the rejection crisis has often been reported (Ooi and Kincaid-Smith 1970), but morphological identification of urinary lymphocytes is not easy because of morphological changes of cells. In this regard, this immunopathological procedure using AHTS makes it easy to identify the excreted
T-lymphocytes in urinary sediments. Cell-debris in renal tubules and lymphoid cells in urine were both stained by AHTS. The detailed process of discharge of AHTS reacting cells into urine is still unknown. Further examination of urinary sediments using AHTS and immunofluorescent staining may also bring useful information upon the changes of grafts at various immunological status.

Acknowledgments

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References