INTERSTITIAL MONONUCLEAR CELL POPULATIONS
IN RENAL GRAFT REJECTION
Identification by Monoclonal Antibodies
in Tissue Sections*

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Humoral and cellular mechanisms have been implicated in the pathogenesis of acute renal allograft rejection in man (1, 2). Regardless of the relative role of either process in individual patients, the dominant histopathologic lesion is one of mononuclear cell infiltration of the interstitium. Investigations of this lesion have focused on analysis of tissue sections by light and electron microscopy and on functional characteristics of cell suspensions derived from rejected tissue (3-5).

Monoclonal antibodies have been developed that identify antigens expressed on functionally defined sets of mononuclear cells. Recently, these reagents have been used to determine the location and quantity of mononuclear cells of thymus (6), lymph node (7), bone marrow (8), and tonsil (9). In the present study, we used six hybridoma-derived monoclonal antibodies that recognize T cells (10, 11), T cell subsets (12, 13), B cells (14), and monocytes and null cells (15) to investigate the mononuclear cell populations of tissue from renal grafts undergoing rejection. Double-layer immunofluorescent enhancement and fluorochrome staining of nuclei (16) permitted localization and enumeration of reactive and nonreactive cells in frozen tissue sections. This analysis has demonstrated a pattern of cellular infiltration characteristic of renal transplant rejection.

Materials and Methods

Source of Tissue and Blood. 33 renal tissues were obtained from 31 patients: 22 grafts with interstitial rejection (IR), 6 grafts with interstitial nephritis without rejection (IN), 2 grafts without interstitial cellular infiltration, and 3 normal donor kidneys (at the time of transplantation), obtained by percutaneous needle biopsy, surgical biopsy, or nephrectomy for diagnostic and therapeutic purposes. Specimens were immersed in isopentane precooled in liquid nitrogen and stored at −70°C. With informed consent, 20 ml of heparinized blood was obtained at the

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1 Abbreviations used in this paper: FITC, fluorescein isothiocyanate; IgM,D, IgM and IgD; IN, interstitial nephritis; IR, interstitial rejection; PBS, phosphate-buffered saline; PMN, polymorphonuclear leukocytes; SgM,D, cell surface IgM and/or IgD; TRIC, tetramethylrhodamine isothiocyanate.

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time of biopsy in 7 patients (ages 11–54) with suspected rejection and from 12 healthy volunteers (ages 23–37).

**Monoclonal Antibodies.** Mouse monoclonal antibodies derived from hybridoma and myeloma cell lines were used to define mononuclear cell populations and as controls. The production, characterization, specificity, and source of these antibodies are summarized in Table I. BM11 is a hybridoma-derived antibody reactive only with human tubular basement membrane and Bowman's capsule, which developed after immunization of BALB/c mice with human renal basement membrane (A. Michael, unpublished observation).

**Preparation and Staining of Tissue Sections.** Frozen sections (4 μm) of kidney tissue were prepared in a Lipshaw cryostat and stored at −70°C. After air drying, sections were acetone-fixed for 10 min, washed sequentially three times for a total of 9 min in phosphate-buffered saline (PBS) at pH 7.4, overlaid with 20 μl of an appropriate dilution of the monoclonal reagent for 30 min, and washed briefly with PBS. Slides reacted with mouse monoclonal IgG antibodies were then stained with fluorescein isothiocyanate (FITC)-conjugated F(ab')2 rabbit anti-mouse IgG (heavy and light chains), and those incubated with mouse monoclonal IgM antibodies were stained with FITC-labeled F(ab')2 goat anti-mouse IgM (mu chain) (N. L. Cappel Laboratories, Inc., Cochranville, Pa.) for 15 min and then washed with PBS. Amplification was achieved by the use of a second FITC-conjugated reagent, either F(ab')2 goat anti-rabbit IgG (heavy and light chains) or F(ab')2 rabbit anti-goat IgG (heavy and light chains) (N. L. Cappel Laboratories), which were applied as described above. FITC-conjugated F(ab')2 reagents were absorbed with human plasma and the optimum dilutions determined by staining of appropriate animal tissues (mouse, rabbit, or sheep). After a final PBS wash, 20 μl of ethidium bromide (1:5,000) was applied to stain nuclei (16).

**Standardization of Monoclonal Reagents and Controls.** The appropriate dilutions of the monoclonal reagents were determined using the amplified immunofluorescence method described above on frozen sections of human spleen, tonsil, thymus, and lymph node. The results of these studies were similar to previous reports (6–9) of immunofluorescence on lymphoid organs using monoclonal reagents. In general, the optimum concentration of monoclonal antibody for staining lymphoid cells in tissue was twice that used for staining intact mononuclear cells isolated from blood.

| Monoclonal antibody | Subclass | Specificity                  | References |
|---------------------|----------|------------------------------|------------|
| TA-1*               | IgG2     | T lymphocytes                | 11         |
| OKT3§               | IgG1a    | T lymphocytes                | 10         |
| OKT4§               | IgG2b    | Helper/inducer               | 12         |
| OKT8§               | IgG2b    | Cytotoxic-suppressor         | 13         |
| BA-1*               | IgM      | B lymphocytes; PMN           | 14         |
| OKM-1§              | IgG2b    | Monocytes                    | 15         |
| UPC 10§             | IgG2b    | Levan                        | 17         |
| MOPC 195§           | IgG2b    | None known                   | 17         |
| MOPC 10E§           | IgM      | B1355S                       | 17         |
| BM11*               | IgG2b    | Tubular basement membrane    | Unpublished |

* Obtained from the University of Minnesota.
§§ Obtained from Ortho Pharmaceutical, Raritan, N. J.
§§§ Obtained from Litton Bioincs, Inc., Kensington, Md.
The following immunofluorescence controls were used: (a) each tissue was stained with both FITC-conjugated F(ab')2 reagents without previous application of the monoclonal antibody; (b) sections of human spleen, tonsil, and heavily infiltrated transplant kidneys were reacted with serial dilutions of mouse myeloma-derived Ig of the same subclass as the hybridoma antibodies used in this study (Table I) followed by the two layers of the appropriate FITC-conjugated F(ab')2 reagents; and (c) a monoclonal antibody of subclass IgG2b (ascites fluid) with specificity for noncellular elements of human kidney (BM11) was applied to heavily infiltrated transplant kidneys followed by double layer FITC enhancement.

Enumeration of Mononuclear Cell Populations in Tissue. Sections were examined with a Zeiss universal microscope (Carl Zeiss, Inc., New York) equipped for epifluorescence with appropriate filters and dichroic mirrors through a X63 plan apochromat oil immersion objective and a 10 x 10-mm ocular indexing grid. The number of positive cells, interstitial cells, and total cells (excluding glomeruli and arteries) were counted in each of 30–55 fields, and the percent of interstitial and total cells that were positive was calculated for each section. Generally, sequential fields were examined in each section, although glomeruli and large arteries were excluded. Cells with a distinctly apple-green fluorescent plasma membrane surrounding a red-orange fluorescent nucleus (ethidium bromide) were considered positive. Cells containing no nuclear material were rarely observed and were not counted. Also excluded were positive cells within glomeruli, arteries, tubular walls, and lumina as well as BA-1-positive and OKM1-positive cells having the nuclear or cytoplasmic characteristics of granulocytes.

Total and interstitial cell counts were performed using optics optimal for rhodamine immunofluorescence (546-nm band pass exciter filter, 580-nm reflector filter, and LP 590 barrier filter), and the percent of interstitial cells was calculated according to the expression, 100 × (number of interstitial cells + number of total cells). Nuclear fragments and cells overlapping the right and lower margins of the grid were not counted. The percentage of interstitial cells was also evaluated by examination of companion frozen-sections stained with PAS-hematoxylin made in 17 of the 33 tissues. The ratios determined by these two techniques were in close agreement with a mean difference of 5.1 ± 4.0% (P > 0.29). Difficulty in distinguishing interstitial cells from tubular cells was occasionally encountered in heavily infiltrated tissues, and, therefore, the number of positive cells as a percent of total cells was determined for each tissue as reported herein.

Preparation, Staining, and Enumeration of Blood Mononuclear Cells. Mononuclear cells were separated from whole blood on a conventional Ficoll-Hypaque gradient, suspended in RPMI medium, and washed with medium consisting of RPMI and 20% fetal horse serum. The appropriately diluted monoclonal antibody (100 μl) was added to 100 μl of cell suspension (10⁷ cells/ml), incubated at 4°C for 45 min, and washed. 50 μl of FITC F(ab')2 goat anti-mouse IgG (Kallestad Laboratories, Inc., Austin, Tex.) or of FITC F(ab')2 goat anti-mouse IgM (N. L. Cappel Laboratories) was added, and the cells were agitated and incubated for 30 min at 4°C. The optimum dilution of FITC-labeled reagents was determined by staining of normal mononuclear cell preparations. Cell suspensions were washed three times with RPMI and mounted wet. The number of positive cells per 200 total cells was determined by examination of random fields. Control slides were prepared for each specimen as described, omitting addition of monoclonal antibody.

Enumeration of Surface IgD-IgM (SIgD,M)-positive Cells. Tissue sections were stained with a mixture at optimum end dilutions of FITC-conjugated F(ab')2 goat anti-human IgM and F(ab')2 goat anti-human IgD (N. L. Cappel Laboratories) followed by FITC-conjugated F(ab')2 rabbit anti-goat IgG (N. L. Cappel Laboratories). Positive cells, interstitial cells, and total cells were enumerated with appropriate controls, as described above.

To determine the relationship of BA-1 positivitiy to SIgD,M positivity, human tonsil and transplant kidney sections were each stained sequentially with BA-1, FITC F(ab')2 goat anti-mouse IgM, FITC F(ab')2 rabbit anti-goat IgG, and tetramethylrhodamine isothiocyanate (TRIC)-conjugated F(ab')2 goat anti-human IgD,M. To exclude binding of the fourth layer (goat anti-human IgD,M) to the third layer (rabbit anti-goat IgG), two further controls were carried out: (a) goat serum applied after the third layer FITC F(ab')2 rabbit anti-goat IgG did not inhibit binding of TRIC F(ab')2 goat anti-human IgD,M; (b) IgM deposits in sections of NZB/NZW mouse kidney identified by FITC F(ab')2 goat anti-mouse IgM followed by FITC
F(ab')2 rabbit anti-goat IgG did not react with TRIC F(ab')2 goat anti-human IgM,D applied as a third layer. The presence of occasional cells in IR tissue positive for BA-1 and negative with TRIC F(ab')2 goat anti-human IgD,M suggested that the latter did not bind the former.

Clinical and Histopathologic Data. Clinical data were obtained from the medical records of each of the patients studied after tabulation of the immunohistochemical analyses. Histopathological evaluation of each tissue was performed by a pathologist who was neither participating in this investigation nor aware of the findings.

For data analysis, tissues from patients were categorized by pathologic diagnosis based upon the clinical picture and morphologic evaluation of the kidney into three groups: (a) renal grafts with interstitial rejection (IR); (b) renal grafts with interstitial nephritis not related to rejection (IN); and (c) kidneys without interstitial infiltration (two grafts, three normal donor kidneys).

Group 1 consisted of 22 tissues (tissues 1–22) from 21 patients (10 females, 11 males; ages 4–54 yr) with IR. The duration of the graft was 0–4 mo in 13 patients, 4–12 mo in 2 patients, and >12 mo in 6 patients. The diseases leading to renal failure were diabetic nephropathy (8 patients), chronic glomerulonephritis (6 patients), chronic pyelonephritis (2 patients), reflux nephropathy (2 patients), nephrotic syndrome with glomerulosclerosis (1 patient), Fabray’s disease (1 patient), and familial nephritis (1 patient). All patients except one had experienced an acute increase in the serum creatinine before percutaneous biopsy (18 patients) or transplant nephrectomy (3 patients). The histopathologic diagnoses of these tissues include acute IR (11 patients), acute and chronic IR and vascular rejection (4 patients), acute IR and vascular rejection (4 patients), IR with acute and chronic vascular rejection (1 patient), chronic IR and vascular rejection (1 patient), and chronic vascular rejection (1 patient). The latter patient with chronic vascular rejection had a clinical course consistent with acute interstitial rejection and histologic evidence of substantial interstitial infiltration. Of 17 patients treated for rejection after biopsy, 13 experienced improvement of renal function and 4 (all of whom had vascular rejection) did not. Two biopsies were performed on one tissue 6 wk and 2 mo after engraftment; the first (tissue 20) revealed early acute IR and the second (tissue 21) revealed severe acute IR and moderate vascular rejection. Both tissues were studied.

Group 2 included 6 patients (tissues 23–28; 3 male, 3 female, 11–41 yr) with allografts showing IN without clinical or histopathologic evidence of rejection. The diseases leading to transplantation were diabetic nephropathy (2 patients), nephrotic syndrome with focal glomerulosclerosis (3 patients), and chronic glomerulonephritis (1 patient). The grafts had been present for 8 d to 7 mo. Three of these patients (tissues 24, 25, and 27) were felt to have cyclosporin A toxicity, and their renal function improved as the dosage of this agent was decreased. One patient (tissue 26) had severe IN of unknown etiology, characterized by interstitial infiltration of polymorphonuclear and mononuclear cells without vascular involvement. Improvement in renal function occurred without specific therapy. Another patient (tissue 28) had acute tubular necrosis 3 wk after transplantation and experienced slow spontaneous improvement. The final patient in this group had IN and glomerular exudation with end-stage renal disease (tissue 23). A biopsy (tissue 22) from this patient 2 wk previously revealed prominent interstitial edema, modest interstitial infiltration, and normal glomeruli, which, when compared to another two prior biopsies, was felt to be consistent with resolving IR. Although rejection might have contributed to the histopathologic findings in tissue 23, the predominant morphologic change and the clinical circumstances were those of end-stage renal failure due to recurrence of the primary glomerular disease. For these reasons, this tissue is included in group 2.

Group 3 consisted of tissues lacking interstitial infiltration from a 37-yr-old male (tissue 29) who had had chronic glomerulonephritis and had an allograft for 1 mo; from a 27-yr-old female (tissue 30) with diabetes who had been engrafted for 18 mo; and from three normal donors (tissues 31–33) obtained at the time of transplantation.

Statistical analysis was performed using Student’s t test for dependent or independent means. The data given in the text are expressed as mean ± standard deviation (SD).

Results

The use of two layers of FITC-labeled F(ab')2 antibody provided sufficient amplification and duration of fluorescence to permit the counting of all positive cells in each field examined (Fig. 1). Plasma membrane staining with TA-1, OKT4, OKT8,
and BA-1 was of linear character and uniform intensity, with OKT4 being the weakest. The fluorescence of OKT3- and OKM1-positive cells varied in intensity and was both linear and granular. Nuclear staining with ethidium bromide provided a dull orange contrast with the FITC filter system and a brilliant red-orange fluorescence with rhodamine filters. Fine granular BA-1 positivity was noted in the cytoplasm but not on the plasma membrane of some tubules in most sections.

**Interstitial Cell Quantitation.** The variability in the number of interstitial cells as a percent of total cells in sections from each tissue as determined by immunofluorescence was relatively low. The percent of interstitial cells was significantly higher in IR (50 ± 8.2%) than in the five kidneys with normal interstitium (24 ± 3.4%, $P < 10^{-6}$) but was not different from that of grafts with IN (42 ± 15%).

(a) **Positive Cells as Percent of Interstitial Cells.** In the 22 tissues with IR, the largest

Fig. 1. Photomicrographs of mononuclear cell infiltrates in renal grafts with interstitial rejection identified by nuclear staining (A) and monoclonal antibodies using F(ab')2 double fluorochrome indirect immunofluorescence (B–F): (A) ethidium bromide reacting with nuclei of tubule surrounded by cellular infiltrate; (B) a T cell is identified by OKT3 between two segments of tubule (T); (C) two helper/inducer cells are recognized by OKT4 within the modestly enlarged interstitium separating five tubules; (D) nodular accumulation of cytotoxic/suppressor cells stained with OKT8; (E) nodular accumulation of B cells stained with BA-1; (F) two cells identified by OKM1 antibody (monocytes/null cells) surrounded by tubules (out of focus), one of which contains autofluorescent granules. Ethidium bromide was not applied to sections B–F. (B, × 1,400; all others, × 590).
percentage (35 ± 9.8%) of interstitial cells was T cells (OKT3 positive). This percentage was substantially higher than that observed in three normal donors (3.1–3.3%), two normal grafts (3.3 and 12%), and in grafts with IN unassociated with rejection (21 ± 16%, \( P < 0.02 \)) (Fig. 2). In IR, the percent of interstitial cells positive for OKT4 (10 ± 5.0%) was similar to that in IN (15 ± 13%) and the five tissues with normal interstitium (4.2 ± 3.1%). In IR, however, the percent of OKT8-positive interstitial cells (26 ± 7.7%) was significantly higher than that observed in IN (9.3 ± 6.2%, \( P < 10^{-6} \)) or in normal kidneys (3.0 ± 2.4%, \( P < 10^{-6} \)).

(b) Positive Cells as Percentage of Total Cells. The data on individual tissues calculated according to the percent of total cells is indicated in Tables II and III. The statistically significant differences are similar to those noted above. In each tissue, the percent of cells reacting with OKT4 plus OKT8 was approximately equal to OKT3. The mean ratio of OKT8/OKT4 in allografts with rejection (3.2 ± 1.4) is strikingly increased \((P < 0.0007)\) when compared with that of kidneys with IN without rejection (0.82 ± 0.39) or with the two transplanted and three donor kidneys without interstitial infiltration (0.75 ± 0.25) (Fig. 3). The ratio of OKT8 to total T cells (OKT3 or TA-1) similarly differentiated the groups \((P < 0.01)\). No specific differences were observed in the ratios of BA-1 to OKT4, nor in OKM1 to OKT4, OKT8, OKT3, and BA-1.

In general, BA-1-positive cells as a percent of total or interstitial cells was low in all tissues examined. The percent of interstitial or total cells reactive with OKM1 was higher in kidneys with interstitial infiltration than in those without infiltration \((P < 0.02)\) (Tables II and III, Fig. 2).

Focality of Infiltration. The infiltration of mononuclear cells was focal in all diseased tissues studied but appeared particularly so in acute IR. Nodular accumulations of mononuclear cells on most sections consisted mainly of T cells. Separate and mixed foci of OKT4- and OKT8-positive cells were noted. On two occasions (tissues 13 and
TABLE II
Mononuclear Cell Populations of Renal Grafts with Interstitial Rejection as a Percent of Total Cells

| Tissue number* |Interstitial cells† | Interstitial mononuclear cells identified by immunofluorescence using monoclonal antibodies |
|----------------|-------------------|------------------------------------------------|
|                |                    | OKT3 | OKT4 | OKT8 | BA-1 | OKM1 |
| 1              | 42 ± 6.6           | 15   | 1.6  | 8.3  | 0.36 | 6.5  |
| 2              | 36 ± 8.1           | 7.3  | 2.9  | 4.4  | 0.54 | 1.6  |
| 3              | 62 ± 7.1           | 36   | 15   | 20   | 2.5  | 17   |
| 4              | 55 ± 1.2           | 23   | 6.2  | 19   | 0.89 | 8.7  |
| 5              | 53 ± 3.9           | 20   | 8.5  | 14   | 2.2  | 5.6  |
| 6              | 46 ± 2.4           | 20   | 6.3  | 16   | 0.30 | 6.3  |
| 7              | 51 ± 3.9           | 13   | 3.6  | 10   | 1.5  | 4.4  |
| 8              | 48 ± 5.4           | 20   | 3.0  | 17   | 1.1  | 6.5  |
| 9              | 43 ± 4.8           | 13   | 2.1  | 10   | 0.59 | 2.4  |
| 10             | 43 ± 5.2           | 14   | 3.6  | 12   | 0.28 | 4.0  |
| 11             | 47 ± 2.9           | 16   | 2.3  | 12   | 0.23 | 6.2  |
| 12             | 49 ± 3.5           | 15   | 2.5  | 13   | 0.71 | 7.1  |
| 13             | 65 ± 6.9           | 29   | 1.1  | 26   | 7.2  | 5.0  |
| 14             | 67 ± 4.6           | 32   | 1.9  | 28   | 2.7  | 6.6  |
| 15             | 59 ± 2.3           | 24   | 5.6  | 19   | 4.1  | 10   |
| 16             | 42 ± 0.82          | 11   | 5.2  | 6.8  | 1.1  | 7.6  |
| 17             | 47 ± 1.9           | 11   | 2.7  | 9.0  | 0.52 | 12   |
| 18             | 46 ± 2.7           | 13   | 2.6  | 9.2  | 1.2  | 6.3  |
| 19             | 39 ± 4.1           | 11   | 3.1  | 6.7  | 0.35 | 5.8  |
| 20             | 56 ± 3.6           | 20   | 6.2  | 19   | 1.5  | 13   |
| 21             | 53 ± 2.1           | 25   | 4.3  | 22   | 0.68 | 5.5  |
| 22             | 49 ± 2.7           | 12   | 3.2  | 11   | 0.38 | 2.9  |
| Mean ± SD      | 50 ± 8.2           | 18 ± 7.5 | 5.1 ± 3.3 | 14 ± 6.4 | 1.4 ± 1.6 | 7.1 ± 3.6 |

* Tissues 20 and 21 were obtained from the same patient.
† Interstitial cells as percent of total cells in sections of kidney tissue.

15), nodules of cells principally reactive with BA-1 were encountered. In tissue 15, several sections of the nodule were stained with each of the monoclonal reagents, and >120 cells were counted in each field; 50% were positive for BA-1, 33% for TA-1, 37% for OKT3, 13% for OKT4, 19% for OKT8, and 3% for OKM1. The percent of BA-1-positive cells was approximately equivalent to the percent of cells shown to be SIgM,D+. Peripheral Blood Mononuclear Cell Populations. Comparison of blood mononuclear cell populations in 7 patients with IR to those of 12 controls demonstrates a similar percent of cells in each group reacting with OKT8 and BA-1 (Table IV). Allograft recipients had fewer OKT4-positive (P = 0.001) and more OKM1-positive (P < 0.001) cells than controls. The ratio of OKT8/OKT4-positive cells in kidney exceeded that present in the blood in five of six determinations, the only exception (patient 17) being a patient who had received intensive therapy for rejection for 2 d before biopsy (Table V).

Specificity of Monoclonal Antibody Reactivity. To confirm the specificity of monoclonal antibodies for human T and B cells, two monoclonal antibodies reactive with T cells were used (TA-1 and OKT3). The number of cells as a percent of total cells reacting with TA-1 and OKT3 was 20 ± 9.6 and 18.2 ± 7.5, respectively in IR; 5.8 ± 2.8 and 6.1 ± 4.6 in IN; and 0.88 ± 0.38 and 0.78 ± 0.04 in uninfilt rated kidney tissue.
**TABLE III**

Mononuclear Cell Populations of Renal Grafts without Rejection and Normal Donor Kidneys as a Percent of Total Cells

| Tissue number* | Interstitial cells‡ | Interstitial mononuclear cells identified by immunofluorescence using monoclonal antibodies |
|----------------|---------------------|------------------------------------------------------------------------------------------------|
|                |                     | OKT3    | OKT4    | OKT8    | BA-1   | OKM1   |
| Grafts with interstitial nephritis other than rejection | % of total cells | % of total cells | % of total cells | % of total cells | % of total cells | % of total cells |
| 23             | 47 ± 3.7            | 13      | 5.2     | 7.5     | 0.27   | 7.3    |
| 24             | 30 ± 3.7            | 2.9     | 2.1     | 2.0     | 0.78   | 3.3    |
| 25             | 31 ± 4.3            | 1.2     | 0.83    | 0.92    | 0.06   | 1.3    |
| 26             | 69 ± 6.6            | 30§     | 24      | 12      | 2.2    | 16     |
| 27             | 36 ± 5.0            | 6.8     | 5.0     | 2.3     | 0.07   | 1.5    |
| 28             | 38 ± 3.7            | 6.8     | 4.8     | 2.4     | 0.08   | 11     |
| Mean ± SD     | 42 ± 15             | 10 ± 11 | 7.0 ± 8.3 | 4.5 ± 4.3 | 0.30 ± 0.84 | 6.7 ± 5.9 |

Tissues without interstitial infiltrates

|                | % of total cells | % of total cells | % of total cells | % of total cells | % of total cells | % of total cells |
|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                |                 |                 |                 |                 |                 |                 |
| Renal grafts   |                 |                 |                 |                 |                 |                 |
| 29             | 30 ± 2.9        | 3.3§            | 2.9             | 2.3             | 0               | 5.0             |
| 30             | 23 ± 1.8        | 0.80            | 0.66            | 0.66            | 0.48            | 1.7             |
| Donor tissue   |                 |                 |                 |                 |                 |                 |
| 31             | 21 ± 1.9        | 0.78            | 0.55            | 0.34            | 0               | 0.66            |
| 32             | 24 ± 2.7        | 0.73            | 0.94            | 0.37            | 0.13            | 0.43            |
| 33             | 25 ± 2.1        | 0.83            | 0.33            | 0.31            | 0               | 0.87            |
| Mean ± SD     | 24 ± 3.4        | 1.3 ± 1.1       | 1.1 ± 1.0       | 0.80 ± 0.85     | 0.12 ± 0.21     | 1.7 ± 1.9       |

* Tissues 22 (see Table II) and 23 were obtained from the same patient.
‡ Interstitial cells as percent of total cells in sections of kidney.
§ TA-1 value given, OKT3 not done.

Comparison of the percent BA-1-positive cells with the percent of cells with SIgD,M reactivity was carried out in seven renal grafts with IR, five grafts with IN, one normal transplant and one donor biopsy. In these 14 tissues, the percent of total cells that were BA-1 positive was 0.96 ± 0.80, whereas the percent of cells with SIgD,M was 1.2 ± 1.0. Dual label staining for BA-1 (FITC) and SIgD,M (TRIC) revealed that cells positive for the former were also positive for the latter.

**Fc Receptor Binding Controls.** Mouse myeloma-derived IgG2a, IgG2b, and IgM were applied to normal human spleen and transplant kidney tissue followed by a double layer of FITC-conjugated F(ab')2 reagents. At dilutions of mouse Ig similar to those used with hybridoma-derived reagents (mg/ml), no cell membrane staining was observed. At concentrations of myeloma proteins 20–100-fold higher than those at which hybridoma antibodies were used, occasionally very weakly staining cells were noted. Because OKT4-positive cells at times stain weakly, we used a second control; a hybridoma-derived mouse IgG2b antibody with specificity for human Bowman's capsule and tubular basement membrane was applied in excess to transplant rejection tissue at various dilutions followed by a double fluorochrome layer. At no concentration of this antibody was cell membrane positivity noted, whereas tubular basement membranes and Bowman's capsule stained intensely at all concentrations.

**Clinical Correlations.** In interstitial rejection, no relationship was evident between the mononuclear cell populations including the OKT8/OKT4 ratio and the severity...
of rejection, the histopathologic type of rejection, the longevity of the graft, and the response to therapy.

In two instances serial biopsies were examined. Tissue 20, with early acute IR, was obtained 2 wk before tissue 21, with severe acute IR and vascular rejection. Only minimum differences in mononuclear cells were observed between the two specimens, although the OKT8/OKT4 ratio changed from 3.1 to 5.1. The second patient (tissues 22 and 23) had resolving acute IR 1 mo after transplantation and 2 wk later had IN with end-stage renal failure from recurrence of original disease. Changes in the interstitial cell populations in this kidney were characterized by a slight increase in
OKT8-positive cells and a decrease in OKT8-reactive cells. The OKT8/OKT4 ratios in the two tissues were 2.1 and 1.4, respectively.

Discussion

The introduction of monoclonal antibodies with specificity for cell surface determinants of various mononuclear cells has spurred investigation of peripheral blood cell populations in a number of diseases, including leukemia (18), lymphoma (19), infectious mononucleosis (20), and interstitial nephritis (21). The application of these antibodies to tissues has helped define the cellular constituents of several lymphoid organs (6-9). However, more extensive immunohistologic use has been hindered by weak fluorescence using conventional indirect staining (9). In this study, we found that double fluorochrome amplification with F(ab')2 reagents and counterstaining of nuclei allowed quantitation of cell populations in frozen sections of tissue.

Of identifiable interstitial cells in 22 tissues from 21 renal grafts undergoing rejection, T cells were most numerous, and the greater fraction of these were OKT8 positive. The mononuclear cell infiltrates of grafted kidneys with IN also contained a preponderance of T cells; however, the OKT8-positive fraction was significantly less than in IR, and, as a consequence, the OKT8/OKT4 ratio was significantly lower. Indeed, the only tissue in the IN group whose OKT8/OKT4 ratio overlapped those of the rejection group was tissue 23, whose group designation was most difficult. Monocytes and null cells (defined by monoclonal antibody OKM1) represented a substantial and approximately equal proportion of infiltrating cells in both types of interstitial disease. B cells did not account for >10% of interstitial cells in any tissue studied and did not distinguish IR from IN.

T cell populations were enumerated with OKT3 and TA-1 antibodies. The latter reagent reacts with T cells and monocytes (11) and may recognize natural killer cells and ~10% less than the sum of OKT4- and OKT8-positive cells in blood (T. LeBien, unpublished observations). The similar range of interstitial cells reacting with TA-1 and OKT3 (with allowance for the broader specificity of TA-1) tends to substantiate
the proportions of T cells present in the tissues examined. Confirmation of BA-1 reactivity for B cells in tissue is suggested by the similar percentages of cells having \( \text{S}_{\text{lgD,M}} \) reactivity. By staining tissue with both BA-1 and \( F(ab') \) goat anti-human IgD,M, the overlapping specificities of these reagents was confirmed.

Although the functional correlates of blood cells reacting with OKT3, OKT4, OKT8, OKM1, and BA-1 are well defined, there is no certainty that these apply to cells in tissue. However, studies of cell suspensions made from lymphoid organs (22) and transplant kidneys (23) suggest that some T and B cell properties in tissue resemble those in blood. Furthermore, the paucity of positive cells in donor and histologically normal transplant tissue sections suggests that the specificities of the antibodies used is not broader in tissue than in blood. In this regard, it is reasonable to ask whether substantial numbers of T cells in the kidney may express determinants recognized by both OKT4 and OKT8, thus beguiling attempts to use a ratio of the two. The data of this study tend to oppose such a hypothesis because the sum of OKT4 and OKT8 usually approached that of OKT3 or TA-1.

Others have found (4, 5) larger numbers of B cells in renal transplant rejection tissue than our investigation revealed. However, the differences may be methodologic. These studies were performed on cell suspensions made from tissue obtained at transplant nephrectomy, whereas only three of the tissues examined for our study were obtained from irreversibly rejected kidneys. The mean percent of interstitial B cells in these three kidneys, though low (5.3%), was twice that of the IR group mean. Further, it has been shown that by 96 h after pokeweed mitogen stimulation, 80% of B cells lose BA-1 positivity (14). Because neither these cells nor plasma cells react notably with BA-1, it is possible that activated \( \text{S}_{\text{lg}+} \) cells may account for the apparent low percentage of recognizable B cells. Still, most in vitro activated B cells react with anti-human IgM antiserum (22). It is possible that there are substantial numbers of activated \( \text{S}_{\text{lg}+} \text{S}_{\text{lgM,-}} \) cells in transplant kidneys. However, in blood (24), spleen (25), and activated lymphoid tissue (22) these cells represent only a small proportion of \( \text{S}_{\text{lg}+} \) cells. Further, our method of counting interstitial cells includes all cells not attached to tubules, arteries, and glomeruli. Thus, our definition is more inclusive than that of other investigators. If one applies data obtained from the normal kidney tissue examined, a significant percentage of the interstitial cells counted in the rejection group may be normal tissue elements. To these nonreactive cells must be added plasma cells, some null cells, PMN, eosinophils, and mast cells, which were not enumerated. Further, the aggregate percent of interstitial cells identified by monoclonal antibodies in IR tissue exceeded those in IN by \( \sim 10\% \). Lastly, the process of harvesting mononuclear cells from tissue, as performed in other studies, results in the selective loss of some cell populations and enrichment of others (23, 26). Thus, results obtained by analysis of cell suspensions and tissue sections may differ (27).

No systematic attempt was made to survey all types of interstitial nephritis in the transplant kidney nor to examine tissue that had infiltration comparable with that of the transplant group. The percentage of total cells located in the interstitium of IR tissue was not significantly different from that in IN, although the mean of the former exceeded that of the latter by \( \sim 15\% \). Further, there was no correlation between the OKT8/OKT4 ratio and degree of tissue infiltration. Indeed, it was the type of cells rather than the number of cells that best distinguished these two groups.

The relative proportions of interstitial cells reacting with the various monoclonal
antibodies did not appear to distinguish acute from chronic nor vascular from interstitial rejection. In addition, there appeared to be no correlation with severity. On the other hand, in the two instances in which serial biopsies were examined, the progression of rejection was accompanied by an increasing ratio of OKT8/OKT4 and resolution by a decreasing ratio. This relationship warrants further investigation.

The mononuclear blood cell populations of seven patients undergoing rejection appeared to have a smaller proportion of cells reactive with OKT3 and OKT4 and a greater proportion of cells reactive with OKM1 than did normal controls. However, the proportions ranged broadly in the patients with allograft rejection, and specific values did not appear to correlate with the relative percentages of mononuclear cells in corresponding kidney tissue nor with consistent increase in OKT8 cells. These results are consistent with previous observations demonstrating a much higher percentage of cytotoxic cells derived from rejecting kidneys than from peripheral blood

There is evidence (28) suggesting that the proportions of peripheral blood T cell subsets may define groups of allograft recipients at low and high risk for rejection. The T cell data reported herein were determined on blood obtained at the time of allograft biopsy in consecutive unselected patients with established rejection and thus are not completely comparable with this report.

Renal allograft rejection would appear to be characterized by interstitial infiltration consisting primarily of T cells, the majority of which have determinants recognized by OKT8. These OKT8 cells may have importance in the mediation of transplant rejection.

Summary

The interstitial mononuclear cell populations of 22 renal grafts with interstitial rejection (IR), 6 grafts with interstitial nephritis without rejection (IN), and 5 kidneys without infiltration (3 donor kidneys, 2 grafts) were identified and quantitated by monoclonal antibodies recognizing T cells (TA-1, OKT3), helper inducer cells (OKT4), cytotoxic/suppressor cells (OKT8), B cells (BA-1), and monocytes and null cells (OKM1). Double-layer fluorochrome enhancement using F(ab')2 reagents and nuclear counter staining with ethidium bromide enabled quantitation of the number of positive mononuclear cells, interstitial cells, and total cells on each of 30–55 microscopic fields per tissue section. T cells were the most abundant infiltrating cell in tissues with IR (35 ± 9.8%), significantly higher than that seen in IN (21 ± 16%) or in kidneys without infiltration (5.0 ± 3.9%). The percentage of T cells identified by TA-1 or OKT3 was approximately equivalent to the summation of OKT4 plus OKT8. Although no differences were observed in the percentage of OKT4 cells, the percentage of OKT8 was significantly higher in IR (26 ± 7.7%, P < 10^-4) than in IN (9.3 ± 6.2%) or in kidneys with normal interstitium (3.0 ± 2.4%). The ratio of OKT8/OKT4-positive T cells in 22 graft tissues with IR (3.2 ± 1.4) was greater (P < 0.0007) than 6 graft tissues with IN without rejection (0.82 ± 0.39) and the 5 kidney tissues without interstitial infiltration (0.75 ± 0.25). There was no significant difference between the groups in the relatively low percentage of interstitial cells identified as B cells reacting with BA-1 or containing SIgD,M. The percentage of interstitial cells recognized by OKM1 was similar in rejection and interstitial nephritis, with both being greater than controls (P < 0.02). The relative numbers of blood mononuclear
cells identified by the monoclonal antibodies was generally not predictive of the proportions present in kidney tissue, although OKT4-positive blood cells were less numerous and OKM1+ blood cells were more numerous than in controls ($P < 0.002$).

Quantitative analysis of identifiable interstitial cells in graft rejection reveals that most infiltrating cells are T cells, the greater proportion of which are recognized by OKT8. OKT8-positive cells may play an important role in mediating renal graft rejection.

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