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Serum markers of T cell activation in relapses of Wegener's granulomatosis

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SUMMARY

Levels of soluble IL-2 receptor (sIL-2R), soluble CD4 (sCD4) and CD8 (sCD8) were measured by sandwich ELISA as markers for T cell activation in serial serum samples from 16 patients showing 18 histologically proven relapses of Wegener's granulomatosis (WG). Levels of sIL-2R increased from 1065 U/ml (median, range 373–2345 U/ml) 6 months before the relapse to 1684 U/ml (median, range 486–3404 U/ml) at the moment of relapse for the whole group ($P=0{\cdot}10$). The eight major relapses showed a profound rise in sIL-2R levels, from 1008 U/ml (median, range 686–1553 U/ml) 6 months before the relapse, to 1994 U/ml (median, range 1469–3404 U/ml) at the moment of relapse ($P<0{\cdot}01$). The levels of sIL-2R at the moment of relapse were significantly higher at the eight major relapses than at the time of the 10 minor relapses ($P<0{\cdot}05$). Minor relapses were not accompanied by a significant rise in sIL-2R levels. Titres of antineutrophil cytoplasmic antibodies (ANCA) rose by two or more titresteps or from negative to positive in 15/18 patients during the 6 months period before the relapse. In all seven cases with both a rise of the ANCA titre and an at least 25% increase in sIL-2R levels, the rise in ANCA preceded the rise in sIL-2R by at least 1 month. The level of sIL-2R at the moment of relapse correlated with the level of C-reactive protein ($r=0{\cdot}488$, $P<0{\cdot}05$) and with the disease activity score ($r=0{\cdot}824$, $P<0{\cdot}002$). There were no significant changes in levels of sCD4 or sCD8, although the levels of sCD4 tended to be higher at the time of major relapses. We conclude that major relapses of Wegener's granulomatosis are accompanied by systemic T cell activation. T cell activation, however, does not appear to precede the rise in ANCA titre.

Keywords Wegener's granulomatosis relapse soluble IL-2 receptor T cell activation antineutrophil cytoplasmic antibodies

INTRODUCTION

Wegener's granulomatosis (WG) is a systemic disease characterized by necrotizing granulomatous inflammation of the upper and lower airways and necrotizing vasculitis of small arteries and veins [1]. Antineutrophil cytoplasmic antibodies (ANCA) directed against lysosomal enzymes of neutrophils and monocytes, in particular Proteinase 3 and myeloperoxidase, have been found to be strongly associated with this disease and other forms of idiopathic necrotizing vasculitis and crescentic glomerulonephritis [2–6]. Changes in levels of these antibodies correlate closely with disease activity, which may suggest a pathophysiological role for these autoantibodies [2,7,8].

Apart from humoral mechanisms, cell-mediated immunity may also be involved in the pathogenesis of WG [9]. A role for T cell involvement in WG is suggested by the presence of granulomas, which points to T cell-mediated delayed type hypersensitivity reactions to an as yet unknown stimulus. Activated T cells, especially of the CD4 subtype, are also found in biopsies from the upper and lower airways and the kidneys in necrotizing non-granulomatous lesions [10–12]. Also, elevated levels of cytokines such as IL-2 and interferon-alpha (IFN-α) have been reported in active vasculitis [13]. The target antigens of these T cells remain unknown. However, preliminary data have been reported that show *in vitro* proliferative responses of peripheral blood mononuclear cells (PBMC) from patients with WG to neutrophil antigens [14].

The IL-2 receptor (IL-2R) is expressed on and released by, predominantly, activated T cells [15]. The level of soluble IL-2R (sIL-2R) in serum is considered an estimate for T cell activation and has been reported to be elevated in a variety of autoimmune diseases, e.g. rheumatic [16–19], vasculitic [20,21], and granulomatous disorders [22,23]. Also, correlations between disease activity and levels of sIL-2R have been reported in some of these diseases [16,18,24–26]. Levels of soluble CD4 (sCD4) and CD8

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(sCD8) antigen in serum have also been reported to reflect T cell activation in several different clinical autoimmune conditions [27–30].

Since clear rises in IgG class ANCA levels, a B cell response probably driven by antigen(s), often precede clinical manifestations of relapsing disease activity [7,8,31], we tried to evaluate the possible role of T cell activation in the initiation and expression of disease activity of WG. For this purpose, stored consecutive serum samples of patients before and during a strictly defined relapse of WG were analysed for levels of sIL-2R, sCD4 and sCD8 antigen. Disease activity was evaluated by a clinical scoring system and by measuring C-reactive protein levels [32].

PATIENTS AND METHODS

Patients

Patients were seen at the out-patient clinic at least every 3 months. Blood samples were taken every month and an aliquot was stored at −20°C. The diagnosis of WG was established according to clinical and histological criteria [33]. Relapses were defined as previously described [8]. Only relapses occurring without clinical and microbiological evidence of infection during the 6 months before relapse were analysed.

Methods

Disease activity was scored using a disease activity index and a distinction into major and minor relapses was made as described previously [34]. Briefly, minor relapses were defined as active lesions of WG in the upper or lower airways generally without major vasculitic activity in other organs. Major relapses were defined by renal involvement with deteriorating renal function with erythrocyte casts or biopsy-proven necrotizing glomerulonephritis, pulmonary involvement with impending respiratory failure, definite cerebral vasculitis, or acute abdomen or massive gastrointestinal haemorrhage due to vasculitis.

Samples taken at 6, 3, 2, and 1 month before and at the moment of relapse were analysed for levels of sIL-2R, sCD4 and sCD8 by sandwich ELISA according to the instructions of the manufacturer (T-cell Sciences, Cambridge, MA). In brief, microtitre plates were coated with a murine IgG MoAb against one epitope on IL-2R, CD4, and CD8, respectively. After incubation of patient sera or standards, a horseradish peroxidase-conjugated murine IgG MoAb recognizing a second epitope on IL-2R, CD4, and CD8, respectively, was added. After colour reaction, the plates were read on an automated multiscanner. Normal values of IL-2R in our laboratory are below 650 arbitrary units/ml (U/ml). C-reactive protein (CRP) serum levels were measured by ELISA [35]. ANCA were measured by indirect immunofluorescence on ethanol-fixed granulocytes [2]. The cytoplasmic fluorescence pattern had to be granular with a decrease in fluorescence intensity towards the periphery of the cells. If the test was positive, serum titrations were performed in two-fold dilutions starting at 1:16 to a maximal dilution of 1:512. Slides were read independently by two observers.

Statistical analysis

Data are given as median values and range in parentheses. For comparison of variables within one group, Friedman's two-way ANOVA was used. For comparison between groups, Wilcoxon's test for unpaired data or Kruskal–Wallis ANOVA was used. Duncan's method was applied to correct for multiple comparisons [36]. A two-tailed P value $<0{\cdot}05$ was considered significant. Correlations were studied by Spearman's rank test.

Table 1. Organ involvement at the moment of major and minor relapses in 16 patients with Wegener's granulomatosis

	Major relapses ($n=8$)	Minor relapses ($n=10$)
Nasal lesions	8	10
Pulmonary lesions	4	2
Progressive glomerulonephritis	8	—
Mononeuritis multiplex	3	1
Episcleritis	5	3
Vasculitis with granulomatous inflammation of the skin	2	—

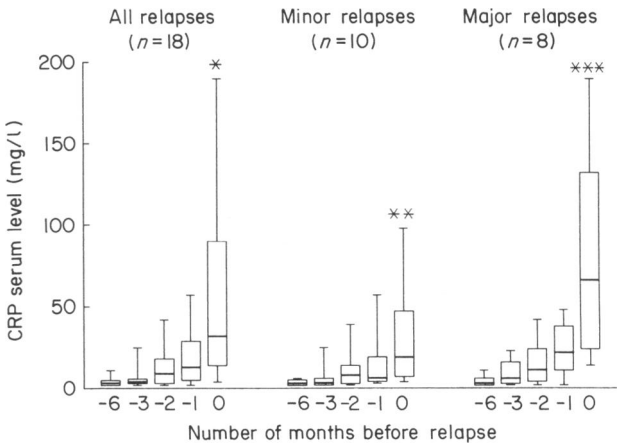

Fig. 1. Median C-reactive protein (CRP) serum levels with 25–75% (□) and total range (error bars) from 6 months preceding the relapse until the moment of clinical relapse of Wegener's granulomatosis in 18 relapses. (−6, −3, −2, −1 denotes 6, 3, 2 and 1 months before the relapse; 0 denotes moment of relapse.) * $P<0{\cdot}05$ compared with −6, −3, −2, and −1 in the same group. ** $P<0{\cdot}05$ compared with −6 and −3 in the same group. *** $P<0{\cdot}05$ compared with −6, −3, and −2 in the same group.

RESULTS

Eighteen relapses occurring in 16 patients were identified in the period from 1989 to 1991. Eight were major relapses, all with renal involvement, and 10 were minor relapses. Patients with major relapses did not differ from those with minor relapses with respect to gender, age, time since diagnosis and number of previous relapses. Clinical manifestations of the relapses are shown in Table 1.

CRP levels had risen significantly at the moment of relapse compared with values recorded before relapse (Fig. 1). Also, c-ANCA titres rose by more than two titre steps (four-fold) or from negative to positive in 15 of the 18 relapses in the 6 months before the relapse. In two cases ANCA levels were persistently

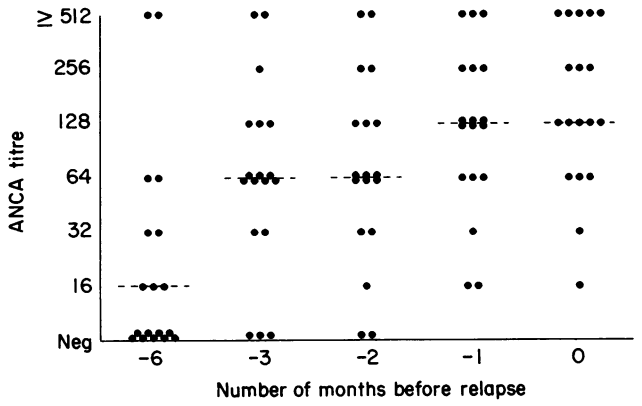

Fig. 2. Titres of antineutrophil cytoplasmic antibodies (ANCA) from 6 months before the relapse until the moment of clinical relapse of Wegener's granulomatosis in 18 relapses. ●, Individual serum ANCA titres; horizontal lines represent median titres at the different points in time. (−6, −3, −2, −1 denotes 6, 3, 2 and 1 months before the relapse; 0 denotes moment of relapse.)

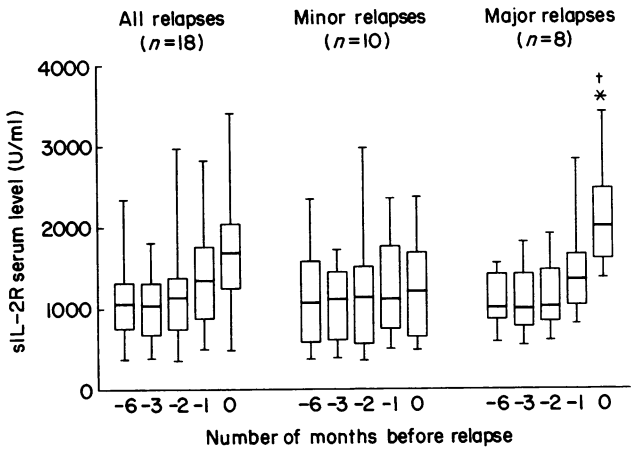

Fig. 3. Median sIL-2R serum levels with 25–75% (□) and total range (error bars) from 6 months preceding the relapse until the moment of clinical relapse of Wegener's granulomatosis in 18 relapses. (−6, −3, −2, −1 denotes 6, 3, 2 and 1 months before the relapse; 0 denotes moment of relapse.) * $P<0.05$ compared with −6, −3, and −2 in the same group. † $P<0.05$ compared with the group with minor relapses at 0.

positive at the maximum of the serum dilution used in this study (Fig. 2). In nine cases the rise in c-ANCA occurred between 6 and 3 months before the relapses, and in six cases between 3 months before the relapse and the moment of relapse. c-ANCA titres at the moment of relapse did not differ between the group with major and the group with minor relapses.

Levels of serum sIL-2R at the moment of relapse were significantly higher in the group of major relapses compared with the group of minor relapses ($P<0.05$, Fig. 3). Levels of serum sIL-2R at the moment of relapse correlated strongly with the disease activity scores at the moment of relapse for the whole group of 18 relapses, as shown in Fig. 4 ($r=0.824$, $P<0.002$). At the moment of relapse, there was also a significant correlation

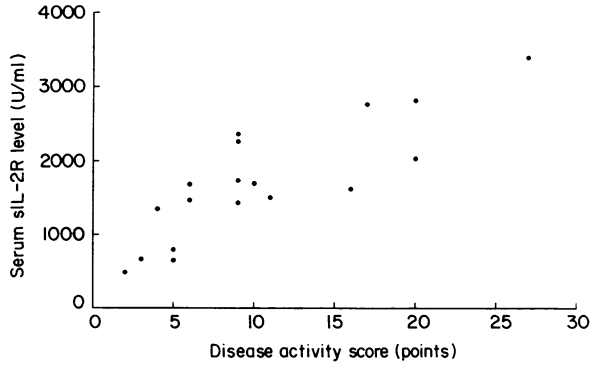

Fig. 4. Relation between serum sIL-2R level and disease activity scores at the moment of relapse in 18 relapses of Wegener's granulomatosis. A highly significant correlation is found (Spearman's rank correlation: $r=0.824$, $P<0.002$).

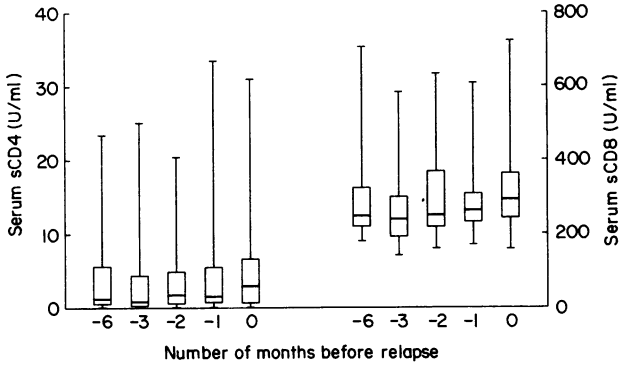

Fig. 5. Median sCD4 (left) and sCD8 (right) serum levels with 25–75% (□) and total range (error bars) from 6 months preceding the relapse until the moment of clinical relapse of Wegener's granulomatosis in 18 relapses. (−6, −3, −2, −1 denotes 6, 3, 2 and 1 months before the relapse; 0 denotes moment of relapse.) No significant changes are seen.

between serum CRP levels and serum sIL-2R levels ($r=0.488$, $P<0.05$), and between serum CRP levels and disease activity scores ($r=0.691$, $P<0.01$).

Serum levels of sIL-2R rose from 1065 U/ml (median, range 373–2345) 6 months before the relapse to 1684 U/ml (median, range 486–3404) for the whole group of 18 relapses ($P=0.10$, Fig. 3). Serum sIL-2R levels in the group of 10 minor relapses did not rise. In contrast, the group of eight major relapses showed a profound rise in serum sIL-2R levels from 1006 U/ml (median, range 686–1553) 6 months before the relapse to 1994 U/ml (median, range 1469–3404) at the moment of relapse ($P<0.05$). Within the group of major relapses, seven out of eight patients showed an increase of at least 25% in serum sIL-2R levels compared with only two out of the 10 patients with minor relapses. In one of the nine patients showing a ≥25% increase in sIL-2R levels, the increase had occurred already 2 months before the moment of relapse. In the remaining eight patients it was first detected 1 month before ($n=3$) or at the moment of relapse ($n=5$). In seven out of the nine cases, both a rise of more than 25% in sIL-2R levels and a four-fold rise in ANCA titre were found before the relapse. In all those cases the rise in

ANCA titre preceded the rise in sIL-2R levels by at least 1 month. In all but two patients, levels of serum sIL-2R were persistently above the upper limit of normal of 650 U/ml. Both patients had a minor relapse and did not show an increase of sIL-2R at the moment of relapse.

Analysis of the levels of serum sCD4 and sCD8 showed no significant changes in the period from 6 months before the relapse to the moment of relapse (Fig. 5). Analysing the group with major relapses and the group with minor relapses separately did not change the results, although levels of serum sCD4 at the moment of relapse tended to be higher in the group with major relapses compared with the group with minor relapses ($P < 0{\cdot}10$). The ratio of serum sCD4 to serum sCD8 did not show significant changes either (data not shown).

DISCUSSION

Studying 18 relapses in 16 patients with WG, we found a significant rise in serum sIL-2R levels in the group with major relapses ($n=8$) occurring within 2 months before the relapse, whereas no significant change in sIL-2R levels occurred in the group with minor relapses ($n=10$). Also, levels of sIL-2R correlated with disease activity scores at the moment of relapse. Recently, das Neves *et al.* also reported elevated levels of sIL-2R in serum samples from patients with different forms of renal vasculitis taken in the acute phase of the disease [37]. In all but two patients (88%), serum levels of sIL-2R were above the upper limit of normal during the whole period of 6 months preceding the clinical diagnosis of relapse.

These findings suggest involvement of T cell-mediated immunity in the process of active WG, as is also suggested by the abundant presence of activated T cells in granulomas and renal lesions of patients with WG [10–12]. A significant rise of sIL-2R levels before or at the moment of relapse was found, however, in the group with major disease activity only. We cannot exclude the possibility that, given the probably limited half life of sIL-2R, intermittent transient changes in sIL-2R could have occurred, since only monthly serum samples were available. Otherwise, the occurrence of rises of sIL-2R in patients with major disease activity only suggests that changes in serum sIL-2R levels are rather insensitive as indicators for localized T cell activity. Significant increases of serum levels of sIL-2R have, however, been reported in more or less localized granulomatous disorders such as sarcoidosis and Crohn's disease, suggesting spill over of sIL-2R into the circulation occurs [22,23]. Our data, thus, indicate that other immune effector cells, in particular polymorphonuclear granulocytes and/or monocytes, are probably more prominent in the effector phase of active WG. Although retention of sIL-2R with impaired renal function has been suggested [38], our findings can not be explained as a result of progressive renal failure during major disease activity, since there was no correlation between sIL-2R levels in those patients and creatinine clearance (data not shown). Others also have suggested overproduction of sIL-2R as a more important factor than metabolic retention to explain the observed high levels of sIL-2R in patients with renal vasculitis [37].

Rises in titre of ANCA have been demonstrated to precede the occurrence of relapses of WG [7,8,31]. The specific increase of these IgG-class antibodies suggests a T cell-dependent B cell activation. We tried to elucidate a possible initiating effect of T cell activation in the production of ANCA by comparing changes over time of serum ANCA and sIL-2R levels in the period preceding the relapse. Since rises in serum sIL-2R never preceded rises in ANCA in our patients, our results do not support an initiating role of T cell activation, as measured by serum sIL-2R levels, in the autoantibody response in WG. Comparable kinetics with respect to specific autoantibody response and serum sIL-2R levels have been reported in relapses of systemic lupus erythematosus (SLE) [26]. The finding that rises in sIL-2R are a rather late phenomenon in the process of disease activation and are closely related with the disease activity score and the acute phase response as measured by serum CRP levels, suggests that T cell activation is only a secondary and limited event in the activation of WG, and therefore may only be a reflection of a more general activation of the immune sytem. However, as in SLE [16,26], persistent elevated serum levels of sIL-2R without discernible disease activity have been found in most of our WG patients. This could indicate a low level of persistent T cell activation even in the absence of clinical disease activity.

In order to gain some insight into the subsets of T cells involved, levels of sCD4 and sCD8 were analysed. No discernible changes in those levels were found before the relapses. This is in contrast to recent findings in Sjögren's syndrome, SLE, scleroderma and rheumatoid arthritis [27–30]. Both in primary Sjögren's syndrome and active rheumatoid arthritis, elevated levels of sCD4 with concurrent decreased levels of sCD8 have been reported [27,29,30]. In active SLE and scleroderma both elevated sCD4 and sCD8 levels were found [28,30]. Our data, however, do not support a central role for either a CD4- or CD8-positive subset of lymphocytes in initiating or maintaining active WG. However, lack of correlation between the presence of activated T cell subsets in the peripheral blood and the levels of sCD4 and sCD8 in serum has been reported [30,39]. Otherwise, the elevated levels of sIL-2R in our study might not be derived from activated T cells, but from activated B cells and/or monocytes. Activated B cells can express IL-2R on their cell surface, but do so in amounts that are far less than on activated T cells [15]. Also, B cell activation as manifested by increase in ANCA levels preceded the rise in levels of sIL-2R. This does not support a B cell origin for the elevated sIL-2R levels as observed in our patients. Since we did not analyse activation of monocytes, we can not exclude the possibility that the elevated sIL-2R levels are derived from these cells [40,41]. In the future, studies using flowcytometric analysis of lymphocyte subsets and monocytes may give more insight into the origin of sIL-2R in active WG.

Given the late occurrence of a discernible rise in sIL-2R levels in the induction of disease activity and its presence only in patients with major disease activity, clinical application of serial sIL-2R determination in the follow up of patients with WG is of limited value. Clinical usefulness is further hampered by the fact that elevation of sIL-2R is probably a non-specific phenomenon, and also occurs during acute and chronic infections [42,43], the clinical conditions which often pose a diagnostic problem in patients with WG.

In conclusion, elevated levels of sIL-2R in patients with WG suggest T cell activation. Rises in levels of sIL-2R occur during major relapses of the disease, and are probably a reflection of a more generalized immune activation. The clinical usefulness of serial measuring of serum sIL-2R seems to be restricted.

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REFERENCES

1 Fauci AS, Haynes BF, Katz P. The spectrum of vasculitis: clinical, pathologic, immunologic and therapeutic considerations. Ann Intern Med 1989; **89**:660–76.

2 Woude van der FJ, Rasmussen N, Lobatto S *et al.* Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. Lancet 1989; **i**:425–9.

3 Falk RJ, Jennette JC. Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing crescentic glomerulonephritis. N Engl J Med 1988; **318**:1651–7.

4 Nölle B, Specks V, Lüdemann J, Rohrbach MS, De Remee DA, Gross WL. Anticytoplasmic antibodies: their immunodiagnostic value in Wegener's granulomatosis. Ann Intern Med 1989; **111**:28–40.

5 Cohen Tervaert JW, Goldschmeding R, Elema JD *et al.* Association of autoantibodies to myeloperoxidase with different forms of vasculitis. Arthritis Rheum 1990; **33**:1264–72.

6 Cohen Tervaert JW, Limburg PC, Elema JD, Huitema MG, Horst G, The TH, Kallenberg CGM. Autoantibodies against myeloid lysosomal enzymes in systemic necrotizing vasculitis: a useful adjunct to classification of patients. Ann J Med 1991; **91**:599–66.

7 Cohen Tervaert JW, Woude van der FJ, Fauci AS *et al.* Association between active Wegener's granulomatosis and anticytoplasmic antibodies. Arch Intern Med 1989; **1499**: 2461–5.

8 Cohen Tervaert JW, Huitema MG, Hené RJ, Sluiter WJ, The TH, Hem van der GK, Kallenberg CGM. Relapses of Wegener's granulomatosis: prevention by treatment based on antineutrophil cytoplasmic antibody levels—a controlled prospective study. Lancet 1990; **336**:709–11.

9 Kallenberg CGM, Cohen Tervaert JW, van der Woude FJ, Goldschmeding R, von dem Borne AEGKr, Weening JJ. Autoimmunity to lysosomal enzymes: new clues to vasculitis and glomerulonephritis? Immunol Today 1991; **12**:61–64.

10 Gephardt G, Ahmad M, Tubbs R. Pulmonary vasculitis (Wegener's granulomatosis). Immunohistochemical studies of T and B cell markers. Am J Med 1983; **74**:700–3.

11 Rasmussen N, Petersen J, Ralfkiaer E, Avnstrøm S, Wiik A. Spontaneous and induced immunoglobulin synthesis and anti-neutrophil cytoplasm antibodies in Wegener's granulomatosis: relation to leukocyte subpopulations in blood and active lesions. Rheumatol Int 1988; **8**:153–8.

12 Brouwer E, Cohen Tervaert JW, Weening JJ, Kallenberg CGM. Immunohistopathology of renal biopsies in Wegener's granulomatosis (WG): clues to it's pathogenesis? *Kidney Int* 1991; **39:** 1055–6.

13 Grau GE, Roux-Lombard P, Gysler C, Lambert C, Lambert PH, Dayer JM, Guillevin L. Serum cytokine changes in systemic vasculitis. Immunology 1989; **68**:196–8.

14 Woude van der FJ, Es van LA, Daha MR. The role of c-ANCA antigen in the pathogenesis of Wegener's granulomatosis. A hypothesis based on both humoral and cellular mechanisms. Neth J Med 1990; **36**:169–71.

15 Rubin LA, Kurman CC, Fritz ME, Biddison WE, Boutin B, Yarchoan R, Nelson DL. Soluble interleukin 2 receptors are released from activated human lymphoid cells *in vitro*. J Immunol 1985; **135**:3172–7.

16 Wolf RE, Brelsford WG. Soluble interleukin-2 receptors in systemic lupus erythematosus. Arthritis Rheum 1988; **31**:729–35.

17 Keystone EC, Snow KM, Bombardier C, Chang C-H, Nelson DL, Rubin LA. Elevated soluble interleukin-2 receptor levels in the sera and synovial fluids of patients with rheumatoid arthritis. Arthritis Rheum 1988; **31**:844–9.

18 Campen DH, Horwitz DA, Quismorio FP Jr, Ehresmann GR, Martin WJ. Serum levels of interleukin-2 receptor and activity of rheumatic diseases characterized by immune system activation. Arthritis Rheum 1988; **31**:1358–64.

19 Clements PJ, Peter JB, Agopian MS, Telian NS, Furst DE. Elevated serum levels of soluble interleukin 2 receptor, interleukin 2 and neopterin in diffuse and limited scleroderma: effect of chlorambucil. J Rheumatol 1990; **17**:908–10.

20 Manoussakis MN, Papadopoulos GK, Drosos AA, Moutsopoulos HM. Soluble interleukin 2 receptor molecules in the serum of patients with autoimmune diseases. Clin Immunol Immunopathol 1989; **50**:321–32.

21 Hamzaoui K, Ayed K. Soluble interleukin 2 receptors in patients with Behçet's disease. J Rheumatol 1989; **16**:852–6.

22 Keicho N, Kitamura K, Takaku F, Yotsumoto H. Serum concentration of soluble interleukin-2 receptor as a sensitive parameter of disease activity in sarcoidosis. Chest 1990; **98**:1125–9.

23 Brynskov J, Tvede N. Plasma interleukin-2 and a soluble/shed interleukin-2 receptor in serum of patients with Crohn's disease. Effect of cyclosporin. Gut 1990; **31**:795–9.

24 Degiannis D, Seibold JR, Czarnecki M, Raskova J, Raska K. Jr. Soluble interleukin-2 receptors in patients with systemic sclerosis: clinical and laboratory correlations. Arthritis Rheum 1990; **33**:375–80.

25 Rubin LA, Snow KM, Kurman CC, Nelson DL, Keystone EC. Serial levels of soluble interleukin 2 receptor in the peripheral blood of patients with rheumatoid arthritis: correlations with disease activity. J Rheumatol 1990; **17**:597–602.

26 Borg ter EJ, Horst G, Limburg PC, Kallenberg CGM. Changes in plasma levels of interleukin-2 receptor in relation to disease exacerbations and levels of anti-dsDNA and complement in systemic lupus erythematotus. Clin Exp Immunol 1990; **82**:21–25.

27 Symons JA, Wood NC, di Giovine FS, Duff GW. Soluble CD8 in patients with rheumatic diseases. Clin Exp Immunol. 1990; **80**:354–9.

28 Kahaleh MB. Soluble immunologic products in scleroderma sera. Clin Immunol Immunopathol 1991; **58**:139–44.

29 Symons JA, McCulloch JF, Wood NC, Duff GW. Soluble CD4 in patients with rheumatoid arthritis and osteoarthritis. Clin Immunol Immunopathol 1991; **60**:72–82.

30 Sawada S, Sugai S, Iijima S *et al.* Increased soluble CD4 and decreased soluble CD8 molecules in patients with Sjögren's syndrome. Am J Med 1992; **92**:134–40.

31 Jayne D, Heaton A, Brownlee A, Evans DB, Lockwood CM. Sequential antineutrophil cytoplasm antibody titres in the management of systemic vasculitis (abstract). Nephrol Dial Transplant 1990; **5**:309–10.

32 Hind CRK, Winearls CG, Lockwood CM, Rees AJ, Pepys B. Objective monitoring of activity in Wegener's granulomatosis by measurement of serum C-reactive protein concentration. Clin Nephrol 1984; **21**:341–5.

33 Fauci AS, Haynes BF, Katz P, Wolff SM. Wegener's granulomatosis: prospective clinical and therapeutic experience with 85 patients for 21 years. Ann Intern Med 1983; **98**:76–85.

34 Kallenberg CGM, Cohen Tervaert JW, Stegeman CA. Criteria for disease activity in Wegener's granulomatosis: a requirement for longitudinal clinical studies. APMIS 1990; **98**:37–40.

35 Leeuwen van MA, Rijswijk van MH, Marrink J. CRP measurements in rheumatic disorders. Protides of the biological fluids. Oxford: Pergamon Press 1986; **34**:315–8.

36 Duncan DB. Multiple range and multiple F-tests. Biometrics 1955; **11**:1.

37 das Neves FC, Suassuna J, Leonelli M, Hartley B, Cameron JS. Cell

activation and the role of cell-mediated immunity in vasculitis. Contrib Nephrol 1991; **94**:13–21.

38 Donati D, Degiannis D, Homer L, Gastaldi L, Rsakova J, Raska K. Immune deficiency in uremia: interleukin-2 production and responsiveness and interleukin-2 receptor expression and release. Nephron 1991; **58**:268–75.

39 Reddy MM, Lange M, Grieco M. Elevated soluble CD8 levels in sera of human immunodeficiency virus infected populations. J Clin Microbiol 1989; **27**:257–60.

40 Nelson DL, Rubin LA, Kurman CC, Fritz ME, Boutin B. An analysis of the cellular requirements or the production of soluble interleukin 2 receptors *in vitro*. J Clin Immunol 1986; **6**:114–9.

41 Holter W, Goldman CK, Casabo L, Nelson DL, Greene WC, Waldmann TA. Expression of functional IL-2R receptors by lipopolysaccharide and interferon-gamma stimulated human monocytes. J Immunol 1987; **138**:2917–22.

42 Stolc V, Krause Jr. Interleukin-2 receptor levels are increased in blood of heart transplant recipients during infections. Diag Clin Immunol 1987; **5**:171–4.

43 Wong KL, Wong RPO. Serum soluble interleukin 2 receptor in systemic lupus erythematosus: effects of disease activity and infection. Ann Rheum Dis 1991; **50**:706–9.