

Differential B- and T-cell activation in Wegener's granulomatosis

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Background: Autoimmune mechanisms are postulated to play a role in the development and progression of Wegener's granulomatosis (WG), a form of systemic, idiopathic necrotizing vasculitis.

Objective: We investigated the relation between lymphocyte activation and disease activity in patients with WG.

Methods: B- and T-lymphocyte activation was studied by cytometric assessment of the expression of the activation markers CD38 on B cells and CD25 and HLA-DR on CD4⁺ and CD8⁺ T-cell subsets, respectively. Activation at the cellular level was related to serum levels of antineutrophil cytoplasmic antibodies and soluble IL-2 receptor, which can be regarded as soluble activation markers of B and T cells.

Results: Percentages of CD38^{bright} activated B cells were higher in patients with active WG than in patients experiencing disease remission ($P < .05$) or in healthy control subjects ($P < .05$). Percentages of activated CD4⁺ and CD8⁺ T cells were higher in patients with active WG (CD4 subset, $P < .0001$; CD8 subset, $P < .005$) than in healthy individuals. An increased percentage of activated T cells of both subsets was also seen in patients whose condition was in remission, as compared with healthy control subjects (CD4 subset, $P < .0005$; CD8 subset, $P < .001$). Lymphocyte activation at the cellular level did not correlate with plasma levels of antineutrophil cytoplasmic antibodies or soluble IL-2 receptor.

Conclusion: In WG, B-cell activation is related to active disease, whereas T-cell activation persists during remission of the disease, which points to an intrinsic disordered immune system in this disease. (*J Allergy Clin Immunol* 1999;103:885-94.)

Key words: Wegener's granulomatosis, immunology, B cells, T cells, activation

Wegener's granulomatosis (WG) is a form of idiopathic necrotizing vasculitis of presumed autoimmune origin.^{1,2} The disease is characterized by granulomatous inflammation of the airways, systemic vasculitis, and glomerulonephritis. Autoantibodies against granule constituents of myeloid cells (antineutrophil cytoplasmic antibodies [ANCA]) are a sensitive and specific marker of the

Abbreviations used

ANCA:	Antineutrophil cytoplasmic antibodies
BVAS:	Birmingham Vasculitis Activity Score
CRP:	C-reactive protein
FACS:	Fluorescence-activated cell sorter
IIF:	Indirect immunofluorescence
PR3:	Proteinase 3
RF:	Rheumatoid factor
sIL-2R:	Soluble IL-2 receptor
WG:	Wegener's granulomatosis

disease^{3,4} and are postulated to play a pathophysiologic role.^{1,5,6} Several studies^{3,7-9} have reported that ANCA serum levels rise before disease exacerbation, which is suggestive of an antigen-specific activation of autoreactive B cells. However, disease relapses occurring in the absence of titer rises of ANCA and persistent high ANCA titers during disease remission have also been observed.⁹ In view of these findings, the relation between disease activity and activation of B cells in WG is not clear.

In WG, ANCA are particularly of the IgG1 and IgG4 subclasses,¹⁰ which indicates a possible role for T cells, especially of the CD4 subset, as helper cells for B-cell activation. The presence of granulomatous inflammation and of activated T cells in the peripheral blood¹¹ and lesions¹²⁻¹⁴ further supports the idea that T cells may play a role in WG. The relation between T-cell activation and disease activity has not been studied in depth. So far, persistently increased serum levels of the activation marker soluble IL-2 receptor (sIL-2R) have been suggested to reflect chronic activation of T cells in this disease.^{15,16} sIL-2R, however, is not a T cell-specific marker, because it can be released by B cells and monocytes as well.¹⁷

The aim of this study was to gain more insight into the relation between disease activity and lymphocyte activation in WG. Because ANCA and soluble markers of T-cell activation do not offer conclusive clues to the activation of the adaptive immune system in this disease, we investigated the relation between activation of B and T cells at the cellular level and disease activity. Understanding the mechanisms that underlie the regulation of immunity in WG may be of importance for prognosis and treatment.

METHODS

Patients and clinical parameters

Thirty-three consecutive patients (22 men and 11 women; mean age, 55 years; range, 19 to 86 years) who fulfilled the classification

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TABLE I. Clinical data of patients with WG

Patient no.	Age (y)	Sex	Diagnosis of WG	Disease duration (mo)	Disease activity*	BVAS	ANCA titer†
1	58	M	Generalized	NP	Active	27	1:320
2	65	M	Generalized	NP	Active	39	1:640
3	33	M	Generalized	79	Active	28	1:320
4	51	M	Generalized	58	Active	12	1:640
5	71	M	Generalized	NP	Active	36	1:320
6	68	M	Generalized	52	Active	2	1:160
7	55	M	Generalized	NP	Active	7	1:80
8	50	M	Limited	71	Active	17	1:160
9	50	F	Limited	NP	Active	15	1:40
10	47	M	Limited	NP	Active	19	1:320
11	45	M	Limited	NP	Active	14	1:160
12	19	M	Limited	NP	Active	10	1:160
13	41	M	Generalized	40	Complete remission	0	1:640
14	86	M	Generalized	58	Complete remission	0	1:320
15	62	F	Generalized	130	Complete remission	0	1:320
16	62	M	Generalized	170	Complete remission	0	1:40
17	31	F	Generalized	110	Complete remission	0	1:40
18	66	M	Generalized	33	Complete remission	0	1:80
19	51	M	Generalized	253	Complete remission	0	1:80
20	27	F	Generalized	74	Complete remission	0	1:640
21	66	M	Generalized	41	Complete remission	0	1:20
22	26	F	Generalized	48	Complete remission	0	<1:20
23	49	M	Generalized	75	Complete remission	0	<1:20
24	57	F	Generalized	256	Complete remission	0	1:160
25	70	F	Generalized	124	Complete remission	0	<1:20
26	64	F	Generalized	67	Complete remission	0	1:40
27	69	M	Generalized	221	Complete remission	0	1:20
28	68	F	Generalized	120	Complete remission	0	1:40
29	78	M	Limited	93	Complete remission	0	1:20
30	77	M	Limited	127	Complete remission	0	1:160
31	86	F	Limited	97	Complete remission	0	1:160
32	78	M	Limited	110	Complete remission	0	<1:20
33	53	F	Limited	79	Complete remission	0	<1:20

NP, New patient.

*For definitions of active disease and complete remission, see text.

†ANCA titers were measured by IIF.

criteria of the American College of Rheumatology for the diagnosis of WG¹⁸ were included in this cross-sectional study. All patients had ANCA with specificity for proteinase 3 at diagnosis. Eight of the 33 patients were studied at first examination of the disease. All other patients were diagnosed 33 months or longer (mean, 103 months; range, 33 to 256 months) before the disease was studied (Table I). Patients were considered to have active disease in case of 1 or more of the following conditions: (1) active glomerulonephritis, as shown by a decrease of at least 30% in renal function in combination with microscopic hematuria with red-cell casts or evidence of necrotizing lesions on renal biopsy; (2) pulmonary infiltrates with or without cavitation with rising serum C-reactive protein (CRP) concentrations in the absence of infections or cancer; (3) sinusitis, otitis, ulceration of the nasal mucosa, or a proliferative nasal mass, in combination with necrotizing granulomatous inflammation on biopsy; and (4) progressive mononeuritis multiplex, cranial-nerve palsy, cerebral vasculitis, necrotizing scleritis, orbital pseudotumor, progressive tracheal stenosis with active disease on biopsy, or peripheral gangrene or necrotizing vasculitis of medium-sized or small muscular arteries.^{7,19} Complete remission of the disease was defined as the complete absence of disease symptoms. Disease extent at diagnosis was defined as "limited"

when upper and/or lower airway involvement was not accompanied by glomerulonephritis and as "generalized" when disease was also extended to the kidneys.²⁰ According to these criteria, at analysis 12 patients had active WG (7 patients with generalized disease; 5 patients with limited disease) and 21 patients were experiencing complete remission of the disease (16 patients with generalized disease; 5 patients with limited disease at diagnosis; Table I). Disease activity was quantified by the Birmingham Vasculitis Activity Score (BVAS), as previously described.²¹ None of the patients received immunosuppressive or corticosteroid treatment at the time of analysis.

Sixteen healthy volunteers (11 men and 5 women; mean age, 40 years; range, 19 to 77 years) served as control subjects.

mAbs

mAbs conjugated with FITC or phycoerythrin were used for the detection of the following lineage-specific or activation markers: CD45 (FITC)/CD14 (phycoerythrin; leukogate), CD19 (FITC), CD3 (FITC), CD4 (FITC), CD8 (phycoerythrin), CD25 (phycoerythrin), HLA-DR (FITC; IQP Diagnostics, Groningen, The Netherlands), CD38 (phycoerythrin; Becton Dickinson, Erembodegem-Aalst, Belgium).

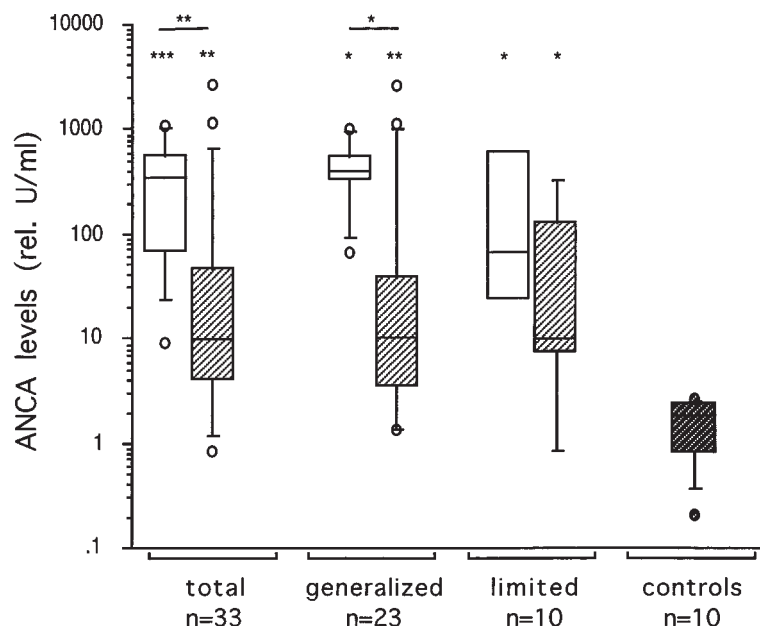


FIG 1. Plasma levels of anti-PR3 ANCA in relation to disease activity and extent. *P* values for each patient group were determined relative to healthy control subjects (*) or relative to another patient group (* above line). Indicated are the 10th, 25th, 50th, 75th, and 90th percentiles (horizontal lines) and outliers (circles). *Gen*, Generalized disease; *lim*, limited disease. **P* < .05; ***P* < .005; ****P* < .0005. Open bars, Active patients (*n* = 12; generalized, 7 patients; limited, 5 patients); light hatched bars, patients experiencing remission of disease (*n* = 21; generalized, 16 patients; limited, 5 patients).

Immunofluorescence staining and flow cytometry

Blood was drawn in heparinized vacutainers (Becton Dickinson). Cell surface expression of lymphocyte lineage and activation markers was analyzed by single or dual immunofluorescence cytometry with the use of a standard whole blood staining procedure.²² Briefly, 100 μ L of whole blood was incubated with appropriate dilutions of each conjugated mAb for 15 minutes at room temperature in the dark. Erythrocytes were lysed with fluorescence-activated cell sorter (FACS) lysing solution (Becton Dickinson) for 10 minutes, according to the manufacturer's instructions. The samples were washed with PBS/0.3% heparin and analyzed on a FACS-STAR cytometer (Becton Dickinson). Lymphocytes were gated on the basis of forward- and right-angle scatter characteristics and dual staining for CD45/CD14 (leukogate). For the detection of CD38-expressing B cells, a live gate was set on the CD19⁺ cell population.

Population percentages were calculated with the WinList 3.0 software package (Verity Software House, Topsham, Me).

Determination of ANCA

From patients and controls, both serum and plasma samples were obtained. For plasma isolation, heparinized blood was centrifuged for 10 minutes at 1000 rpm. Plasma was transferred to a fresh tube and recentrifuged for 10 minutes at 3100 rpm to remove platelets. Plasma was stored at -20°C until further use. ANCA titers were measured in serum samples by a standard indirect immunofluorescence (IIF) protocol on ethanol-fixed neutrophils, as previously described.²³ ANCA titers lower than 1:20 were defined as negative. Levels of anti-PR3 ANCA were determined by direct ELISA. Briefly, Maxisorp plates (Nalge Nunc, Rochester, NY) were coated for 2 hours at 37°C with 5 $\mu\text{g/mL}$ phenylmethylsulfonyl fluoride-inactivated PR3 that was isolated from human granulocytes, as previously described.²⁴ After washing, the plates were

incubated with serial dilutions of patient plasma samples or a standard reference sample for 2 hours at 37°C , washed again, and incubated with an F(ab')₂ goat-anti-human IgG alkaline phosphatase conjugate (Am Qualex, San Clemente, Calif) for 1 hour at 37°C . The plates were developed with p-nitrophenol phosphate in 10% diethanolamine buffer. The reaction was blocked with 5 mol/L NaOH, and optical densities were measured at 405 nm wave length with an Emax ELISA reader (Molecular Devices, Sunnyvale, Calif). The concentration of ANCA was calculated relative to the standard serum with SOFTmaxPro 1.2.0 software (Molecular Devices). ANCA concentrations higher than the mean plus 2 SD of the control values were considered increased.

Soluble factors

Plasma levels of CRP, rheumatoid factor (RF), IgM, and IgG were measured by nephelometry (Behring Diagnostics, Frankfurt, Germany).²⁵ Plasma levels of sIL-2R were measured by the automated Immulite (DPC, Apeldoorn, The Netherlands) chemiluminescent immunoassay,²⁶ following the manufacturer's instructions.

Statistical analyses

Mann-Whitney *U* test, Fisher's R to Z transformation and exact test, and Spearman's rank test analyses were performed with StatView software (Abacus, Berkeley, Calif). Two-tailed *P* values of less than .05 were considered significant. Percentages of lymphocyte subsets differing from the control group by the mean plus 2 times the SD were considered significantly increased.

RESULTS

Clinical parameters

Serum titers of ANCA, as determined by IIF, are shown in Table I. Anti-PR3 ANCA levels, as measured

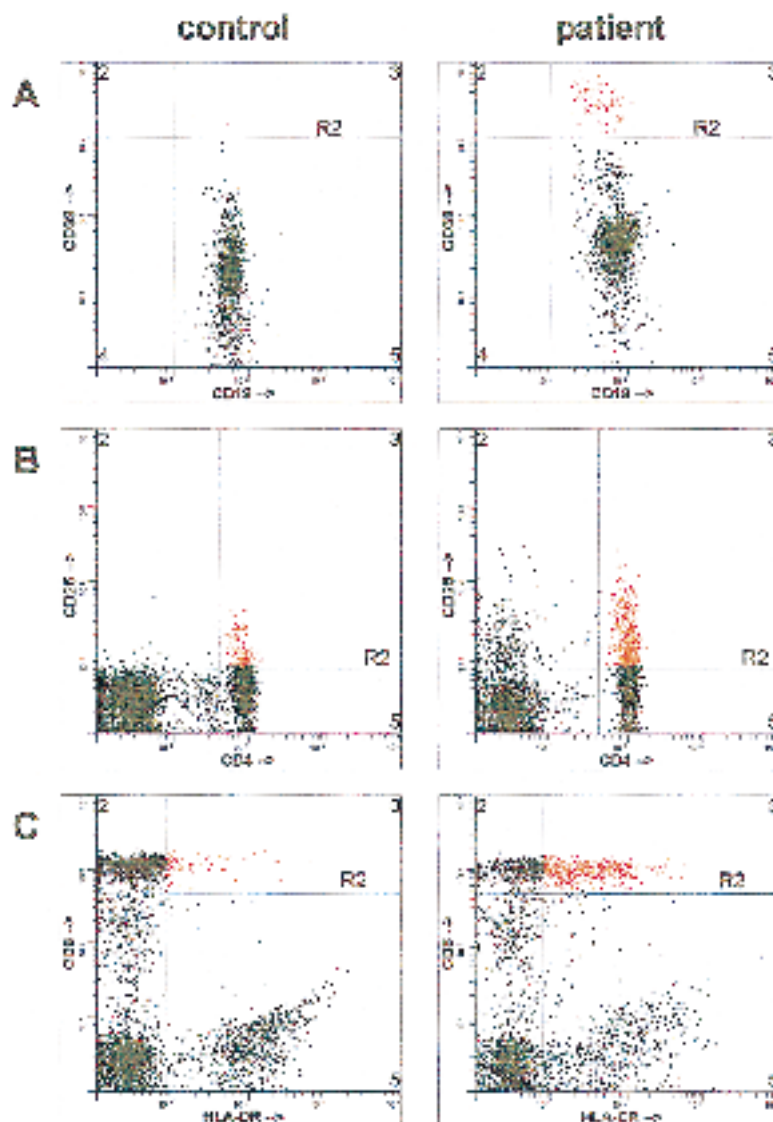


FIG 2. Lymphocyte activation profiles. Whole peripheral blood was incubated with combinations of mAbs against lymphocyte lineage and activation markers. After lysis of erythrocytes and washing, the expression of activation markers on the surface of B and T cells was measured by FACS. For the detection of CD38-expressing B cells, a live gate was set on the CD19⁺ population. **A**, B-cell activation profile; **B**, activation profile of CD4⁺ T cells; **C**, activation profile of CD8⁺ T cells.

by direct ELISA, were significantly higher in patients with active WG than in patients with quiescent disease ($P < .005$; Fig 1). This difference was seen in the group of patients with generalized disease but not in the group of patients with limited disease (Fig 1). Levels of CRP and RF were significantly higher in the group of patients with active disease (CRP median, 80 mg/L; range, 4 to 307 mg/L; RF median, 23.0 mg/L; range, 10.0 to 211.0 mg/L) than in patients experiencing remission of the disease (CRP median, 4.0 mg/L; range, 2.0 to 38 mg/L; $P < .0005$; RF median, 11.0 mg/L; range, 10.0 to 129.0 mg/L; $P < .05$). The median plasma levels of IgM and IgG in patients with WG did not differ from those of healthy control subjects.

Lymphocyte subsets

The number of circulating leukocytes in patients with WG was not different from healthy control subjects ($8.5 \times 10^6/\text{mL}$ in patients with active disease; $7.0 \times 10^6/\text{mL}$ in patients experiencing remission of disease; and $7.5 \times 10^6/\text{mL}$ in healthy control subjects). In contrast, the percentage of lymphocytes (CD45⁺/CD14⁻ cells) was decreased in patients with active disease (median, 11.0%; range, 3.5% to 23.0%; $P = .0005$) and patients experiencing remission of disease (median, 17.0%; range, 8.0% to 28.5%; $P < .05$), as compared to control subjects (median, 22.0%; range, 6.0% to 34%). The percentages of CD3⁺, CD4⁺, and CD8⁺ T-cell subsets and of CD19⁺

TABLE II. Percentages of CD38-expressing B-cell subsets in the peripheral blood of patients with WG and healthy control subjects

B-cell subset	Control subjects (n = 16)	Patients (n = 33)	
		Active (n = 12)	Remission (n = 21)
CD19 ⁺ /CD3 ⁻ (%)	20.58 (4.83-36.90)	13.05* (6.25-78.85)	16.88* (6.30-60.98)
CD19 ⁺ /CD38 ⁺ (%)	78.84 (62.80-95.82)	86.35* (8.75-93.41)	82.37* (32.25-93.86)
CD19 ⁺ /CD38 ⁺⁺ (%)	0.31 (0.07-0.81)	0.82 (0.10-7.00) <i>P</i> < .05	0.40* (0.10-1.29)

Frequencies are expressed as percentage of the CD19⁺ lymphocyte population. *P* values for each patient group and lymphocyte subset were calculated relative to healthy control subjects.

*Not significant.

B cells were within the normal range, irrespective of disease activity (not shown). The CD4/CD8 ratio in patients with WG also did not differ from that of control subjects.

B-cell activation

The expression pattern of the activation and differentiation marker CD38 on peripheral blood B cells from patients with WG (Fig 2, A) allowed for the subdivision of these cells into 3 subsets of CD19⁺/CD38⁻, CD19⁺/CD38^{dull}, and CD19⁺/CD38^{bright} cells, respectively. In patients with either active or quiescent WG, the median percentage of CD19⁺/CD38⁻ B cells and of CD19⁺/CD38^{dull} B cells did not differ from that of healthy control subjects (Table II). However, the percentage of CD38^{bright} cells was markedly higher in the group of patients with active disease than in the group of patients experiencing remission of disease (*P* < .05) or in healthy control subjects (*P* < .05; Table II; Fig 3, A). This increased population of CD38^{bright} B cells was present more often in patients with active disease (6 of 12 patients) than in patients experiencing remission of disease (1 of 21 patients; *P* < .005). Of the 6 patients with active WG and increased numbers of activated B cells, 4 patients were studied at the time of primary diagnosis. The frequency of CD38^{bright} B cells in patients with active WG correlated with BVAS (*r* = 0.74; *P* = .0048). Furthermore, CD38^{bright} B cells had forward/sideward scatter characteristics of blast cells (not shown).

We further asked whether the presence of CD38^{bright} B cells in the peripheral blood of patients with active WG correlated with disease extent as defined by organ involvement. The median percentage of CD38^{bright} peripheral blood B cells was higher in the group of patients with generalized, active WG than in patients with WG with limited active disease (*P* < .05), in patients with limited, quiescent disease (*P* < .01), or in healthy control subjects (*P* < .05; Fig 3, A).

Relation between B-cell activation and ANCA levels

In WG, sudden rises in serum ANCA titers often precede a relapse episode.⁹ Because ANCA are produced by activated, autoreactive B cells, we asked whether B-cell activation correlated with plasma levels of ANCA in the group of patients with active disease. No such correlation was present. Further subdivision of the patient groups

according to organ involvement (generalized/limited) had no effect on this finding. We further reasoned that B-cell activation in patients with active WG disease may be polyclonal rather than antigen specific. We therefore investigated possible correlations between the percentage of CD38^{bright} peripheral blood B cells and plasma levels of RF, IgM, and IgG. There was no correlation between these parameters.

T-cell activation

To study the activation of CD4⁺ T cells, we used the activation marker CD25 (IL-2Rα chain; Fig 2, B). The percentage of CD4⁺/CD25⁺ cells was higher in both the group of patients with active disease (median, 29.5% of the CD4⁺ T cells; range, 13.5% to 60.5%; *P* < .0001) and in the group of patients experiencing remission of disease (median, 17.0%; range, 8.0% to 77.5%; *P* < .0005) than in healthy control subjects (median, 9.0%; range, 4.0% to 22.5%; Fig 3, B). The number of patients displaying an increased percentage of CD4⁺/CD25⁺ T cells did not differ between active (8 of 12 patients) and quiescent patients (8 of 21 patients; *P* > .05). No difference was seen in the percentage of CD4⁺/CD25⁺ T cells between the group of patients with active disease and the group of patients experiencing remission of disease (Fig 3, B).

Activation of CD8⁺ T cells was studied by analyzing the expression of the HLA-DR molecule (Fig 2, C). The median percentage of CD8⁺/HLA-DR⁺ T cells was higher in both active (median, 20.0% of the CD8⁺ T cells; range, 8.5% to 35.0%; *P* < .005) and quiescent patients (median, 20.5%; range, 7.0% to 79.0%; *P* < .001) as compared with control subjects (median, 9.0%; range, 0.1% to 22.5%; Fig 3, C). There was no difference between the percentage of CD8⁺/HLA-DR⁺ cells in patients with active disease as compared with patients experiencing remission of disease. The number of patients displaying an increased percentage of CD8⁺/HLA-DR⁺ cells did not differ between the group of active (5 of 12 patients) and quiescent patients (11 of 21 patients; *P* > .05).

We further investigated the relationship between the extent of T-cell activation and disease extent. The median percentage of CD4⁺/CD25⁺ T cells was higher in patients with generalized or limited disease, as compared with healthy control subjects, irrespective of disease activity (generalized [active], *P* = .001; limited [active], *P* < .005; generalized [quiescent], *P* < .0005; limited

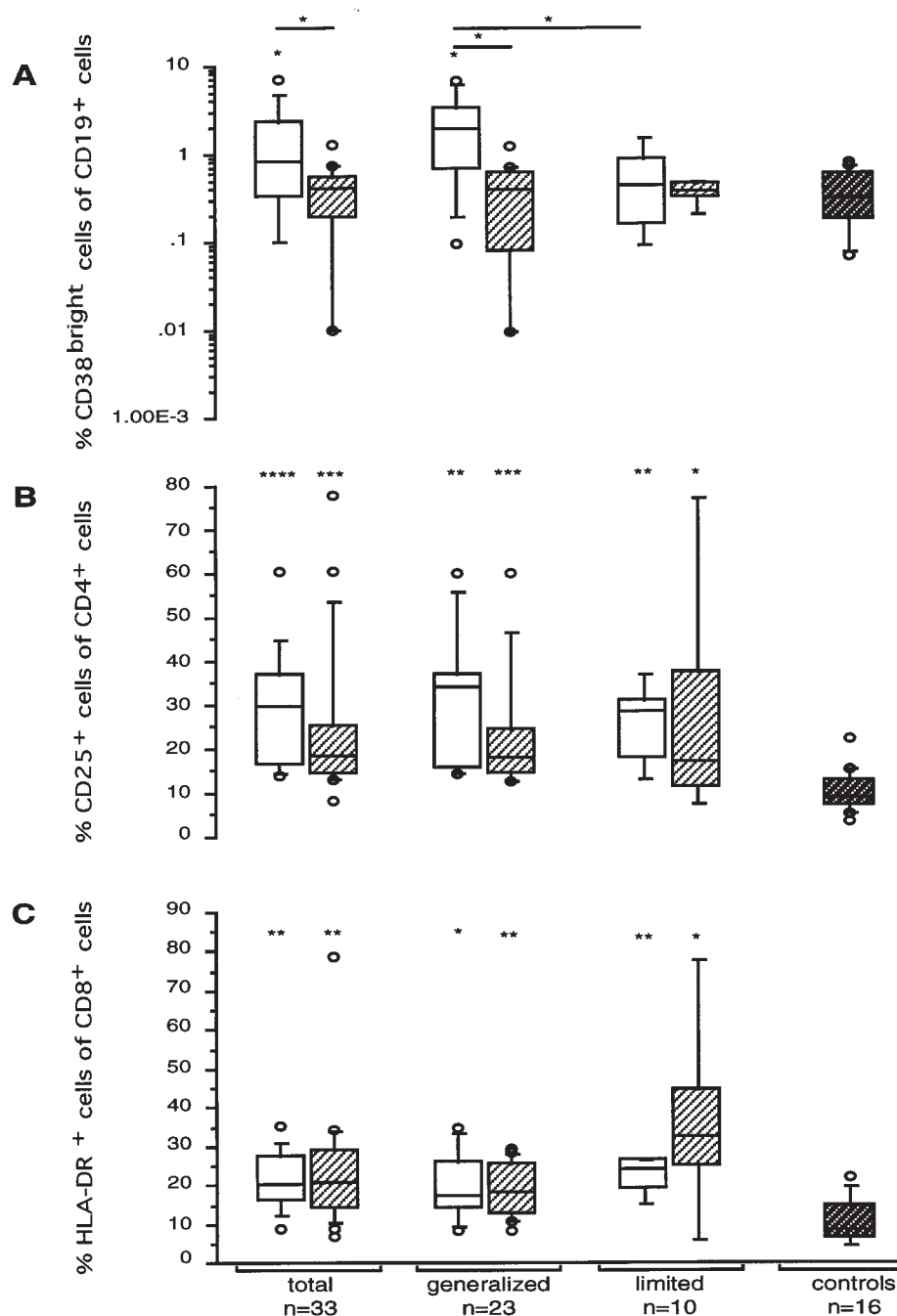


FIG 3. Frequency of activated B and T cells in the peripheral blood of patients with WG and healthy control subjects in relation to disease activity and extent. **A**, Frequency of CD38-expressing cells within the CD19⁺ population; **B**, frequency of CD25-expressing cells within the CD4⁺ T-cell population; **C**, frequency of HLA-DR-expressing cells within the CD8⁺ T-cell population. Indicated are the 10th, 25th, 50th, 75th, and 90th percentiles (*horizontal lines*) and outliers (*circles*). *P* values for each patient group were determined relative to healthy control subjects (*) or relative to another patient group (* *above line*). **P* < .05; ***P* < .005; ****P* < .0005; *****P* < .0001. Open bars, Active patients (n = 12); light hatched bars, patients experiencing remission of disease (n = 21).

[quiescent], *P* < .05; Fig 3, B). The percentage of CD8⁺/HLA-DR⁺ T cells exceeded that of the control group in patients with both generalized and limited disease, irrespective of disease activity (Fig 3, C; generalized [active], *P* < .05; limited [active], *P* < .005; general-

ized [quiescent], *P* = .001; limited [quiescent], *P* < .05). For both T-cell subsets, the percentage of activated T cells in either subset did not differ between patients with generalized and limited disease.

There was no correlation between the percentage of

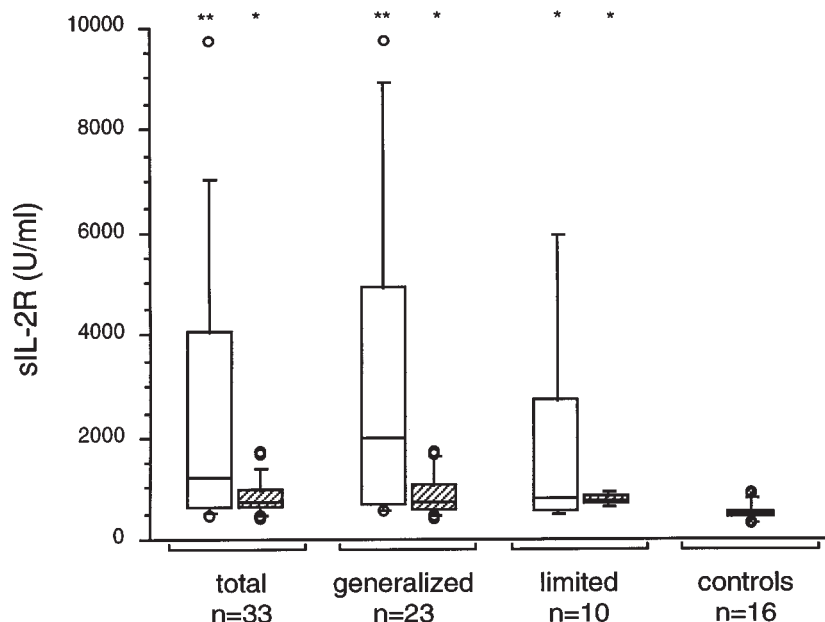


FIG 4. Plasma levels of sIL-2R in relation to disease activity and extent. Indicated are the 10th, 25th, 75th, and 90th percentiles (horizontal lines) and outliers (circles). *P* values were calculated with respect to healthy control subjects. **P* < .05; ***P* < .005. Open bars, Active patients (*n* = 12); light hatched bars, quiescent patients (*n* = 21).

activated T cells of either subset and levels of CRP, ANCA, RF, IgM, and IgG. Also, there was no correlation between T-cell activation and the BVAS.

Arguing that in patients with active WG, T-cell help would be required for the activation of the observed CD38^{bright} B-cell subset, we investigated the correlation between the percentages of CD38^{bright} B cells and CD4⁺/CD25⁺ T cells in this patient group. No correlation between these 2 parameters could be demonstrated.

sIL-2R

Levels of sIL-2R were increased in patients with WG (active WG median, 1219.0 U/mL; range, 506.0 to 9696.0 U/mL; quiescent WG median, 720.0 U/mL; range, 417.0 to 1732.0 U/mL) as compared with control subjects (median, 474.0 U/mL; range, 321.0 to 895.0 U/mL; Fig 4). Levels of sIL-2R did not differ between patients with active and quiescent disease or between patients with generalized and limited disease.

To find the possible source of sIL-2R, we investigated the relation between sIL-2R levels and the percentage of activated T and B cells from the peripheral blood of patients with WG. No such correlation was found.

DISCUSSION

In this study we investigated the relation between cellular and humoral immunity in WG in relation to disease activity and the interrelationship of B- and T-cell activation. We found a differential activation pattern of lymphocytes where B-cell activation correlated with active disease, whereas increased T-cell activation persisted during complete remission.

CD38 was used as a marker of B-cell activation and differentiation.²⁷ CD19⁺/CD38^{bright}, blast-like B cells were present at a higher frequency and with a higher prevalence in patients with active, generalized WG than in patients experiencing remission of disease or in healthy control subjects. The coexpression of CD19 and CD38 on these cells suggests that they have not yet attained the plasma-cell stage. However, Harada et al²⁸ reported CD19⁺/CD38⁺ plasma cells in the bone marrow of healthy individuals. In line with this study, it is possible that the CD38^{bright} B cells found in patients with active WG are plasma cells.

The frequency of activated B cells in the peripheral blood of patients with active WG correlated with disease activity as measured with the BVAS, indicating a link between the activation of humoral immunity and disease exacerbation. More specifically, this link appears to be with the occurrence of systemic vasculitis particularly with renal involvement, because increased numbers of activated B cells were present in patients with active, generalized WG but not in patients with active, limited WG, characterized mainly by local granulomatous inflammations.

There was no correlation between the percentage of activated B cells in patients with active WG and plasma levels of anti-PR3 ANCA. Several possibilities may explain this finding: (1) the observed CD38^{bright} peripheral blood B cells have been recently activated and have not yet attained their plasma cell (antibody-producing) potential; (2) the peripheral blood B cells are not the major source of secreted antibody; ANCA detected in the blood probably originate from plasma cells residing in the bone marrow; (3) the half-life of ANCA may exceed

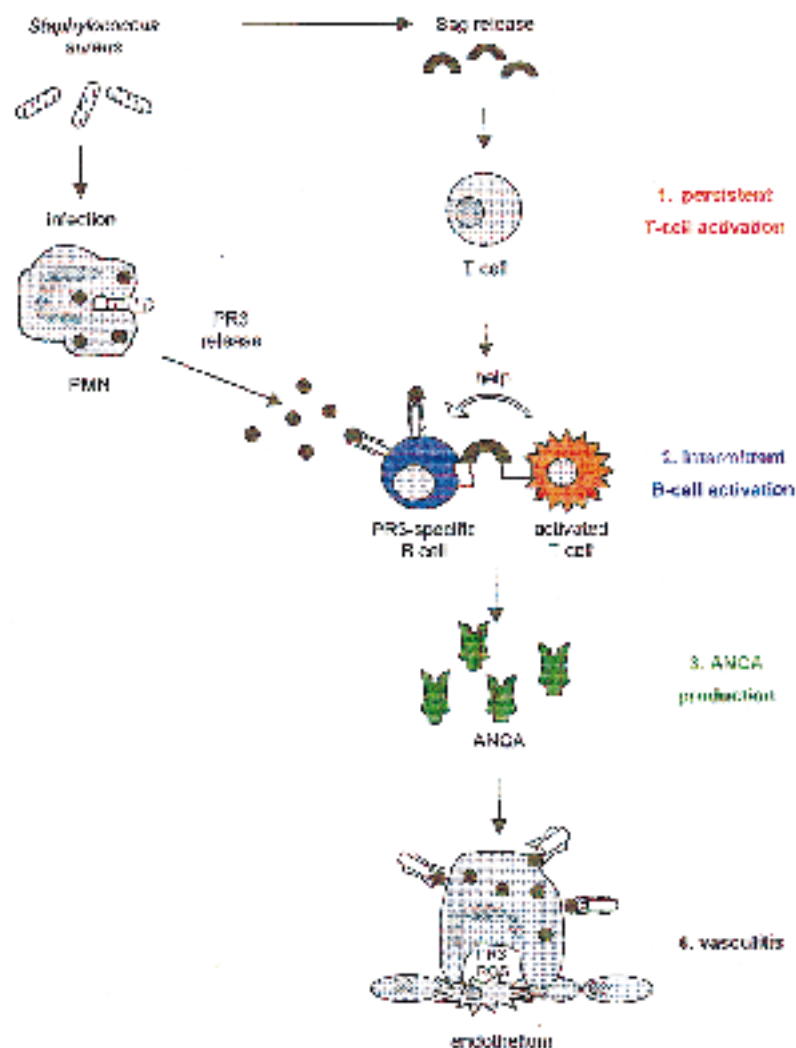


FIG 5. Postulated cause for and effects of lymphocyte activation in WG. Chronic carriage of *S aureus* in patients with WG is associated with the release and persistent presence of superantigens (SAGs). These potent stimulators of the immune system can lead to chronic activation of T cells (phase 1, persistent T-cell activation). In the event of a staphylococcal infection, PMN phagocytose *S aureus*, a process leading to neutrophil activation and release of proteinase 3 (PR3), the major antigen in WG. In the presence of the released autoantigen and costimulatory help from persistently activated T cells, PR3-specific B cells can be activated (phase 2, intermittent B-cell activation) to produce autoantibody (ANCA; phase 3, ANCA production). ANCA have a pathogenic potential by activating neutrophils. In this process reactive oxygen species (ROS) and PR3 are released, contributing to endothelial damage and vasculitis (phase 4, vasculitis). Components of this scenario from the present study are depicted in color.

temporary changes in peripheral blood lymphocyte subsets; and (4) the observed CD38^{bright} peripheral blood B cells are not PR3-specific. However, we also found no correlation between B-cell activation and serum levels of RF, IgM, and IgG, which are markers of a polyclonal rather than an antigen-specific immune response. A longitudinal study will help clarify the relation between B-cell activation and autoantibody levels in the blood.

We found a positive correlation between ANCA levels and active disease and between increased percentages of activated B cells and active disease but not between percentages of activated B cells and ANCA levels. It is possible that the presence of active disease is more closely

associated with the occurrence of ANCA with an increased affinity. Also, the percentage of activated B cells may more strongly correlate with the occurrence of ANCA with increased affinity. The elucidation of the role of the activated B cells in the peripheral blood of patients with WG awaits the characterization of the specificity of these cells. Matthieson et al²⁹ have previously demonstrated the presence of low numbers of ANCA-secreting B cells in the peripheral blood of patients with WG, using an ELISpot assay. We are presently attempting to demonstrate the specificity of activated cells using a similar assay.

In our study percentages of CD4⁺ T cells were not

decreased in patients with WG. This finding is in contradiction with that of others³⁰ who reported decreased percentages of peripheral blood CD4⁺ T cells and might be explained by the fact that we included only patients who were not treated with immunosuppressive drugs or corticosteroids at the time of sampling. T-cell activation was studied by assessing the expression of CD25 (IL-2R α) and HLA-DR on the surface of these cells. The cell surface expression of both markers is upregulated upon activation³¹⁻³³ and has been used in monitoring T-cell activation in a number of autoimmune diseases such as SLE,^{22,34} Sjögren's syndrome,³⁵ and WG.¹¹ Surprisingly, we found a persistently increased percentage of activated CD4⁺ and CD8⁺ T cells in patients with WG during complete remission. We offer 2 possible explanations for this finding: (1) general T-cell activation in WG is dysregulated or (2) a persistent stimulus is leading to ongoing stimulation of T cells. Recently, we reported on the correlation between the presence of T-cell superantigens from *Staphylococcus aureus* in bacterial isolates from patients with WG and an increased frequency of disease exacerbations in these patients.³⁶ It is conceivable that staphylococcal superantigens might be involved in the persistent stimulation of T cells.

Previously, we reported that serum levels of the sIL-2R are elevated in patients with WG and increase significantly before or during a disease relapse.¹⁶ We were able to demonstrate increased levels of sIL-2R in patients with WG, irrespective of disease activity and extent. However, there was no statistical difference between sIL-2R levels in patients with active disease and patients experiencing remission of disease.

T cells are not the only source of sIL-2R, which can be released by proteolytic cleavage¹⁷ by activated monocytes and B cells as well. In our attempt to find the source of sIL-2R in WG, we found no correlation between plasma levels of sIL-2R and the frequency of activated B or T cells in patients with WG. Therefore another source for sIL-2R, possibly monocytes, may be responsible for the increased plasma levels of this molecule.

In conclusion, we propose a scenario in which T-cell activation in WG is sustained during remission by a chronically persisting stimulus, possibly a bacterial molecule, whereas B-cell activation is triggered by a stimulus present during active disease only. We propose this stimulus to be released during an inflammatory response and to be either PR3, shed by activated neutrophils, or a bacterial factor (Fig 5).

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