CLINICAL INVESTIGATION

Lupus nephritis: Correlation of interstitial cells with glomerular function

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Lupus nephritis: Correlation of interstitial cells with glomerular function. Mononuclear inflammatory cells were studied using monoclonal antibodies in the interstitium and glomeruli of 35 renal biopsy specimens from patients with lupus nephritis already taking immunosuppressants. The aims of this study were to assess the composition and significance of the infiltrate, and to assess correlations with immediate glomerular function and ability to predict the future course of the disease. The majority of interstitial cells were T lymphocytes and monocytes/macrophages. The number of interstitial CD4 +ve T helper/inducer lymphocytes was greater than that of CD8 +ve T cytotoxic/suppressor cells in only 19 out of 35 biopsies, the mean CD4:CD8 ratio being only 1.5 ± 1.2. NK cells and B lymphocytes were a minor component only. Some expression of IL-2, transferrin and C3b receptors was seen on interstitial cells, but HLA-DR expressing cells were much in excess of controls and the number of tubular cells expressing HLA-DR was also increased. The number of interstitial T cells, CD4 +ve cells and monocytes/macrophages was highly correlated with the extent of chronic damage judged by optical microscopy. There was also an association between glomerular function at biopsy and numbers of interstitial T cells, CD8 +ve cells, monocytes/macrophages and DR expressing cells. Subsequent decline in renal function, however, was associated only with numbers of monocytes/macrophages and the rather small number of C3b receptor-positive cells. The presence of tubulointerstitial immune aggregates of Ig and/or C in 63% of patients was associated with greater numbers of NK cells. As previously described, the degree of renal function at biopsy correlated with a chronicity index based on optical microscopy. No correlations were found between numbers or types (mostly monocyte/macrophages) of intraglomerular leukocytes and clinical or biopsy features, except that more proliferative types showed greater leukocyte numbers. One hypothesis consistent with our findings is that interstitial T cells and monocytes may be important determinants of pathogenesis and progression of lupus nephritis. While several mechanisms may play an initial role, interstitial monocytes may be the major factor in chronic injury.

The mechanisms of formation and the significance of tubulointerstitial lesions in lupus nephritis remain speculative. It has been suggested that tubules are equally involved along with the glomerulus, and that immune complex deposition may be an important factor leading to the tubulointerstitial damage [1–7]. Interstitial mononuclear inflammatory cells are a frequent finding in biopsies, and their severity was found to correlate with the pattern of glomerular injury, the histologic activity, and the

clinical severity of the disease [3, 5]. In addition, the intensity of the interstitial inflammation was also found to be a useful predictor of renal functional outcome [5]. The presence of these infiltrating mononuclear cells in the interstitium has led also to the suggestion that cell-mediated immune mechanisms may operate in the pathogenesis of the lesions [8, 9].

With the advent of monoclonal antibodies, it is possible to phenotype these cells into functionally distinct groups. However, recent reports have given divergent results concerning the composition of interstitial infiltrates in lupus nephritis [10–13]. Also, D'Agati et al [12] failed to demonstrate an association between individual types of interstitial cells and any of the histological or clinical parameters they studied. Moreover, no attempts have been made to determine what the prognostic value of the cell subsets might be, and information on their possible implications in the long-term outcome of the disease are scanty.

The aim of the present study was to characterize the types and the functional status (activated or not) of the mononuclear cells infiltrating the kidneys in patients with lupus nephritis. In addition, we attempted to correlate individual cell species with histopathological findings, and with renal function both at the time of biopsy and after long-term follow-up.

Methods

Patients

Thirty-five patients satisfying the criteria of the American Rheumatism Association for the diagnosis of SLE [14] were studied retrospectively. They consisted of 30 females and 5 males who had a mean age of 28 years (range 8 to 60 years). Twenty-seven patients with complete follow up have been included in the long-term prognosis study. Glomerular filtration rate (GFR) was estimated by a single injection of 51Cr-EDTA [15]. In five adult patients who did not have a GFR estimation at the end of the follow-up period, GFR was calculated from plasma creatinine according to a formula [16]. All patients had proteinuria but none was severely nephrotic. Thirty-four patients were treated with a combination of prednisolone with azathioprine or cyclophosphamide, and only one with prednisolone alone.

Tissue studies

Renal tissue obtained by percutaneous biopsy from all patients and eight normal cadaveric kidneys used for transplantation, was processed for light microscopy, electron microscopy

Table 1. Monoclonal antibodies employed and their characteristic specificity

Monoclonal antibody	CD reactivity	Specificity	Source
2D1	CD45	All leucocytes	Dr. P. Beverley
UCHT1	CD3	Pan T cells	Dr. P. Beverley
Leu 3a	CD4	T helper/inducer cells	Becton Dickinson
UCHT4	CD8	T suppressor/cytotoxic cells	Dr. P. Beverley
FMC32	CD14	Monocytes/macrophages	Serotec
Leu 7	—	Natural killer cells/ large granular lymphocytes	Becton Dickinson
M708	—	B lymphocytes	DAKO
T05	CD35	C3b receptors	DAKO
DK25	—	HLA-DR related antigen	DAKO
Anti-TAC	CD25	IL-2 receptors	DAKO
Anti-human transferrin receptor antibody	—	Transferrin receptors	Becton Dickinson

and immunoperoxidase detection of immune deposits according to standard techniques. Renal biopsy data were analyzed and classified in a semiquantitative way by two independent observers (E.A. and R.B.H.) without information of patient identity.

Histologic patterns of glomerular involvement were determined according to the WHO classification [17]. The "activity" and "chronicity" of the histological appearance of disease were assessed by grading the presence and the severity of specific histologic features as described by Austin et al [18]. Each parameter was graded on a scale of 0 (absent), 1 (mild), 2 (moderate) and 3 (severe). Two active lesions, fibrinoid necrosis and cellular crescents, were each arbitrarily weighted by a factor of two on the assumption that these features were disproportionately more ominous that the other active lesions [18]. Composite scores derived from the sum of scores for individual active lesions (activity index) and chronic, irreversible lesions (chronicity index) were finally calculated. According to this scoring system, maximum score of activity index is 24 and of chronicity index 12.

Snap-frozen tissue sections were cut at 4 μm and stained by a direct immunoperoxidase method using antisera for IgG, IgA, IgM, C3, C4 and C1q. The intensity of the deposits in the glomeruli was expressed as negative (0), mild (+), moderate (++) and heavy (+++). In addition, the number of patients with tubulointerstitial immune deposits was recorded.

Monoclonal antibodies

The monoclonal antibodies (McAb) used and their characteristics as defined at the Third Conference on Leukocyte Typing [19] are listed in Table 1. An indirect immunoperoxidase technique was used throughout the study. In brief, serial sections of snap frozen material were cut at 6 μm and fixed in cold acetone at 4°C for 10 minutes. This was followed by sequential 90-minute incubation with the mouse antihuman McAb, at the appropriate dilutions. Finally the sections were treated with diaminobenzidine (DAB) (0.5 mg/ml in phosphate buffered saline, PBS, plus 0.01% H_2O_2) for two minutes. After extensive washing in PBS, sections were counterstained with

Meyer's Haemalum, dehydrated and mounted in DPX (Raymond Lamb, London, UK). In 28 biopsies, where sections were stained for interleukin-2 (IL-2) and transferrin receptors, intensification of the DAB reaction product was achieved by treating the sections with gold salts (0.25% $NaAuCl_4 \cdot 2H_2O$ in PBS) for 30 seconds [20]. Positive controls run for each antibody (tonsil section) and an internal negative control were processed.

Quantitative studies of cells

Only cells with a distinctly brown and continuously stained plasma membrane surrounding a clear identifiable nucleus were counted. Positive cells were separately counted in the interstitium and the glomeruli.

Interstitial cells. Cells in the interstitium were counted using an eye piece graticule to identify 10 microscopic fields, each 0.058 mm^2. Thus a total area of 0.58 mm^2 was counted. The sections were counted in a sequence of adjacent fields without adjustments, except to avoid glomeruli or major vessels. Finally the numbers were expressed as cells per mm^2.

Glomerular cells. The number of glomeruli in the biopsies varied from 5 to 16 per section. For each section, both the number of the intraglomerular positive cells and the number of glomeruli were counted. Cells in Bowman's space or in crescents were not included in the count. Also, C3b-receptor and DR-expressing cells were not enumerated in the glomeruli because epithelial and endothelial glomerular cells are normally stained by these two antibodies [21, 22], from which infiltrating cells cannot readily be distinguished. Finally the number of positive cells was expressed as number of cells per glomerular cross section.

Clinicopathologic correlations

Correlation coefficients were obtained between the numbers of individual species of cells, both in the interstitium and the glomeruli, and the optical microscopy findings, as expressed by the activity and chronicity index. Also, correlations were assessed between the types of cells and the intensity of immune deposits on immunoperoxidase technique. In addition, we correlated the numbers of each type of cells and the severity of tubulointerstitial lesions with the clinical data of the patients at the time of biopsy, including renal function parameters. In 28 patients, where follow up was available, a similar correlation was performed with renal function at the end of the observation period.

Statistical analysis

Student's t-test was used for paired and unpaired data, and Pearson's and Spearman's correlation coefficient for the analysis of parametric and non-parametric data. $P < 0.05$ was accepted as statistically significant.

Results

Patients

Age of the patients ranged from 8 to 60 years at biopsy (28.6 ± 14.2 yrs). Proteinuria of variable amounts was present in all the patients. Thirteen patients (37%) presented with nephrotic syndrome at the time of biopsy. Mean proteinuria was 3.2 ± 3.1 g/24 hr and mean plasma albumin 30.0 ± 7.3 g/liter. Microscopic hematuria was observed in 18 patients (51%) and hypertension
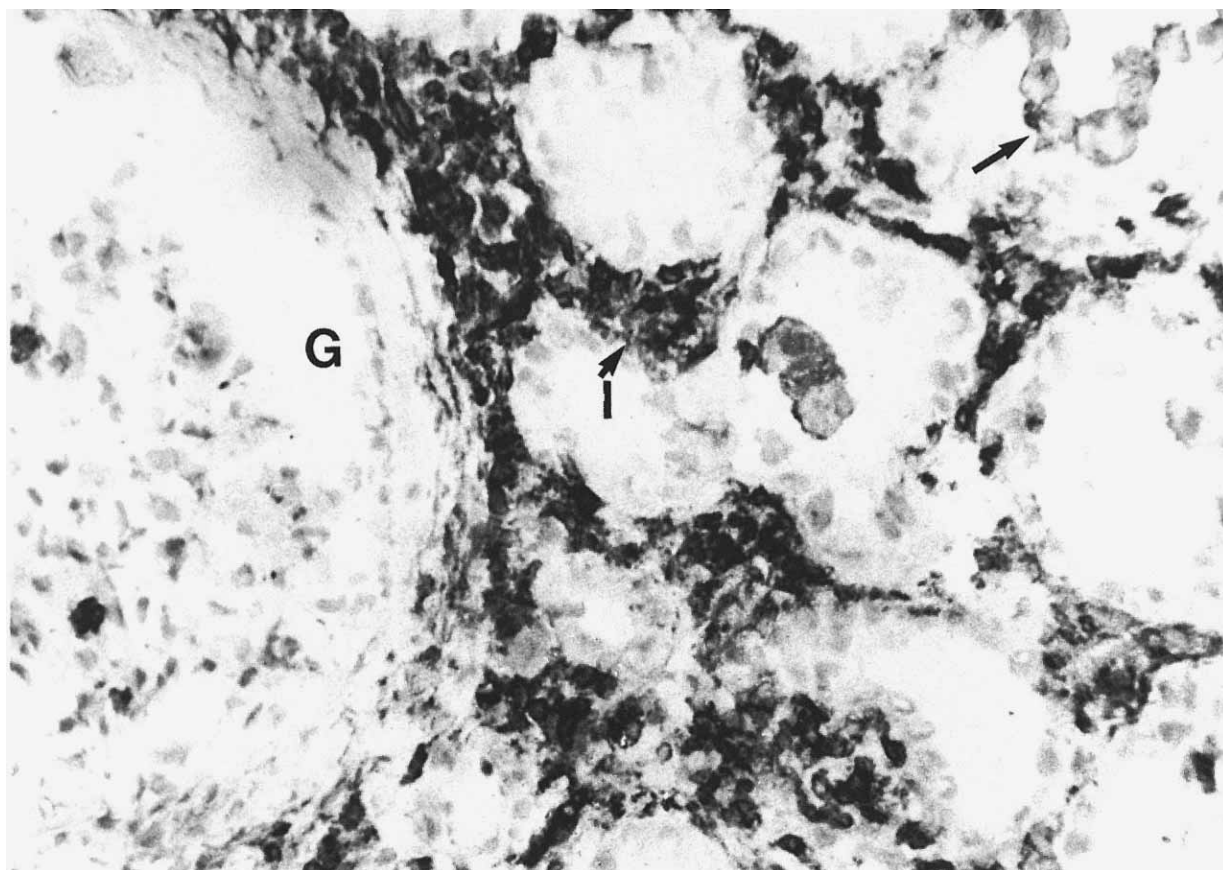
Fig. 1. *Leucocytes (CD45+ve) in the interstitium (I) and the glomerulus (G) of a patient with WHO Class II lupus nephritis.* Note the relatively small number of intraglomerular leucocytes and the positive staining of a cluster of foam cells (arrow) by this antibody (×250).

in 12 (34%). Twenty-three patients (66%) presented with impairment of renal function at the time of biopsy (GFR < 90 ml/min). The lowest GFR observed was 14 ml/min. Mean plasma creatinine and GFR at presentation were 117 ± 84 μmol/liter and 76 ± 34 ml/min, respectively. The mean follow up of 28 patients who were included in the long-term study was 44.6 ± 30.5 months. During the observation period mean plasma creatinine increased significantly from 112 ± 84 to 211 ± 281 μmol/liter ($P = 0.01$) while GFR dropped from 78 ± 34 to 73 ± 45 ml/min ($P = $ NS). By the end of the follow up four patients (11%, 3 females and 1 male) had reached end-stage renal disease and required chronic hemodialysis. Patients who started on the chronic hemodialysis program were considered as having a GFR of 2 ml/min at that time.

Optical microscopy and immunoperoxidase findings

Glomerular appearances in renal biopsies were classified on optical microscopy as mesangial proliferative (Class IIb) in nine patients, focal proliferative (Class III) in four, diffuse proliferative (Class IV) in 14 and membranous (Class V) in eight. The activity index ranged from 2 to 20 (mean 7.9 ± 5.0) and the chronicity index from 0 to 9 (mean 3.2 ± 2.2). Thirty-one patients showed a variable degree of tubulointerstitial damage while the remaining four had a normal interstitium by the optical microscopy.

Tubulointerstitial deposits were noted in 22 patients (63%).

The deposits were mainly observed in patients with proliferative lesions [Class III (3) and Class IV (10)], they appeared in a finely granular pattern, and were predominantly seen along the tubular basement membrane. The composition of the tubulointerstitial deposits varied greatly. In 16 cases the deposits consisted of one or more immunoglobulins most commonly associated with C3. In six cases C3 but no immunoglobulins was detected. Granular immune deposits of variable intensity were found in all the glomeruli examined. They were distributed in the mesangial area and/or along the capillary wall depending on the class of the lesions.

Analysis of the cells

Interstitial cells. The mean number of total leucocytes was 268 ± 17/mm^2 (Fig. 1), much in excess of control kidneys, with CD3 +ve cells predominating (Fig. 2 and Table 2). The mean number of CD4+ve helper/inducer T-cells exceeded that of CD8 +ve cytotoxic suppressor cells by only a modest margin: in 19 cases CD4+ve cells were in the majority, but in 16 biopsies CD8 +ve cells were the predominant type of T-cell. In four biopsies, there was clear evidence of infiltration of renal tubules by CD8 +ve cytotoxic/suppressor cells.

Monocytes/macrophages were seen in lower numbers than T cells (Table 2) while NK cells and B cells formed only a minority of the interstitial infiltrates. Surprisingly, in view of the numbers of monocytes present, only few CD35 positive cells
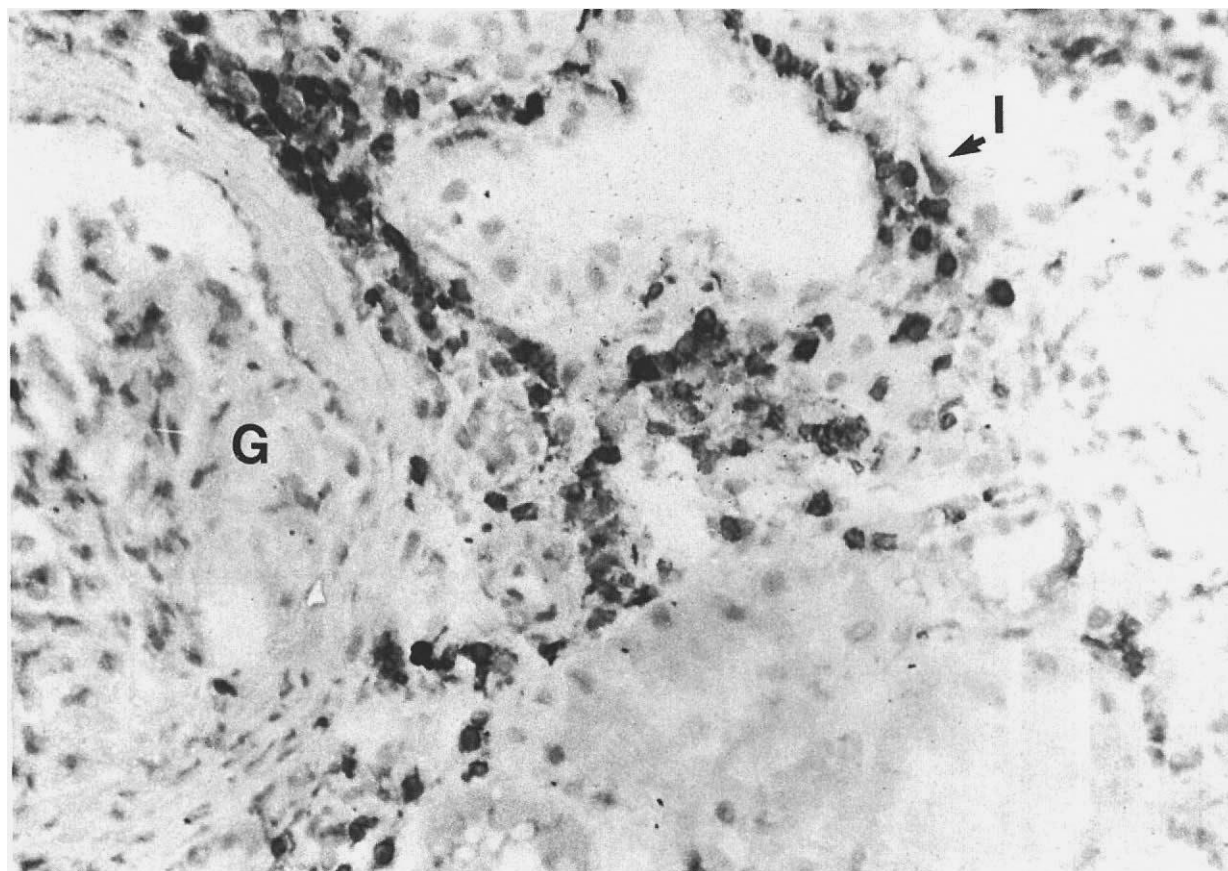
Fig. 2. *T-lymphocytes (CD3+ve) in the same part of the biopsy shown in Figure 1.* The cells are mainly distributed in a peritubular and periglomerular pattern (×250). (G = glomerulus, I = interstitium).

Table 2. A. Interstitial cells in 35 patients with lupus nephritis

	2D1 CD45	UCHT1 CD3	Leu 3a CD4	UCHT4 CD8	CD4:CD8 ratio	FMC32 CD14	M708 DK22	Leu 7	T05 CD35	DR + ve interstitial cells	DR + ve tubular cells
Controls N = 8	113 ± 11	46 ± 7 (41%)	34 ± 11 (30%)	22 ± 7 (19%)	1.8 ± 0.2	75 ± 12 (66%)	4 ± 2 (3%)	3 ± 1 (3%)	7 ± 2 (6%)	109 ± 30 (96%)	18 ± 5
Lupus nephritis N = 35	268 ± 17	172 ± 25 (64%)	84 ± 10 (31%)	77 ± 15 (29%)	1.5 ± 0.3	109 ± 13 (41%)	7 ± 4 (3%)	9 ± 2 (3.3%)	3 ± 2 (1.1%)	158 ± 14 (59%)	28 ± 4
N studied	33	30	34	35	34	35	34	33	35	28	29

Table 2. B. Glomerular cells in 35 patients with lupus nephritis

|	2D1 CD45	UCHT1 CD3	Leu 3a CD4	UCHT4 CD8	CD4:CD8 ratio	FMC32 CD14	M708 DK22	Leu 7
Controls N = 8	2.1 ± 1.1	0.4 ± 0.3 (19%)	0.3 ± 0.3 (14%)	0.2 ± 0.2 (9%)	1.6 ± 0.2	0.9 ± 1.0 (43%)	0.2 ± 0.4 (9%)	0.1 ± 0.1 (5%)
Lupus nephritis N = 35	4.0 ± 0.6	0.6 ± 0.1 (15%)	0.4 ± 0.1 (10%)	0.2 ± 0.1 (5%)	1.3 ± 0.3	1.7 ± 0.4 (42%)	0.02 ± 0.02 (0.5%)	0.1 ± 0.02 (2.5%)
N studied	30	28	32	32		31	32	31

A. Data are mean ± SEM per mm², CD4:CD8 ratio SEM. Numbers in parentheses express the percentage of the mean total leukocytes. Numbers below each column indicate numbers of biopsies studied with each monoclonal antibody. All 8 controls were studied with all antibodies.

B. Data are mean ± SEM per glomerular cross section. Numbers in parentheses express the percentage of the mean total leukocytes. Numbers below each column indicate numbers of biopsies studied with each monoclonal antibody. All 8 controls were studied with all antibodies.

bearing CR-1 (C3b) receptors were seen (Table 2). The numbers of DR expressing cells were higher in controls, as was the case with tubular epithelial cells (Fig. 3, Table 2).

Interestingly, patients with proliferative glomerular lesions (Class III and IV) did not show any difference from those with less proliferative glomeruli (WHO Class II and V). Expression of CD25 55kd IL-2 receptor (tac) and transferrin receptor by some interstitial cells was seen in 6 of 25 and 11 of 25 biopsies
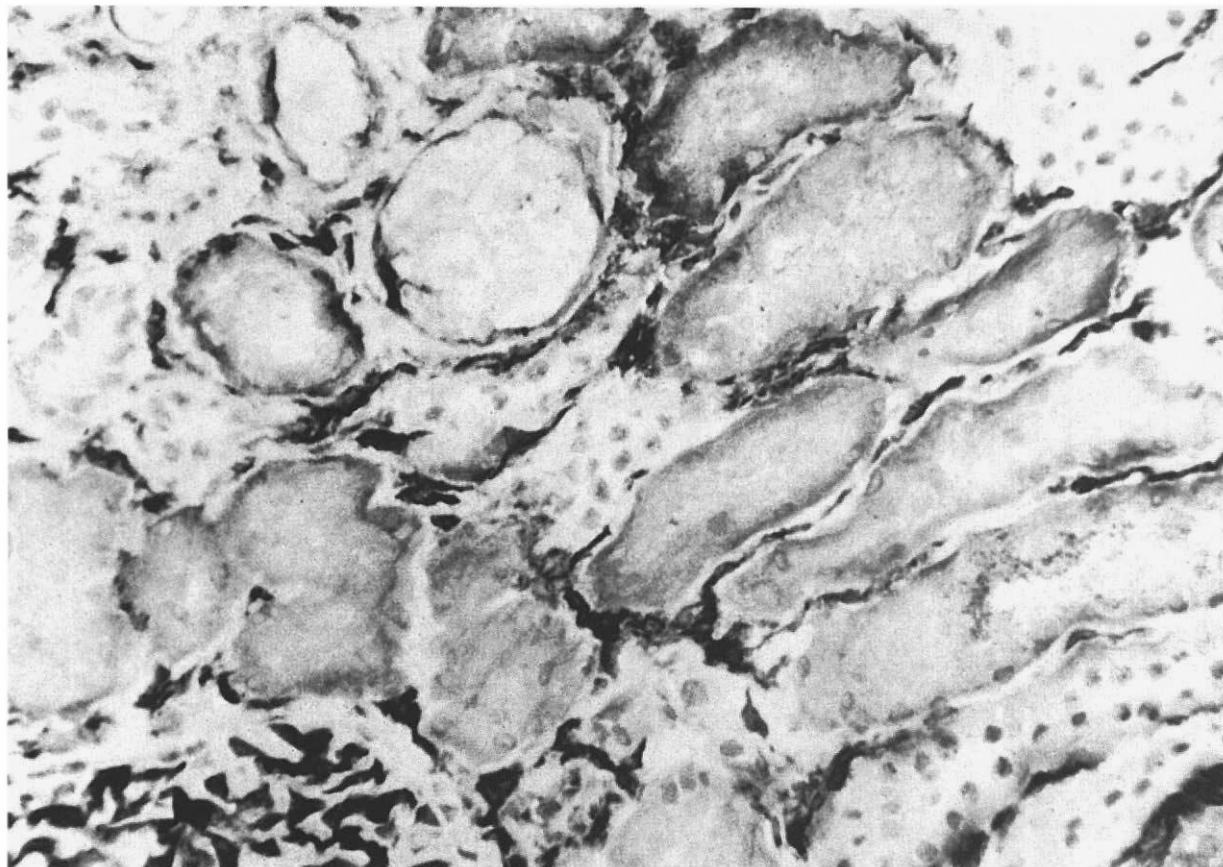
Fig. 3. *DR expression by the tubular epithelial cells.* Note the presence of a great amount of DR positive interstitial cells, surrounding the tubules (×250).
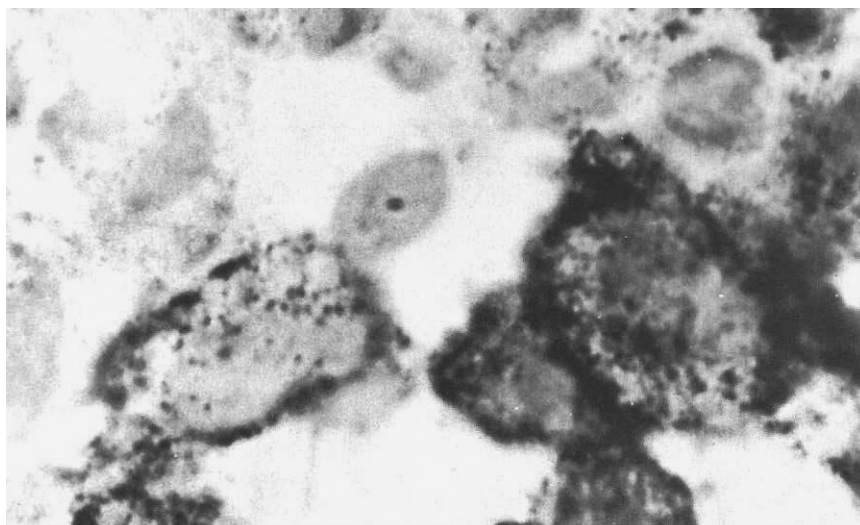
Fig. 4. *Interleukin 2 (CD25) expression by interstitial cells in a patient with diffuse proliferative (Class IV) lupus nephritis (×1000).*

respectively (Fig. 4). Staining of tubular epithelial cells with anti-transferrin McAb was a common feature, but varied greatly in intensity and distribution.

Glomerular cells. There were rather few leukocytes (CD45 +ve) per glomerular cross section (Table 2). Greater numbers of leukocytes were noted in WHO Class IV biopsies (7.6 ± 3.9) than in class III (4.8 ± 3.1), class II (3.9 ± 2.0) or class V (2.2 ± 1.3). Monocyte/macrophages formed the majority of these
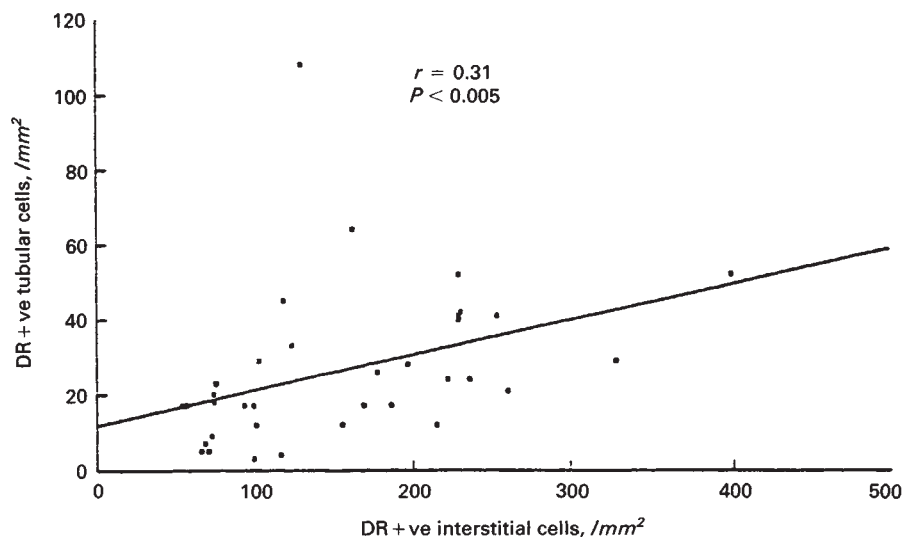
Fig. 5. *Correlation between the DR expressing tubular cells and the numbers of interstitial DR +ve cells (r = 0.31; P = 0.005).*

cells in the glomeruli, with CD3 +ve T cells seen in much lower numbers (Table 2). NK cells and B cells were almost absent from glomeruli (Table 2).

Correlations

Correlations between cell types. As expected, there were highly significant associations between CD45 +ve total leuko-cytes, and CD3 +ve T-cells, CD4 +ve and CD8 +ve T-cells, and CD14 +ve monocytes/macrophages (all $P < 0.001$). Only the numbers of NK cells and CD35 +ve C3b receptor +ve cells did not correlate with those of other cell types or with total cells.

However, neither the ratio of lymphocytes: monocytes nor the CD4:CD8 ratio correlated with total lymphocytes or total cells, and activation markers were sometimes present in biopsies of all intensities of infiltrate. DR expression in interstitial cells did, however, correlate with numbers of other cell types (CD45 +ve, $P < 0.001$; CD3 +ve, $P < 0.025$; CD4 +ve, $P < 0.05$; CD8 +ve, $P < 0.025$; CD14 +ve, $P < 0.005$) except NK cells and C3b receptor +ve cells. The proportion of DR +ve interstitial cells, in contrast, was independent of the intensity of the interstitial infiltrate. Expression of DR on interstitial cells correlated with DR expression in tubular epithelial cells (Fig. 5).

Pathological correlations

The activity index did not correlate with numbers of any type of infiltrating cells (Table 3). In contrast, the chronicity index correlated well with numbers of CD3 +ve T-cells ($P < 0.0005$), the subset of CD4 +ve T-cells ($P < 0.05$) and CD14 +ve monocytes/macrophages ($P < 0.025$; Table 3.). Interestingly, biopsies showing tubulointerstitial immune aggregates of Ig and/or C3 showed significantly higher numbers of interstitial NK cells (11.6 ± 3.4 vs. 3.3 ± 4.9; $P < 0.025$) than those without such aggregates. As expected, the activity index was significantly greater in proliferative biopsies (WHO classes III and IV) compared to those classified as WHO classes II ($P < 0.005$) or V ($P < 0.01$). The activity, but not the chronicity index was highly associated with the glomerular deposition of C3 ($P < 0.05$), C4 ($P < 0.01$) and C1q ($P < 0.05$).

Table 3. Relationship between different types of interstitial cells and the degree of active and chronic tubulointerstitial lesions

No. of cells		Activity Index	Chronicity Index
Reacting with	Displaying		
2D1	CD45	NS	NS
UCHT1	CD3	NS	<0.05 (r = 0.25)
Leu 3a	CD4	NS	<0.05 (r = 0.20)
UCHT4	CD8	NS	NS
FMC32	CD14	NS	<0.025 (r = 0.18)
Leu 7	—	NS	NS
T05	CD35	NS	<0.05 (r = 0.37)
M708	—	NS	NS
DR + ve interstitial cells		NS	NS
DR + ve tubular cells		NS	NS
CD4:CD8 ratio		NS	NS

Correlations with glomerular function

Glomerular function at presentation was significantly correlated with higher numbers of CD3 +ve T-cells (Fig. 6), CD14 +ve monocytes/macrophages and DR expressing interstitial cells (Table 4).

Glomerular function at follow-up was correlated with numbers of monocytes/macrophages (Table 4, Fig. 7) and CD35 +ve cells bearing C3b receptors but not with numbers of CD3 +ve T cells or their subsets (Table 4); the CD4:CD8 ratio was not predictive for GFR or plasma creatinine, either initially or at follow-up. Tubular expression of HLA-DR did not correlate with follow-up GFR. The chronicity index was highly correlated with the degree of renal impairment at the time of biopsy and at the end of the period of observation (Table 5), but not the activity index.

The numbers or types of intraglomerular cells did not relate to the initial or follow-up GFR or plasma creatinine, nor did the WHO classification of glomerular appearances predict outcome in these patients, all of whom received treatment. No clinical parameter (age, sex, proteinuria, presence of hematuria or hypertension) correlated with intraglomerular or interstitial leukocytes.
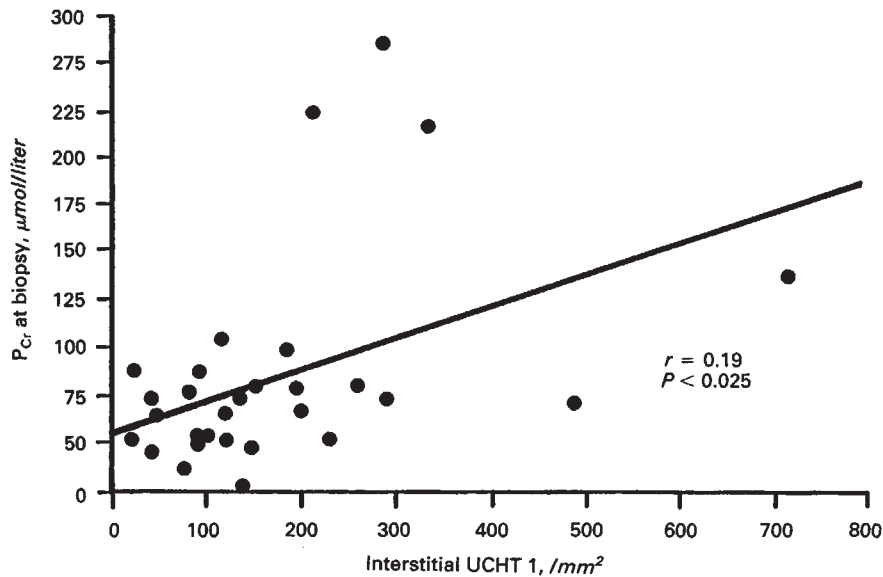
Fig. 6. *Correlation between plasma creatinine at presentation and the numbers of interstitial T cells (CD3 +ve; r = 0.19; P < 0.025).*

Table 4. Correlation between different species of cells infiltrating the interstitium and glomerular function at the time of biopsy, and at end of follow-up

No. of cells		Time of biopsy		Last follow-up	
Reacting with	Displaying	P_{Cr}	GFR	P_{Cr}	GFR
2D1	CD45	NS	NS	NS	NS
UCHT1	CD3	<0.025 ($r = 0.19$)	<0.025 ($r = 0.20$)	NS	NS
Leu 3a	CD4	NS	NS	NS	NS
UCHT4	CD8	NS	<0.05 ($r = 0.11$)	NS	NS
FMC32	CD14	NS	<0.025 ($r = 0.14$)	<0.025 ($r = 0.22$)	<0.025 ($r = 0.18$)
Leu 7	—	NS	NS	NS	NS
T05	CD35	<0.0001 ($r = 0.70$)	<0.01 ($r = 0.14$)	<0.005 ($r = 0.38$)	<0.005 ($r = -0.16$)
M708	—	NS	NS	NS	NS
DR + ve interstitial cells		<0.01 ($r = 0.23$)	<0.025 ($r = -0.19$)	NS	NS
DR + ve tubular cells		NS	NS	NS	NS

Discussion

Interstitial inflammatory cells and tubulointerstitial disease are commonly present in biopsies of patients with lupus nephritis [3]. The severity of interstitial inflammation has been found to correlate with other histological indices of disease activity as well as with plasma creatinine and diastolic hypertension of the patients at initial biopsy [5]. Whether these cells play a primary role in the pathogenesis of tubulointerstitial lesions or they represent a secondary reaction to tissue injury is not known. Recent literature using monoclonal antibodies has yielded rather conflicting results regarding the composition of the infiltrates [10–13].

Some investigators have failed to show a correlation between different types of cells infiltrating the interstitium and histological or clinical parameters, including glomerular function [12]. Also, data on possible relations of interstitial cells and long-term renal function have been lacking. In our study, as in previous work [10–13] the majority of infiltrating interstitial cells in lupus nephritis were T lymphocytes and monocytes/macrophages. CD4 +ve T-cells were the majority in 19 patients, but in 16 CD8 +ve cells predominated. D'Agati et al [12] noted CD8 +ve cells in the majority in 22 of 26 patients studied, as did

Castiglione et al [13]. In contrast, other reports have shown a majority of CD4 +ve cells either in all patients [11], or the majority of cases [23]. These differences could arise from selection of different patients for study, but treatment obviously may be one factor, since it is known that corticosteroid treatment preferentially decreases CD8 +ve cells and would increase the CD4:CD8 ratio [24]. Thus, Castiglione et al [13] noted a mean CD4:CD8 ratio of only 0.7 ± 0.5 in patients who were *untreated* (RC Atkins, personal communication, 1988). It has also been suggested that the interstitial cell population is phase-dependent [25] and may change with the evolution of the disease [8]. Certainly, in most patients with lupus nephritis there are more CD8 +ve cells than in other forms of primary glomerulonephritis (IgA and membranous GN) which we and other workers have studied [10, 23, 25–27].

Monocytes and macrophages were seen in lower numbers, but in a similar distribution within the interstitium as T cells. The low expression of the C3b receptor on these interstitial cells was a surprise, but this receptor is susceptible to both down and up regulation on monocytes [28]. NK cells and B cells were a minor component of the interstitial infiltrates, but it is of interest that NK cells were present in greater numbers in those
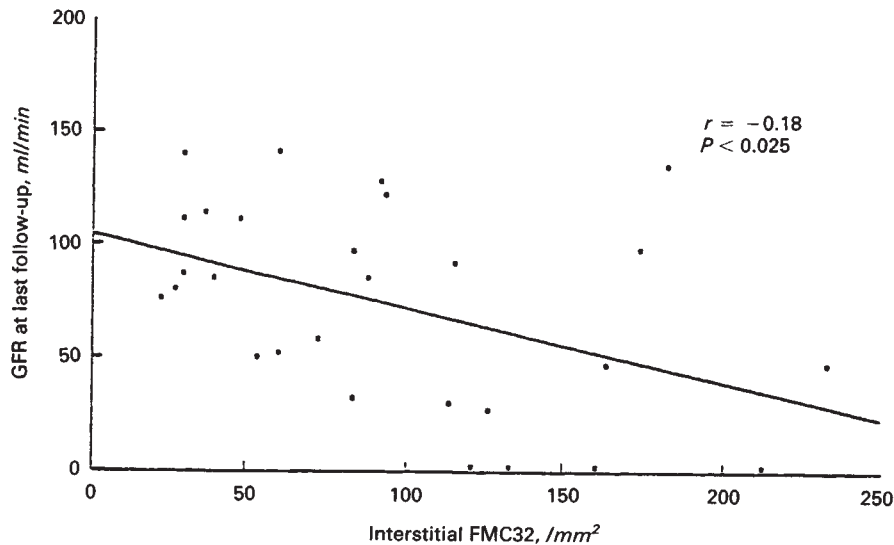
Fig. 7. *Correlation between number of interstitial monocytes/macrophages and GFR at follow-up (r = 0.18; P < 0.025).*

Table 5. Correlation between the activity and chronicity index with renal function parameters at the time of biopsy and at the end of follow-up

	Time of biopsy		End of follow up	
	P_{Cr}	GFR	P_{Cr}	GFR
Activity Index	NS	NS	NS	NS
Chronicity Index	<0.01	<0.05	<0.01	NS
	(r = 0.48)	(r = -0.34)	(r = 0.54)	

biopsies showing tubulointerstitial immune aggregates. HLA-DR was expressed by the interstitial cells irrespective of the presence of deposits or histological tubulointerstitial damage. The presence of DR positivity was correlated highly with all of the types of interstitial cells, in excess of those normally present (dendritic and endothelial cells), suggesting that most of the cells comprising the infiltrate are activated. The relative rarity of IL-2 receptor expression we found does not negate the presence of activated cells, since the expression of IL-2 receptors appears to be a transient early phenomenon [29] and the production of IL-2, as well as the responsiveness of SLE T cells to it, appears to be diminished [30]. The effects of prior treatment, in all but one patient studied, may be important here also.

Tubular DR expression was observed also, and there was a strong association between interstitial and tubular DR expressing cells. The proportion of DR expressing tubular cells was greater in lupus nephritis in comparison to other forms of glomerular diseases, such as IgA nephropathy or membranous nephropathy [26, 27]. This probably indicates the greater degree of tubular cell activation in lupus nephritis than in other glomerular diseases, and Boucher et al [23] noted similar tubular DR positivity in a few patients with lupus. Tubular activation is supported also by the finding that transferrin receptor expression by the tubular cells was a common feature in the majority of the biopsies [26, 27, 31, 32], even in the absence of transferrin receptor expressing interstitial cells [26, 27].

Mechanisms of mononuclear cell accumulation

The mechanisms responsible for the accumulation of mononuclear cells in the interstitum in lupus remain unclear. It has been suggested that the interstitial inflammation in lupus nephritis may occur in response to immunoglobulin and/or complement deposition in the TBM and interstitium [2]. However, our own results and those of others [5] do not support this hypothesis, since we did not find immune deposits in all our cases, and there were no differences in the numbers or types of interstitial cells between the patients with and without interstitial immune deposits, except for the minority of NK cells. Nor did the numbers of infiltrates differ significantly in biopsies with the more active proliferative forms of glomerular disease.

It has been shown recently that renal tubular epithelial cells may express HLA-DR antigens in response to immunological stimuli, and that this permits them to induce T helper lymphocytes [33, 34]. The high degree of association between tubular and interstitial and DR expressing (Fig. 5) may indicate that DR expression by the tubules and the influx of activated mononuclear cells in the interstitium are linked in lupus nephritis.

Functional significance of the interstitial cells

The functional significance of the interstitial cells in the pathogenesis of the tubulointerstitial lesions in lupus nephritis is not clear either. No correlation was found between the "activity index" and any of the types of interstitial infiltrating cells. These data agree in general with the findings of D'Agati et al [12], but in contrast to their data we were unable to show any relationship between CD4:CD8 ratio and the activity index. Further, our data showed a strong association between the chronicity index and the extent of interstitial infiltration by T cells and monocytes/macrophages. Whether this relationship represents a role of cell-mediated immune mechanism in the pathogenesis of the lesions, or is a non-specific secondary response to injury, is unknown.

The lack of differences between patients with and without tubulointerstitial immune deposits with respect to the chronicity index, and the absence of significant correlation with B cells suggests that chronic lesions are probably caused by mecha-

nisms other than antibody-dependent immune mechanisms. Our results support the alternative suggestion made by other workers [11] that activated T cells can produce a local reaction, perhaps via recruited macrophages, and the prevalence of CD4+ T cells suggests a role for a delayed-type hypersensitivity reaction [35]. The role of a direct cytotoxic reaction involving CD8 +ve cells [36] may be secondary, based on the absence of any relationship between CD8+ cells and the extent of chronic damage. However, the predominance of CD8+ cells in almost half the biopsies and the infiltration of tubular epithelium by CD8+ cells does suggest a role for T-cell mediated cytolysis [12, 36]. Finally, the higher numbers of NK cells in biopsies with tubulointerstitial immune deposits could indicate antibody-dependent cell-mediated cytotoxicity (ADCC) also operates in lupus nephritis, as it does in some forms of experimental anti-TBM nephritis [37].

In contrast to the data of D'Agati et al [12], the present study showed that the degree of renal impairment at the time of biopsy was strongly correlated with the density of interstitial infiltration by T cells, monocytes/macrophages and DR expressing interstitial cells. This again supports an important role for cellular immune mechanisms in the pathogenesis of the disease. The exact pathogenetic relationship between activated components of cellular immunity, chronic tubulointerstitial damage and renal function impairment at presentation awaits further elucidation.

Long-term prognosis

The long-term prognosis of the disease seemed to be predicted by both the major degree of monocyte/macrophage and the minor C3b receptor-expressing cell infiltration. It is known that monocytes can be attracted by C3b receptor-expressing cells [38]. Whether macrophages result primarily in tissue destruction by releasing proteolytic enzymes or by phagocytosis [39, 40] leading finally to renal failure or they represent a secondary phenomenon to tissue damage [41] is not clear. In addition, the chronicity index was highly associated with the initial renal function, in agreement with the data of Austin et al [18]. They also pointed out that relatively small set of patients with a markedly elevated activity index were clearly at increased risk for development of end-stage renal disease. No other correlations emerged in our study. In particular, no glomerular feature correlated with GFR or plasma creatinine, either at time of biopsy or follow-up.

In conclusion, one explanation for our data is that T cells and monocytes/macrophages may play an important role in the pathogenesis of chronic tubulointerstitial lesions in lupus nephritis, and that the immune deposits, commonly absent, may play a minor role. The chronicity index [18] is valuable in predicting renal function impairment at presentation and the progression of the disease. A variety of humoral and cell-mediated immune mechanisms probably determine the degree of renal impairment early in the course of the disease, while long-term outcome seems to be influenced by the extent of macrophage infiltration in the initial biopsy.

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