Serum soluble interleukin 2 receptor levels in anti-neutrophil cytoplasmic autoantibodies – positive systemic vasculitis

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Summary: Systemic vasculitis is characterized by the presence of autoantibodies to neutrophil cytoplasmic antigens (ANCA). The role of T-lymphocytes in systemic vasculitis remains uncertain. In the present study, we attempted to explore the role of T-lymphocytes in systemic vasculitis by measuring the serum soluble interleukin 2 receptor (sIL2R) levels in seven vasculitic patients and comparing the sequential measurements with the titres of ANCA which satisfactorily reflect the disease activity. The serum levels of both ANCA and sIL2R were elevated at clinical presentation. Contrary to ANCA, the serum sIL2R remained elevated in most patients despite clinical remission following immunosuppressive therapy.

These findings suggest that T-lymphocytes may be activated in the acute phase of the disease. The finding of elevated serum sIL2R levels in most patients during clinical remission indicates that it is not a good measure of the disease activity and tends to argue against the role of T-lymphocytes as a major effector mediating inflammatory injuries in systemic vasculitis.

Introduction

Wegener's granulomatosis (WG) and microscopic polyarteritis (MPA) are systemic vasculitides of uncertain aetiology characterized by chronic polymorphonuclear leucocyte infiltration within and around vessel walls at various sites throughout the body. Recent demonstration of circulating autoantibodies to neutrophil cytoplasm antigens (ANCA) has implicated autoimmune mechanisms in the pathogenesis of systemic vasculitis.¹ The role of ANCA in pathogenesis remains uncertain: deposition of immunoglobulin is not common in affected tissues, and this has led to the speculation that cellular mechanisms may be at least as important as humoral abnormalities in the development of tissue injury in these conditions.² The role of T-lymphocytes in these conditions has not been extensively studied. One would argue that a breakdown in tolerance at the T-lymphocyte level has occurred as T-lymphocyte help is required for most antibody production, and ANCA have the characteristics of T-lymphocyte-dependent antibodies (IgG isotype and high affinity). Indirect evidence of a role for T-lymphocytes is provided by their presence in involved tissues,^{3,4} although a definitive

role for T-lymphocytes in pathogenesis is hard to prove.

Activation of T-lymphocytes not only leads to the expression of interleukin 2 receptor (IL2R) molecules on the cell surface⁵ but also releases soluble IL2R (sIL2R) molecules into the circulation.⁶ Previous studies have confirmed a strong association of serum sIL2R levels with the activation of T-lymphocytes *in vitro*, and have indicated the sIL2R production is directly proportional to cellular IL2R expression.^{6,7} Markedly elevated levels of sIL2R have been reported in diseases involving T-lymphocytes such as haematological malignancy,⁸ systemic lupus erythematosus,⁹ and IgA nephropathy.¹⁰ In the present study, we attempted to explore the role of T-lymphocytes in systemic vasculitis by measuring the sIL2R and to compare the levels with the titres of ANCA which satisfactorily reflect the disease activity.

Materials and methods

Patients

Seven patients with systemic vasculitis were studied. Each patient was studied for at least 6 months (mean 12.7 months). Three suffered from WG and the remaining four had MPA based on the

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clinicopathological findings (independently of ANCA results).¹¹ Serological examination revealed that the three patients with WG had ANCA against serine proteinase 3 and the other four patients with MPA had ANCA against myeloperoxidase. Their clinical data are shown in Table I.

Treatment

Induction therapy comprised oral prednisolone and cyclophosphamide in all patients with systemic vasculitis. Hyperimmune globulin was given in one patient with WG. For the four patients with MPA, 2 litre plasma exchange was performed six to eight times during the acute phase of the disease.

Clinical assessment and diagnosis of relapse

Diagnosis of relapse was made on the basis of clinical and laboratory findings. 'Renal' relapse was diagnosed in the presence of a rising serum creatinine, with increasing proteinuria, and microscopic haematuria. The features of 'non-renal' relapse included fever, arthralgia, cutaneous vasculitis and pulmonary haemorrhage. Erythrocyte sedimentation rate or C-reactive protein was measured during clinical follow-up.

In all cases, the clinical and pathological evidence for relapse was considered sufficient to

justify an increase or reintroduction of immunosuppressive therapy.

Assays

Sera were collected for measurement of ANCA and sIL2R at each outpatient attendance (and twice weekly during initial hospital admission) and stored at -20°C until assayed. ANCA was tested in the sera by both indirect immunofluorescence¹ and solid-phase radioimmunoassay.¹² Serum sIL2R levels were measured by an ELISA kit available commercially (Cellfree, T cell Science, Boston, MA, USA). The normal range of the ANCA radioimmunoassay was previously determined to be 0-15.4%¹² whereas the upper normal limit of sIL2R was 747 U/ml. These values represent the values of mean + 2 standard deviations obtained from 25 healthy controls. The assay was carried out on the same day in identical conditions to avoid variability. The intra-assay and interassay coefficients of variation were 3.7% and 10%, respectively.¹³

Results

The serum ANCA levels were elevated in all vasculitic patients at first clinical presentation

Table I Clinical features of the seven vasculitic patients

Patient identification age (years)	Clinical characteristics	Diagnosis	ANCA specificity	ANCA level at presentation (%)	sIL2R at presentation (U/ml)
1. M, 61	Perforated eardrums, epistaxis, fever, chest lesions	WG	c-ANCA anti-PR3	110	1,146
2. M, 67	Nasal granuloma, productive cough	WG	c-ANCA anti-PR3	101	902
3. M, 52	Epistaxis, fever	WG	c-ANCA anti-PR3	33	760
4. M, 58	Haematuria, proteinuria, renal impairment	MPA	p-ANCA anti-MPO	53	1,588
5. F, 47	Haemoptysis, haematuria, proteinuria	MPA	p-ANCA anti-MPO	65	1,090
6. F, 53	Haemoptysis, haematuria, proteinuria, renal impairment	MPA	p-ANCA anti-MPO	74	1,232
7. F, 72	Haemoptysis, chest infection, heart failure, renal impairment	MPA	p-ANCA anti-MPO	118	1,068

WG = Wegener's granulomatosis; MPA = microscopic polyarteritis; PR3 = serine proteinase 3; MPO = myeloperoxidase; ANCA = anti-neutrophil cytoplasmic autoantibodies; sIL2R = serum soluble interleukin 2 receptor.

(median 74%, range 33–118%). The serum ANCA levels fell with clinical improvement following immunosuppressive therapy with or without plasma exchange. During clinical remission, the serum ANCA levels were often within the range of normal control. Nonetheless, the serum ANCA progressively rose before or immediately following clinical exacerbation. The characteristic profile of ANCA in these patients is illustrated in Figure 1. Similarly, the serum sIL2R levels were elevated in all vasculitic patients at first clinical presentation (median 1,090 U/ml, range 760–1,588 U/ml). Nevertheless, the serum sIL2R levels frequently remained elevated despite apparent clinical improvement following immunosuppressive therapy with or without plasma exchange. During clinical remission, the serum sIL2R levels not infrequently remained elevated or at the upper limit of the normal range. An obvious rise of serum sIL2R level detected before or immediately following clinical exacerbation was not frequently

observed. Examples of these phenomena are illustrated in Figure 1.

There was no significant correlation between the serum ANCA and sIL2R levels either during clinical exacerbation or in clinical remission.

Three of these seven patients had renal impairment (creatinine clearance < 65 ml/minute/1.73 m²) at first clinical presentation. Only one had renal relapse. The serum sIL2R failed to reflect the disease activity in the remaining two patients with impaired but stable renal function.

A rise of erythrocyte sedimentation rate or C-reactive protein exceeding the upper limit of normal value twofold was observed in five patients following clinical relapse yet a clear-cut correlation between these measurement and serum ANCA levels was lacking.

Discussion

In this study, we examined the role of T-lymphocytes in systemic vasculitis by monitoring the serum sIL2R levels as an indirect measure of T-lymphocyte activation. That T-lymphocytes may play a contributory role in the inflammatory injuries of systemic vasculitis is suggested by the fact that ANCA have the characteristics of T-lymphocyte-dependent antibodies and their presence in involved tissues.^{3,4} Furthermore, there is growing support for the notion that neutrophils, the targets for the ANCA, may be controlled by T-lymphocytes.¹⁴

We have observed elevated serum levels of both ANCA and sIL2R in our seven vasculitic patients at clinical presentation before receiving immunosuppressive therapy. Similar to the experience reported by other investigators,^{11,15} we found the ANCA titre served as a good indicator of the disease activity. Serial monitoring of serum ANCA levels will be helpful in the adjustment of therapy according to the disease activity. On the contrary, the serum sIL2R remained elevated in most patients despite clinical remission following immunosuppressive therapy. These findings suggest that sIL2R is not a good indicator of disease activity in systemic vasculitis although serum sIL2R levels correlate well with the disease activity in other T-lymphocyte-mediated autoimmune disorders.^{8–10} It is interesting to note that, although sIL2R level may be dependent on renal function, the serum sIL2R failed to reflect the disease activity in the two patients with impaired renal function who developed non-renal relapse.

The elevated serum sIL2R levels in patients with systemic vasculitis suggests that the T-lymphocytes are often activated even during apparent clinical inactivity. Nonetheless, it remains difficult to

Figure 1 (a) The serum ANCA and sIL2R levels in a patient with Wegener's granulomatosis (patient no. 3). (b) The serum ANCA and sIL2R levels in a patient with microscopic polyarteritis (patient no. 7).

confirm whether T-lymphocytes play an important effector role in these diseases. T-lymphocyte autoactivity has been reported in systemic vasculitis.¹⁶ Van der Woude and coworkers reported impressive proliferation of patients' peripheral lymphocytes in response to neutrophil antigen extracts but not with peripheral lymphocytes from healthy controls.¹⁶ Nevertheless, a similar study with a larger patient population by Mathieson and coworkers¹⁷ failed to demonstrate any difference in T-lymphocyte response to neutrophil extract *in vitro* between patients and controls. Serum cytokine changes have been studied in three patients with WG by Grau and coworkers.¹⁸ Serum interleukin 2 (IL2) and alpha-interferon levels were elevated, whereas interleukin 1- β , gamma-interferon, and tumour necrosis factor-alpha levels remained within normal limits. In view of the high IL2 levels, it was tempting for these authors to speculate that vasculitis might be a T-lymphocyte-

dependent immunopathological reaction, although the origin of these cytokines was not studied.

In conclusion, these preliminary data reveal that serum sIL2R levels are often elevated in systemic vasculitis during clinical exacerbation suggesting T-lymphocytes may be activated in the acute phase of the disease. Nonetheless, the finding of elevated serum sIL2R levels in most patients during clinical remission indicates that it is not a satisfactory measure of the disease activity and tends to argue against the role of T-lymphocytes as a major effector mediating inflammatory injuries in systemic vasculitis.

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