

HLA-Dr⁺ T cells of the Leu 3 (helper) type infiltrate the kidneys of patients with systemic lupus erythematosus

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SUMMARY

The lineage and distribution of mononuclear cells infiltrating the kidneys of patients with systemic lupus erythematosus (SLE) have been investigated in cryostat tissue sections of biopsies from 11 patients. The use of heterologous antisera and monoclonal antibodies has revealed that: (1) B lymphocytes and plasma cells are virtually absent in SLE kidney specimens; (2) The vast majority of mononuclear cells which infiltrate the interstitium are activated (HLA-Dr⁺) T cells (Leu 4⁺) presenting the helper (Leu 3⁺) phenotype; (3) T cells are absent in the glomeruli, where HLA-Dr⁺, SiGM⁻, Leu 4⁻ elements with a macrophage like appearance can be observed.

Keywords monoclonal antibodies lymphocytes autoimmunity immune complexes

INTRODUCTION

Human systemic lupus erythematosus (SLE) is a multisystemic autoimmune disease characterized by B lymphocyte hyperactivity, with enhanced production of polyclonal immunoglobulins (Ig) and specific autoantibodies.

Antigen-antibody complexes (immune complexes, IC) are formed and deposit mainly in the glomeruli. Glomerulonephritis is one of the cardinal manifestations of SLE.

The markers and functional activity of T lymphocytes have been investigated in murine SLE, in order to test the possibility that the B cell hyperactivity can be caused by abnormalities of the T cell control. These studies have led to the conclusion that in some strains a B cell differentiation factor is produced by Lyt1⁺, 2⁻ (helper) T cells which induces activated B lymphocytes to differentiate into Ig secreting cells (Prud'homme *et al.*, 1983a), while, in others, B cells give abnormally high responses to the usual signals (Prud'homme *et al.*, 1983b). In human SLE, these investigations have been limited to peripheral circulating T lymphocytes and have yielded conflicting results (Bach & Bach, 1981). However, two observations were made which are the rationale for the present study. (a) Mononuclear cells are present in human SLE kidneys (Appel *et al.*, 1978). (b) a significant infiltration of T lymphocytes has been found in the inflamed tissues of another multisystemic autoimmune disease (rheumatoid arthritis, RA) (Janosy *et al.*, 1981).

On these bases, we have investigated the lineage and distribution of mononuclear cells in cryostat tissue sections of kidney biopsies from 11 patients with histologically proven SLE glomerulonephritis utilizing monoclonal antibodies (MoAb). The studies provide evidence that the vast majority of mononuclear cells which infiltrate SLE kidneys are activated (HLA-Dr⁺) T cells presenting the helper phenotype.

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MATERIAL AND METHODS

Patients. Eleven patients (10 females and one male), age 19–65 (mean 32) satisfying the criteria of the American Rheumatism Association for the diagnosis of SLE (Cohen *et al.*, 1971) were selected for this study. All the patients underwent percutaneous renal biopsy. The samples were divided into two parts: one was processed for conventional histology and the other snap frozen in liquid nitrogen for immunofluorescent studies (see below). The patient's histological findings were classified according to Appel *et al.* (1978) in five classes (Table 1): class I: normal kidney; class II: mesangial changes; class III: focal and segmental glomerulonephritis; class IV: diffuse proliferative glomerulonephritis; class V: membranous glomerulonephritis.

Six normal human kidney specimens, obtained during open surgery, were used as controls in the immunofluorescent studies.

Antibodies. Rabbit (R) antisera to human IgG, IgM, IgA, C3, C1q, C4, fibrinogen (F) directly conjugated with fluorescein isothiocyanate (FITC: Behringwerke, Mannheim, FRG) or tetraethyl rhodamine isothiocyanate (TRITC: Dakopatt, Glostrup, Denmark) were employed in direct immunofluorescence (IF) assays.

The MoAb Leu 2, Leu 3, Leu 4, and Leu 7 were from Becton-Dickinson, Sunnyvale California, USA; RFT1, RFT11, and RFA-HLA-Dr were produced at the Department of Immunology, Royal Free Hospital, London, UK. Leu 4, RFT11 (OKT11 like) and RFT1 (OKT1, Leu 1 like: Caligaris-Cappio *et al.*, 1982) are pan-T reagents; Leu 2 is a T suppressor/cytotoxic marker, Leu 3 a T helper marker and Leu 7 detects an antigen expressed by cells with natural killer activity. RFA-HLA-Dr detects the HLA-Dr (Ia like) structure. MoAb were utilized in indirect IF assays revealed by goat (G)-anti-Mouse (M) IgG or rat (r)-anti-M IgM (both FITC from Becton-Dickinson).

Staining of sections. Cryostat sections 4 μ m thick were cut from frozen tissue biopsies and fixed in cold (4°C) acetone for 5 min. In every case, some fixed sections were incubated with R-anti-human IgG, IgM, IgA, C3, C1q, C4, F FITC for 30 min at room temperature (rt) in a humid chamber, then washed with phosphate-buffered saline (PBS). The intensity of the glomerular

Table 1. Histological and immunofluorescence features of the SLE patients studied.

| Cases | Histological class | Glomerular immunofluorescence deposits* | | | | | | | MoAb reactive cells† | | |
|-------------------|--------------------|---|-----|-----|----|-----|----|----|----------------------|--------|--------|
| | | IgG | IgM | IgA | C3 | C1q | C4 | F‡ | Leu 4+ | Leu 3+ | Leu 2+ |
| 1 | II | +1 | 0 | +1 | +1 | +1 | +1 | 0 | 50–60 | 40–45 | 10–15 |
| 2 | II | +1 | +1 | 0 | +2 | +2 | +1 | +1 | 5–6 | 5–6 | 1–2 |
| 3 | III | +2 | +1 | +2 | +1 | +2 | +1 | +1 | 40–50 | 35–40 | 5–8 |
| 4 | IV | +3 | +1 | +1 | +1 | +3 | +1 | 0 | 50–60 | 45–50 | 8–10 |
| 5 | IV | +1 | 0 | +1 | +1 | +3 | +1 | +1 | 50–60 | 40–50 | 8–10 |
| 6 | IV | +2 | +1 | +1 | +1 | +1 | +1 | +2 | 10–15 | 12–15 | 3–4 |
| 7 | IV | +3 | +1 | 0 | +3 | +3 | +3 | +1 | 20–30 | 20–30 | 0–5 |
| 8 | IV | +4 | +3 | +1 | +2 | 0 | 0 | 0 | 20–30 | 20–25 | 3–4 |
| 9 | IV | +3 | +1 | +1 | +1 | +3 | +1 | 0 | 50–70 | 50–60 | 10–15 |
| 10 | V | +3 | +3 | +3 | +3 | +3 | +2 | +1 | 50–60 | 45–50 | 8–10 |
| 11 | V | +3 | +1 | +3 | +3 | +1 | +1 | +2 | 20–25 | 18–20 | 5–8 |
| Controls (n=6) | I | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0–2 | 0–1 | 0–1 |

* Intensity of glomerular staining graded from 0 to 4.

† Number of positive cells per microscopic field ($\times 400$).

‡ F = fibrinogen.

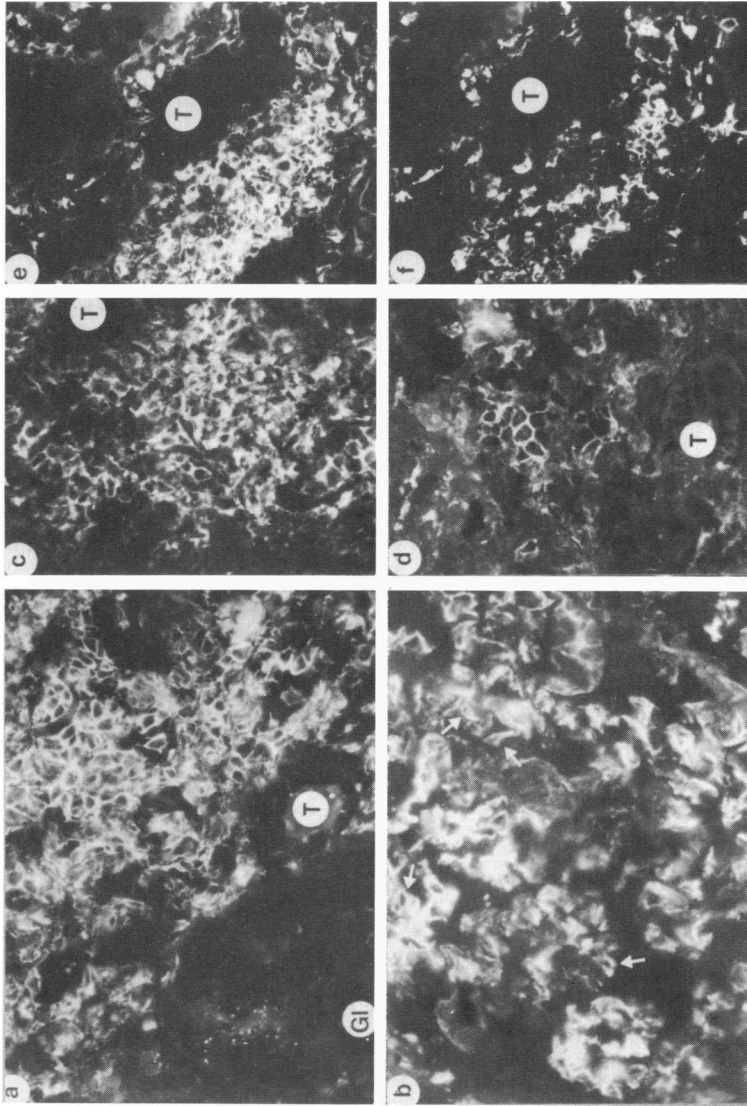


Fig. 1. Cell lineage and distribution of mononuclear elements infiltrating SLE kidney specimens. Gl = glomerulus; T = tubule. (a) Leu 4⁺ cells infiltrate the interstitium but not the glomerulus; (b) HLA-Dr staining of a glomerulus. Cells with an elongated shape can be observed amidst the capillary loops (arrows). (c) Leu 3 staining shows that the majority of interstitial mononuclear cells are positive. (d) Some Leu 2⁺ cells are nevertheless always detectable. (e) (f) The same field stained in double combination with Leu 3 (e) and Ch-anti-Ia (F). Many Leu 3⁺ cells are also Ia⁺.

staining was independently assessed by two observers under a Zeiss fluorescence microscope and graded from 0 to 4. Other sections were incubated with the relevant MoAb for 20 min at rt, washed and restained with G-anti-M IgG FITC or r-anti-M IgM FITC as second layers for 30 min at rt. The sections were then washed and mounted with buffered glycerol. The number of positive cells per microscopic field ($\times 400$) was scored and their anatomical distribution observed.

In some experiments, a double staining procedure was performed (Janossy, Alero Thomas & Habeshaw, 1980) in order to detect the concomitant expression of surface (s) IgM or HLA-Dr and T associated antigens on the same cell. R-anti-human IgM TRITC and chicken (C)-anti-human Ia revealed by sheep (sh)-anti-C Ig TRITC were utilized in double combination with the relevant MoAb plus G-anti-M IgG FITC.

RESULTS

The patients studied had the following histological distribution: two class II, one class III, six class IV and two class V (see Table 1). All patients presented a classical pattern of glomerular deposits of Ig and complement fractions. In two cases (patients 7 and 9, Table 1) granular deposits of IgG and C3 were present also along the tubular basement membrane.

In every kidney biopsy, mononuclear cells were observed. Most of the cells were Leu 4⁺ (Fig. 1a), RFT11⁺, RFT1⁺, sIgM⁻ and therefore bona fide T cells.

Their range varied from five or six to > 50 per microscopic field ($\times 400$). The distribution of T cells was interstitial and periglomerular. They usually presented in clusters and a diffuse even pattern was rarely observed. T cells were never observed in the glomeruli (Fig. 1a), where, instead, rare (four to eight per glomerulus) elements could be found with the sIgM⁻, Leu 4⁻, RFT11⁻, RFT1⁻, HLA-Dr⁺ (Fig. 1b) phenotype. These cells were large and exhibited an elongated shape with sometimes an irregular (stellate) appearance. Their number was proportional to the amount of Leu 4⁺ cells present in the interstitium. Rare elements with the same phenotype and morphology could be observed also amidst the interstitial Leu 4⁺ T lymphocytes.

Most of the infiltrating T cells were Leu 3⁺ (helper type), though a certain number of Leu 2⁺ (suppressor/cytotoxic) elements could be found (Fig. 1 c,d & Table 1). The Leu 3:Leu 2 ratio was 4:1, 5:1 (Table 1). Many T cells among the Leu 3⁺ cell population concomitantly expressed the HLA-Dr antigen (Fig. 1 e,f). Leu 7⁺ cells were virtually absent.

B lymphocytes (sIgM⁺, Leu 4⁻) were rare, usually < 1 microscopic field ($\times 400$) and plasma cells were virtually absent in all the samples examined.

DISCUSSION

A role for T lymphocytes in the triggering of B cell hyperactivity has been demonstrated in murine SLE (Prud'homme *et al.*, 1983a, 1983b). In order to investigate whether a similar mechanism can be operating also in human SLE, we have analysed the lineage and relationships of mononuclear cells infiltrating SLE kidney biopsies.

Our observations clearly show that activated (HLA-Dr⁺) T lymphocytes with the Leu 3⁺ (helper) phenotype represent the majority of mononuclear cells in the interstitium of SLE kidneys. These cells are absent in the glomeruli, where HLA-Dr⁺, sIgM⁻, Leu 4⁻ elements with a macrophage like appearance can be observed. A T helper cell infiltration has been described both in sarcoid lung (Semenzato *et al.*, 1981) and in RA (Janossy *et al.*, 1981). However, in RA synovia the infiltrating T helper cells have a close spatial relationship with HLA-Dr⁺ macrophages and are accompanied by an abundance of B lymphocytes and plasma cells (Janossy *et al.*, 1981). The whole picture in RA can thus be interpreted in terms of local B cell hyperactivity triggered by an exaggerated T cell/macrophage interaction. The situation is clearly not quite so in SLE, where the absence of B lymphocytes and plasma cells suggests that the autoantibodies involved in IC formation are not produced in the kidneys. Rather, our results can be more easily interpreted taking into account the experimental data showing that: (1) in normal rats, HLA-Dr⁺ cells are present in

glomeruli where can function as antigen handling cells capable of presenting antigens to T lymphocytes in a genetically restricted interaction (Schreiner *et al.*, 1981). (2) In rat experimental glomerulonephritis, the injection of IC and T lymphocytes sensitized to the Ig component of the IC (but not of IC alone) leads to glomerular abnormalities with increase in mononuclear phagocytes in mesangial region (Bhan *et al.*, 1979). (3) In rabbit experimental glomerulonephritis, the development of proteinuria can be suppressed by anti-macrophage serum (Holdsworth, Neale & Wilson, 1981; Lavelle, Durland & Yum, 1981).

Therefore, it can be suggested that also in human SLE activated T lymphocytes can (perhaps via recruited macrophages) produce a local reaction. This reaction does not appear to involve B cells. The significance of this mechanism to the IC-mediated damage remains to be evaluated, but, in any case, a local role of T cells in the pathogenesis of SLE has to be taken into account.

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