Circulating Leukocyte Integrin Expression In Wegener's Granulomatosis

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ABSTRACT

Leukocyte adhesion and infiltration are important in the pathogenesis of Wegener's granulomatosis (WG). We tested the hypothesis that the expression of the β1-chain integrin VLA-4 (CD49d/CD29) and the β2-chain integrins LFA-1 (CD11a/CD18), Mac-1 (CD11a/CD18), and gp150,95 (CD11c/CD18) is increased on leukocytes in patients with active WG. Fifteen patients with active WG as defined by positive antineutrophil cytoplasmic autoantibody (cANCA) titers and biopsy, 30 patients with WG in remission as defined by negative cANCA titers and/or immunosuppressive therapy, 25 normal control subjects, and 12 patients with other inflammatory renal and systemic diseases were studied. Surface expression of LFA-1, Mac-1, p150,95, and VLA-4 on neutrophils, lymphocytes, and monocytes was measured by fluorescent antibody cell sorting with monoclonal antibodies against CD11a, CD11b, CD11c, CD18, CD49d, and CD29 respectively. Immunocytochemistry and confocal microscopy were also utilized. β1 (CD29) and β2 (CD18) integrin subunit expression on neutrophils, monocytes, and lymphocytes from patients with acute WG was significantly increased compared with healthy persons and compared with patients with treated vasculitis. Furthermore, the α-integrin subunit CD11b expression was increased on granulocytes and monocytes, but not on lymphocytes. Finally, the α-integrin subunit CD11a expression was increased on monocytes. Immunocytochemistry showed that the increased immunoreactivity on neutrophils was evenly distributed on the plasma membrane and in the cytosol. Immunosuppression resulted in decreased expression of the β1 and β2-integrin subunits. It was concluded that the integrin adhesion molecules, particularly Mac-1 (CD11b/CD18), are upregulated on leukocytes in active WG. This finding suggests a role for integrin expression in the pathogenesis of WG and a possible clue for treatment.

Key Words: Adhesion molecules, integrins, vasculitis, Wegener's granulomatosis, FACS analysis, confocal microscopy

Wegener's granulomatosis (WG) is a necrotizing granulomatous vasculitis that affects the upper and lower respiratory tract and the kidneys (1). Most WG patients have antineutrophil cytoplasmic autoantibodies (cANCA) directed against neutrophil proteinase 3 (2,3). cANCA can activate "primed" neutrophils and cause them to produce free oxygen radicals and release lysosomal enzymes (4). On the basis of these and other findings, a pathogenic model has been developed, whereby neutrophils are primed by low concentrations of cytokines resulting from local infections and then further activated by cANCA (5). The activated neutrophils bind to the endothelium and infiltrate into the vessel wall, leading to vasculitis and glomerulonephritis. The crucial step of leukocyte binding to endothelial cells is a complex adhesion molecule-mediated process (6).

Leukocyte adhesion is mediated by four classes of adhesion molecules: the selectins, the carbohydrate-containing selectin ligands, the integrins, and the immunoglobulin-like (Ig-like) molecules (7,8). The initial attachment of phagocytes to endothelium appears to involve endothelial cell selectins with cognate carbohydrate-containing ligands (9). Selectin-mediated adhesion is insufficient to immobilize phagocytes on endothelium. Rather, it causes the cells to “roll” on the endothelium, where they are subject to local activation signals from endothelium and extravascular tissue (8). These events facilitate activation of phagocyte integrins, immobilization of phagocytes by the interaction of integrins with Ig-like molecules on the endothelium, and eventual migration of the phagocytes through the endothelium into the vascular wall (10). Thus, the interaction between integrins on the surface of leukocytes and Ig-like molecules on endothelium is necessary for immobilization and eventual leukocyte migration (6,11).

The integrins are heterodimeric glycoproteins composed of noncovalently associated α and β subunits and are classified according to the structure of their β subunits. In general, members of each class share a common β subunit and are distinguished by their unique α subunit. The four integrins that appear most important in leukocyte-endothelial adhesion are three β2 integrins, each with a different α subunit, and one β1 integrin. The nomenclature (see Table 1) of these molecules is confusing because of the various naming systems and numerous pseudonyms. The β1 integrin is the very-late activation antigen-4 integrin (VLA-4),

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1046-6673/0071-0040050.00/0
Journal of the American Society of Nephrology
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40 Flag Volume 7 • Number 1 • 1996
TABLE 1. Nomenclature of the leukocyte integrins VLA-4, LFA-1, Mac-1, and gp150,95.*

<table>
<thead>
<tr>
<th>Leukocyte Integrins</th>
<th>VLA-4</th>
<th>LFA-1</th>
<th>Mac-1</th>
<th>gp150,95</th>
</tr>
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<tbody>
<tr>
<td><strong>α4</strong></td>
<td>CD49</td>
<td>CD29</td>
<td>CD11a</td>
<td>CD11c</td>
</tr>
<tr>
<td><strong>β1</strong></td>
<td>CD29</td>
<td>CD11a</td>
<td>CD18</td>
<td>CD18</td>
</tr>
<tr>
<td><strong>αL</strong></td>
<td>CD49</td>
<td>CD29</td>
<td>CD18</td>
<td>CD18</td>
</tr>
<tr>
<td><strong>β2</strong></td>
<td>CD11b</td>
<td>CD18</td>
<td>CD11c</td>
<td>CD18</td>
</tr>
</tbody>
</table>

* Each contains an α and a β subunit. The α (α4, αL, αM, and αX) and β (β1 and β2) subunits are differentiated by their cluster of differentiation (CD) antigens.

also termed α4β1 according to the integrin nomenclature, or CD49/CD29 according to the cluster of differentiation (CD) nomenclature (12). The three β integrins share the β2 chain (CD18) and are named lymphocyte function-associated antigen-1 (LFA-1; also termed αLβ1 or CD11a/CD18), macrophage-1 antigen (Mac-1; also termed αMβ2 or CD11b/CD18) and the glycoprotein p150,95, also termed αXβ2 or CD11c/CD18.

Recently, an upregulated expression of VLA-4 in patients with systemic lupus erythematosus and vasculitis was reported by Takeuchi et al. (13), who suggested that expression of this integrin may be of pathogenic significance. Integrin expression in WG has not been investigated. We studied integrin expression in leukocytes of patients with active and inactive disease by using dual marker cytofluorometry to test the hypothesis that integrin expression is increased in this disease. We confirmed the cytofluorometric results with immunohistochemistry and confocal microscopy.

METHODS

Patients

We studied 45 patients who developed WG between 1984 and 1995. The mean follow-up time was 40 months. The diagnosis was made on the basis of clinical, laboratory, and histological criteria, and fulfilled the diagnostic requirements outlined by Fauci et al. (14). The patients were classified on the basis of their systemic manifestations at the time the diagnosis was made. WG was clinically staged according to the airway, chest, and renal classification criteria outlined elsewhere (14). Only patients with cANCA-positive titers and reactivation of old or development of new symptoms were defined as having active disease. Patients with negative cANCA values and declining or absent clinical disease activity were defined as being in remission. In addition, patients were separated into those not receiving immunosuppressive or antibacterial treatment and into those receiving such therapy. By the use of these criteria, 15 patients were classified as patients with active WG, whereas 30 patients were classified as patients with quiescent disease. Biopsies from various sites that documented a necrotizing granulomatous vasculitis consistent with WG were available in all 45 patients (100%). At the time of first integrin determination, nine (20%) patients with active disease and 23 patients (51%) in remission were receiving immunosuppressive therapy. Forty-five (100%) patients presented with renal involvement, which was followed by upper airway involvement in 39 (89%) patients, joint involvement in 29 (64%) patients, pulmonary involvement in 27 (60%) patients, ocular involvement in 17 (38%) patients, and peripheral nervous system involvement in eight (18%) patients. Twenty-five healthy, age- and sex-matched individuals served as healthy control subjects. For an additional comparison, we measured integrin expression in five patients with systemic lupus erythematosus (SLE), three patients with myeloperoxidase (MPO)-positive systemic vasculitis, two patients with membranous nephropathy, and two patients with sepsis.

Materials and Chemicals

We utilized the following monoclonal antibodies and chemicals purchased from Becton Dickinson (San Jose, CA): CD4/CD8 (Mouse IgG1/IgG1), CD11a (Mouse IgG2a), CD18 (Mouse IgG1), IgG1 Isotype Control, Mouse IgG2a Isotype Control, Cellwash®, CaliBrite®, and FACS Lysing Solution®, CD-Check® was purchased from Streck Laboratories (Omaha, NE). The following monoclonal antibodies were purchased from Dianova (Hamburg, Germany): CD11b (Mouse IgG1), CD11c (Mouse IgG1), CD14 (IgG2a), CD15 (IgGM), CD49d (Mouse IgG1), CD29 (Mouse IgG2a), Mouse IgG1 Isotype Control, and Mouse IgG2a Isotype Control.

Immunofluorescence Analysis

Fluorescent antibody cell sorting (FACS) analysis was carried out as described previously (15). Whole blood was collected in EDTA Vacutainers® and stained with monoclonal antibodies (mAB) conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) within 6 h after collection. Thereafter, the blood samples were treated with FACS Lysing Solution®, realizing the lysis of erythrocytes and partial fixation of leukocytes. The samples were then washed two times with optimized phosphate buffer solution (PBS) (Cellwash®, Becton Dickinson), which resulted in a leukocyte suspension suitable for flow cytometry.

The cells were analyzed on a standard FACScan flow cytometer (Becton Dickinson, San Jose, CA). Flow cytometric standardization was achieved by running 6.6 mm beads (CaliBrite® and CD-Check®). For each sample, 10,000 cells were analyzed on the log fluorescence scale of the flow cytometer. Subsets of white blood cells were differentiated in the forward versus sideward scatter diagram and verified with specific cell markers (CD4 and CD8 for lymphocytes, CD14 for monocytes, and CD15 for granulocytes). The surface antigen expression, the mean log FITC, and PE-fluorescence channel of the positively stained cells were determined from a single parameter histogram.

Immunocytochemistry

For immunocytochemical analysis, the cells were separated using a FACSsort (Becton Dickinson). The techniques for confocal microscopy were utilized as described previously (16). The cells were fixed with 3% paraformaldehyde and...
permeabilized with 80% methanol at -20°C. After incubation with 3% skimmed milk in phosphate buffer solution (SM/PBS) for 60 min, the preparation was incubated for 1 h at room temperature with the integrin antibodies (see above) diluted in PBS with 0.1% BSA (1:80). Washed three times with PBS and then exposed to the secondary antibody (FITC-conjugated antirabbit or antimouse IgG, at 1:100, 1% BSA/PBS; Pierce Chemicals, Oud-Beijerland, The Netherlands) for 60 min. The preparation was washed with 50% glycerol under a glass cover slip on a Nikon-Diaphot (Tokyo, Japan) microscope. A Biorad MRC 600 confocal imaging system (Bio-Rad Laboratories, Freiburg, Germany) with an argon laser was used. At least 30 cells from each patient were examined. The results were reproduced by two separate investigators and multiple experiments were done. The observers were unaware of the patients' names and antibodies used. Quantification of the signal intensity was done with histogram/area functions in the MRC-Comos software. The cellular regions were outlined manually and the calculated mean fluorescence intensity was obtained for the delineated regions.

cANCA Determinations

We used two methods to determine cANCA, namely, an indirect immunofluorescent test (IFT) and an antigen-specific ELISA. For the IFT, ethanol fixed granulocytes (blood group O) were used (Euroimmun, Lübeck, Germany). cANCA (antiproteinase 3) was also measured with a commercially available ELISA. The proteinase 3 antigen for this assay was purified with affinity chromatography (Gesellschaft für Immunchemie und Immunbiologie, Hamburg, Germany).

Statistical Analysis

Statistical analysis was carried out on a Macintosh II computer (Apple Inc., Cupertino, CA) by using a commercially available program (Statview; Cricket Software Inc., Philadelphia, PA). The results (mean ± SE) represent duplicate measurements. For statistical analysis, the nonparametric Wilcoxon test was used. Differences were considered to be significant when the P value was \(\leq 0.05\).

RESULTS

Table 2 shows the comparison of the mean fluorescence intensities as assessed by flow cytometry. Part A of Table 2 shows the integrin expression on neutrophils from normal control subjects, patients with acute WG, and in WG patients in remission. The order of the subunits is \(\alpha L\) (CD11a), \(\alpha M\) (CD11b), \(\alpha X\) (CD11c), \(\beta 2\) (CD18), and \(\beta 1\) (CD29). Increased surface expression, compared with healthy controls was observed for the \(\alpha\) integrin CD11b and the \(\beta\) integrins CD18 and CD29 in active WG. No changes were seen with respect to CD11a and CD11c. In WG patients in remission who were receiving therapy, CD18 and CD29 were also significantly increased compared with the normal controls. The increase in the \(\alpha M\)-Integrin subunit CD11b and the \(\beta 1\)-integrin subunit CD29 in patients with acute disease without therapy was significantly greater than that of the WG group in remission without therapy. In contrast to the increased expression of CD11b, CD18, and CD29 in patients with active WG, we did not find an increase of these integrins in the second control group, which included patients with SLE and other inflammatory diseases (CD11a, 42.5 ± 1.7; CD11b, 350.0 ± 38.4; CD11c, 286.3 ± 38.3; CD18, 195.6 ± 22.9; CD29, 41.2 ± 2.6) compared with healthy controls.

Part B of Table 2 shows the integrin expression on monocytes from normal control subjects, patients with acute WG, and in WG patients in remission. The order of the subunits is \(\alpha L\) (CD11a), \(\alpha M\) (CD11b), \(\alpha X\) (CD11c), \(\beta 2\) (CD18), \(\beta 1\) (CD29), and \(\alpha 4\) (CD49d). The \(\beta 1\) and \(\beta 2\) subunits CD29 and CD18 and the \(\alpha\) subunits CD11a and CD11b showed an increased surface expression in patients with active disease with and without therapy compared with healthy controls and to patients with other forms of inflammation and vasculitis (data not shown). The \(\alpha\) chain CD11c was increased in patients with acute WG under treatment compared with healthy controls. CD29 and CD18 were significantly increased in the remission group compared with healthy controls, but significantly lower than in patients with acute WG \((P < 0.05)\) under treatment, as well as in WG patients without therapy. There was no significant difference observed between healthy controls and patients with other inflammatory diseases.

Part C of Table 2 shows the integrin expression on lymphocytes from normal control subjects, patients with acute WG, and WG patients in remission. The order of the subunits is \(\alpha L\) (CD11a), \(\beta 2\) (CD18), \(\beta 1\) (CD29) and \(\alpha 4\) (CD49d). The \(\alpha\) integrins \(\alpha M\) (CD11b) and \(\alpha X\) (CD11c) are not found on lymphocytes. The \(\beta\) integrins CD18 and CD29 showed an increased surface expression in both groups of WG patients compared with healthy controls. The increase in CD11a subunit showed an increasing trend \((0.1 > P > 0.05)\) compared with healthy controls.

Figure 1 shows representative immunohistochemistry of the \(\beta 2\) subunit CD18 (upper panel) and the \(\beta 1\) subunit CD29 (lower panel) on neutrophils from a patient with acute WG without therapy (left) and a healthy individual (right). The confocal photomicrograph shows pseudocolours. The highest fluorescence values are shown in yellow; the lowest values are demonstrated in blue. The staining for CD18 shows a dense staining within the cytosol, whereas CD29 is accumulated at the cell periphery. For both integrin subunits, the cells from the patient with active WG show a greatly increased immunoreactivity compared with the neutrophils from the healthy individual.

Figure 2 shows CD11b (upper panel) and CD18 (lower panel) expression in cANCA-positive active WG patients and in cANCA-negative WG patients in remission, as well as controls. This CD11b/CD18 complex makes up the integrin Mac-1. Significant increases in this expression in acute WG patients compared with the other two groups is apparent. The patients with diverse inflammatory conditions are not represented. These patients also had no increase in CD11b/CD18 expression.

Figure 3 (upper panel) shows the effect of immunosuppressive therapy on the surface expression of \(\beta 2\) integrin subunits CD18 on neutrophils (upper left
**TABLE 2. Integrin expression in healthy controls, cANCA-positive, and cANCA-negative WG patients**

<table>
<thead>
<tr>
<th>Leukocyte</th>
<th>CD11A</th>
<th>CD11B</th>
<th>CD11C</th>
<th>CD18</th>
<th>CD29</th>
<th>CD49d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Controls (N = 25)</td>
<td>46.7±1.7</td>
<td>368.2±21.3</td>
<td>308.0±15.4</td>
<td>142.7±10.0</td>
<td>34.8±0.6</td>
<td></td>
</tr>
<tr>
<td>cANCA-Positive (N = 15)</td>
<td>53.1±3.11</td>
<td>631.7±99.1</td>
<td>275.4±94.7</td>
<td>584.3±48.0</td>
<td>110.9±26.8</td>
<td></td>
</tr>
<tr>
<td>W/O Treatment (N = 6)</td>
<td>48.5±2.9</td>
<td>524.4±41.8</td>
<td>397.6±7.18</td>
<td>457.1±79.8</td>
<td>76.9±19.1</td>
<td></td>
</tr>
<tr>
<td>W/ Treatment (N = 9)</td>
<td>53.1±3.1</td>
<td>631.7±99.1</td>
<td>275.4±94.7</td>
<td>584.3±48.0</td>
<td>110.9±26.8</td>
<td></td>
</tr>
<tr>
<td>cANCA-Negative (N = 15)</td>
<td>46.0±2.32</td>
<td>365.4±67.3</td>
<td>328.0±79.4</td>
<td>286.7±68.5</td>
<td>90.2±23.6</td>
<td></td>
</tr>
<tr>
<td>W/O Treatment (N = 7)</td>
<td>44.5±1.35</td>
<td>400.8±34.2</td>
<td>334.2±24.9</td>
<td>308.4±40.1</td>
<td>71.0±11.2</td>
<td></td>
</tr>
<tr>
<td>W/ Treatment (N = 23)</td>
<td>142.7±6.0</td>
<td>491.0±75.3</td>
<td>1007.6±377.5</td>
<td>496.8±71.1</td>
<td>301.5±7.8</td>
<td></td>
</tr>
<tr>
<td>cANCA-Negative (N = 30)</td>
<td>153.5±14.8</td>
<td>377.5±93.5</td>
<td>842.0±108.6</td>
<td>266.6±138.7</td>
<td>80.3±12.5</td>
<td></td>
</tr>
<tr>
<td>W/O Treatment (N = 7)</td>
<td>142.7±6.0</td>
<td>491.0±75.3</td>
<td>1007.6±377.5</td>
<td>496.8±71.1</td>
<td>301.5±7.8</td>
<td></td>
</tr>
<tr>
<td>W/ Treatment (N = 23)</td>
<td>195.2±7.7</td>
<td>219.7±22.9</td>
<td>195.2±21.3</td>
<td>95.8±2.91</td>
<td>64.0±6.3</td>
<td></td>
</tr>
</tbody>
</table>

**Panel A** shows the expression on granulocytes (which lack CD11b and CD49d). **Panel B** shows expression on monocytes, and **Panel C** shows expression on lymphocytes (which lack CD11b and CD11c). *P < 0.05 compared with controls, **P < 0.01 compared with controls. Values are given as mean ± SE.

Panel) and monocytes (upper right panel) in four patients with acute WG. The first measurement was carried out before immunosuppressive therapy with corticosteroids and cyclophosphamide (17). Within a mean treatment period of 12 wk, CD18 expression decreased significantly and was in the midrange of the values observed in healthy control subjects. Patients with WG may also show an improvement under antimicrobial therapy with trimethaprim-sulfamethoxazole. The drug was given because of purulent sinusitis. A nasal culture had revealed Staphylococcus aureus. These patients were not receiving prednisone and cyclophosphamide. The first measurement was conducted before or within 2 days of the start of the antimicrobial therapy. Expression of CD18 decreased within a mean treatment period of 12 wk in these patients, compared with pretreatment values.
Integrins in Wegener's Granulomatosis

**DISCUSSION**

We reasoned that leukocyte immobilization on the endothelial surface with integrins and their ligands would be important to the pathogenesis of WG (20). We tested the components of four integrin molecules. VLA-4 (CD49d/CD29) is constitutively expressed by lymphocytes, monocytes, basophils, and eosinophils, but not neutrophils. Its ligand is the inducible Ig-like vascular cell adhesion molecule-1 (VCAM-1) (21). The CD18 group is distinguished by their \( \alpha \) subunits (22). CD11a shares 36% homology with CD11b and CD11c and is expressed by neutrophils, monocytes, and lymphocytes. In contrast, CD11b and CD11c share 63% homology and are expressed by granulocytes and monocytes, but not lymphocytes. LFA-1 (CD11a/CD18) has as its ligand the intercellular adhesion molecules 1 and 2 (ICAM-1 and ICAM-2). Mac-1 (CD11b/CD18) can attach to ICAM-1, C3b, fibrinogen, and Factor X, although the ligands for gp150,95 (CD11c/CD18) have not yet been characterized (23). Endothelial cells are target cells for all four integrins. However, epithelial cells, vascular smooth muscle cells, mesangial cells, and platelets also may express Ig-like integrin ligands (24). We found that the surface expression of the \( \beta2 \) CD18 integrins and the \( \beta1 \) CD29 integrins is increased in patients with active WG. In addition, the \( \alpha \)-integrin subunits CD11b and CD11c were also increased, whereas increased expression of CD11a was only observed on monocytes. CD11b/CD18 or Mac-1 was the integrin most regularly expressed in acute WG. We also found that the increased expression of CD18 and CD29 decreased during treatment, together with an improvement in the clinical symptoms.

These findings suggest that leukocytes in patients with active WG display an increased propensity for

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**Figure 1.** Representative immunohistochemistry CD18 (upper panel) and CD29 (lower panel) on neutrophils from a patient with acute WG (left) and a healthy control subject (right). The confocal photomicrograph shows pseudocolors. The highest fluorescence values are shown in yellow, and the lowest values are in blue. The CD18 staining shows a dense pattern throughout the cytosol, whereas CD29 accumulated at the cell periphery. The cells from the patient with active WG show a greatly increased immunoreactivity compared with the healthy subject.
adherence to the endothelium and that this increased stickiness is reduced during remission. This assumption agrees with the hypothesis of Jennette and Falk (25). They suggested that the local neutrophils and monocyte accumulation in WG is initiated by the priming of circulating neutrophils by low concentrations of cytokines resulting from local infection. Priming causes proteinase 3 and myeloperoxidase to be expressed on the neutrophil membrane, where reaction with antibodies is facilitated. In WG patients, cANCA further activate the neutrophils, which leads to the production of reactive oxygen free radicals and the release of proteolytic enzymes. The activated neutrophils also damage the endothelium, induce up-regulation of ICAM-1, and/or VCAM-1. These effects increase leukocyte adherence and promote their migration into the vessel wall, evoking the initial step of vasculitis. The final pathway of this scheme involves leukocyte-vessel wall adhesion and penetration via cell adhesion-molecule interactions. The adhesion

Figure 2. CD11b/CD18 (Mac-1) expression on granulocytes. CD11b is shown in the upper panel, and CD18 is given in the lower panel. The values in cANCA-positive active WG patients are significantly higher than in healthy controls and in cANCA-negative WG patients in remission. The values for patients with other inflammatory conditions, which were not different from controls, are not shown.
molecules on leukocytes that mediate the binding to ICAM and VCAM are the integrins. Our data show that integrins are upregulated in active WG and decline during treatment and remission. Our findings are consistent with recently published results in which ICAM-1 and VCAM-1 were increased in WG patients (26).

Conceivably, only the integrin affinity was increased and protein levels remained unchanged in our patients. Others have shown that “activation” of the integrin receptors can be achieved by inducing allosteric alterations of the protein (27). Integrin activation may also lead to clustering of the molecules in the plasma membrane surface, thereby enhancing the avidity of cell-cell interactions (27). However, our immunocytochemical results do not support such a mechanism. We found increased expression of CD18 and CD29 which showed a uniform staining pattern. In our study, the β subunits CD18 and CD29 were significantly increased in all three cell types analyzed, whereas the α-integrin subunits CD11a, CD11b, and CD11c showed a more diverse pattern. The different integrin subunits are located on different genes and have promoters that are also different (28,29). Thus, the differential expression of integrins in WG patients is likely determined at the message level. A differential expression of α subunits has been shown in human leukocytes both in vitro (30) and in vivo (31).

The CD29 and CD18 surface expression decreased significantly during treatment in all patients with active WG. Furthermore, in the remission group these integrin β subunits were significantly lower compared with patients with active disease. This finding suggests that integrin expression may be related to the clinical course of the disease. Takeuchi et al. recently reported that the expression of the integrin receptor
VLA-4 (CD49d/CD29) was increased in lupus erythematosus patients with vasculitis, but not in those without the disease (13). They suggested a role for this integrin in the development of more severe vasculitis (13). However, in our study, even the patients with remission demonstrated increased expression of some integrin subunits compared with healthy control subjects. Although our findings during treatment suggest an association between integrin expression and clinical symptoms, a role for integrin expression as a disease activity marker is questionable. However, the correlation between cANCA titers and clinical symptoms and an increased expression of LFA-3 in patients with chronic inflammation of the ear. In the same instance, integrin binding to S. aureus suggests a role for S. aureus nasal carriers were more prone to relapses, suggesting a role for S. aureus in WG pathogenesis. Our patients received trimethoprim-sulfamethoxazole because of sinusitis, and had positive S. aureus cultures. We are unable to distinguish between an indirect effect of infection irradiation, or some as yet unappreciated action of trimethoprim-sulfamethoxazole. However, our data suggest that integrin expression and its remission is involved in the process.

In summary, we observed increased β1 and β2 integrin CD29 and CD18 subunit expression on leukocytes from patients with acute WG. We also showed that the α-integrin subunits CD11a and CD11b were upregulated in WG. Immunosuppression and trimethoprim-sulfamethoxazole therapy resulted in a decrease of the β1 and β2 chain integrins. This decrease also occurred with disease remission. Our findings may have therapeutic implications. In animal models, antiLFA-1 and antiCAM-1 antibodies effectively inhibited rejection, inflammatory disease progression, and reperfusion injury, respectively (44-46). A therapeutic intervention preventing leukocyte integrin-adhesion molecule interaction may also prove to be useful in WG.

ACKNOWLEDGEMENT
This project was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG Ha - 1388/2).

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