Disease-activity in ANCA-associated vasculitis

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2009

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Plasma levels of soluble IL-2R, soluble CD30, IL-10 and BAFF during follow-up in PR3-ANCA-associated vasculitis: associations with disease activity and relapse

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Department of Internal Medicine, Divisions of ¹Clinical Immunology and ²Nephrology, University Medical Center Groningen, The Netherlands

Chapter 4

ABSTRACT

Objective: To evaluate whether T cell activation as reflected by levels of sIL-2R, sCD30, IL-10 and B cell activator of the TNF family (BAFF) at diagnosis and during initial follow-up is predictive for persistent or renewed ANCA-positivity and clinical relapse in PR3-ANCA-associated vasculitis.

Methods: Patients with PR3-ANCA associated vasculitis and at least 2 years of follow-up were included (n=87). C-ANCA titers were detected by IIF; PR3-ANCA, sIL-2R, sCD30, IL-10, and BAFF were assessed by ELISA, at diagnosis, and at 3, 6, 12, 18, and 24 months after diagnosis. Healthy volunteers (n=31) provided plasma samples for comparison. Levels of immune markers were related to ANCA-positivity and relapse during follow-up

Results: Plasma levels of sIL-2R, sCD30, and BAFF were higher in patients than in healthy controls at all time points. Plasma levels of sIL-2R, sCD30 and IL-10 were higher at diagnosis and relapse than during remission. At 18 months, sCD30 (p<0.001) and sIL-2R levels (p=0.01) were significantly higher in patients positive for PR3-ANCA (ELISA) than in PR3-ANCA negative patients. At 24 months, patients who were ANCA positive by ELISA or IIF had significantly higher sCD30 plasma levels (p = 0.02; p = 0.03) as compared to ANCA negative patients.

Conclusion: Increased T cell activation in patients with ANCA-associated vasculitis in remission during and after immunosuppressive treatment is associated with persistent or renewed ANCA positivity.
INTRODUCTION

Antineutrophil cytoplasmic antibodies against myeloperoxidase (MPO-ANCA) and proteinase 3 (PR3-ANCA) are associated with primary vasculitides affecting small to medium sized vessels [1, 2]. With current immunosuppressive induction therapy most patients with these diseases can be brought into stable remission. Patients positive for PR3-ANCA, however, are particularly prone for relapse of disease during long-term follow-up when immunosuppressive therapy is tapered and finally stopped [3]. Recently, we showed that persistence of a positive ANCA-titer in these patients after induction of remission by cyclophosphamide, at the time treatment is switched to azathioprine, is associated with an increased risk for relapse [4].

Additionally we found positive c-ANCA and PR3-ANCA titres at 3, 12, 18 and 24 months after diagnosis were also associated with relapse [5]. We concluded from these data that ANCA positivity reflects smouldering immune-activation, and is, therefore, associated with an increased risk for disease relapse when immunosuppressive therapy is tapered and stopped. Serological markers of immune activation are, indeed, present in active ANCA-associated vasculitis. Notably, serum levels of soluble IL-2 receptor (sIL-2R) and soluble CD30 (sCD30) are elevated during active disease reflecting T cell activation [6-9]. They have been proposed as markers of disease activity. Moreover, not only in active disease but also during remission activated T cells can be present [10]. Recently, Ohlsson et al reported that the presence of low plasma levels of the immunosuppressive Th2 cytokine IL-10 during remission is associated with an increased risk of subsequent relapse in ANCA-associated vasculitis [11]. In addition to activated T cells, patients with active ANCA-associated vasculitis have been shown to have increased numbers of activated B cells. In other autoimmune diseases elevated plasma levels of the polyclonal B cell stimulator BAFF (B cell activating factor of the TNF family) have been related to B cell activation [12-14]. BAFF stimulates B cells promoting their survival and BAFF itself might be produced by activated T cells. As BAFF is produced by activated T cells it might link activated T cells to B cell activation. We hypothesize that patients with PR3-ANCA-associated vasculitis who remain positive for ANCA after induction of remission, might have ongoing smouldering immune-activation with an associated increased risk for relapse. In this study, we measured serial levels of the immune-markers sIL2R, sCD30, IL-10 and BAFF in patients with PR3-ANCA-associated vasculitis up to 24 months after diagnosis. We assessed their association with ANCA-status, disease activity, and relapse rate during long-term follow-up. We observed that patients positive for ANCA during remission had persisting increased T cell activation after tapering of immunosuppressives.
PATIENTS AND METHODS

Patients and ANCA assessment
Eighty-seven patients diagnosed with PR3-ANCA-associated vasculitis at our center between January 1991 and March 2002 and being followed for at least 2 years, were included in this retrospective study. Data of the patients are given in table 1. Patients were followed until March 2004. In all patients, the presence of PR3-ANCA was confirmed by antigen specific enzyme-linked immunosorbent assay (ELISA) [1]. Induction treatment consisted of oral cyclophosphamide (2 mg/kg) and prednisolone (1 mg/kg; maximum dose of 60 mg/day). Doses of cyclophosphamide were adjusted to maintain the white blood cell count above 4 x 10^9/L. After 4 to 6 weeks, the daily prednisolone dose was tapered by 10 mg every 2 weeks until the dose reached 30 mg, and thereafter by 5 mg every 2 to 4 weeks. Once remission was achieved the daily dose of oral cyclophosphamide was tapered by 25 mg every 3 months during the period 1991-1996. From 1997 on, patients were switched to azathioprine (1.5-2 mg/kg body weight daily) after three months of stable remission with tapering by 25 mg every 3 months.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>At diagnosis</th>
<th>All patients (n=87)</th>
</tr>
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<tbody>
<tr>
<td>male/female, n (%)</td>
<td>55 (63%)/32 (37%)</td>
</tr>
<tr>
<td>age (years)*</td>
<td>55 (14-80)</td>
</tr>
<tr>
<td>ANCA titer (IIF)*</td>
<td>1:320</td>
</tr>
<tr>
<td>CRP (mg/l)*</td>
<td>126.3 ± 96.7</td>
</tr>
<tr>
<td>Creatinine (μmol/l)*</td>
<td>219.4 ± 223.5</td>
</tr>
<tr>
<td>BVAS*</td>
<td>25 (7-48)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Organ Involvement</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ear, nose, throat: n (%)</td>
<td>77 (89%)</td>
</tr>
<tr>
<td>Lung: n (%)</td>
<td>46 (53%)</td>
</tr>
<tr>
<td>Kidney: n (%)</td>
<td>67 (77%)</td>
</tr>
</tbody>
</table>

#median (range), *mean ± SD

All patients received Pneumocystis carinii prevention with co-trimoxazole (three times per week 960 mg). An additional 13 patients received maintenance therapy with higher dosages of co-trimoxazole (960 mg twice daily). Fifteen patients were treated additionally
with plasma exchange or methylprednisolone infusions (1000 mg on 3 consecutive days) due to the clinical severity of their disease.

At diagnosis, during follow-up and at relapse, disease activity was scored using the Birmingham Vasculitis Activity Score (BVAS) [15]. At diagnosis and at 3, 6, 12, 18, 24 months after diagnosis, and at relapse, serum ANCA titers were determined by indirect immunofluorescence (IIF) as described previously [16], and EDTA plasma was collected from each patient. Sera were considered positive for ANCA when a cytoplasmic staining pattern (C-ANCA) was present at a dilution of at least 1:40. Plasma samples were spun at 3000 rpm for 10 minutes, and supernatants were stored at -80°C until use. In plasma samples PR3-ANCA levels were determined by direct PR3-ANCA ELISA as described previously [17]. Values were expressed as arbitrary units/ml; values of ≥10 units/ml were considered positive.

Thirty-one sex and age matched healthy laboratory workers (13 females, 18 males; median age 51; range: 44-67) volunteered and provided EDTA plasma specimens for comparison.

Definitions
Remission was defined as the absence of clinical signs and symptoms of active vasculitis (Birmingham Vasculitis Activity Score (BVAS)=0) in combination with a normal C-reactive protein (<10 mg/l). A relapse was defined as clinical signs of vasculitic activity in combination with biopsy proven vasculitic disease activity, or the occurrence of nodular pulmonary lesions after exclusion of infectious or malignant diseases. Renal vasculitic disease was defined as biopsy proven necrotizing glomerulonephritis or a combination of microscopic glomerular erythrocyturia, erythrocyte cell casts, proteinuria, and a decrease in creatinine clearance [18].

Detection of soluble IL-2R, soluble CD30, IL-10, and BAFF
Soluble IL-2R (R&D systems; Oxon, UK), soluble CD30 (Bender MedSystems, Vienna, Austria) and IL-10 (Mabtech, Strand, Sweden) were detected by commercial sandwich enzyme-linked immunosorbent assays (ELISAs) according to the manufacturer’s instructions. Lowest levels of detection of the ELISAs were 32 pg/ml for sIL-2R, 0.5 U/ml for sCD30, and 0.5 pg/ml for IL-10. Plasma levels of BAFF were measured by a sandwich ELISA, developed in our laboratory, using a monoclonal antibody as capturing and a biotinylated polyclonal antibody as detecting antibody. In brief, 96-wells plates (Nunc, Maxisorb) were coated overnight at 4°C with 4 mg/ml mouse anti-human BAFF (clone 36006.211; R&D systems). After washing, plates were blocked with 2% bovine serum albumin (BSA), 0.05% Tween 20 in PBS for 60 minutes. Human plasma samples and recombinant BAFF were diluted in High Performance ELISA (HPE) buffer (Sanquin, Amsterdam, The Netherlands), added to
the plate and incubated at room temperature for one hour. After washing, bound BAFF was detected by a one hour incubation with biotinylated goat anti-human BAFF (PeproTech, NJ, USA). After washing, samples were incubated with 0.125 mg/ml peroxidase conjugated streptavidin (Sanquin, Amsterdam, The Netherlands) for 30 minutes, and the colour reaction was performed with tetramethylbenzidin (TMB) (Roth, Karlsruhe, Germany). The colorimetric reaction was stopped by the addition of 100 ml/well 0.5 M 2 N H$_2$SO$_4$. Adsorption at 450/575 nm was measured with a microplate reader. Sensitivity of this ELISA to detect BAFF was 0.15 ng/ml. Plasma concentrations were determined by interpolation from a standard curve.

**Statistical analysis**

For comparison of paired data the Wilcoxon signed rank test was used, for unpaired data the Mann-Whitney U test was used. For multiple comparisons the non-parametric Kruskal-Wallis test was used, followed by Dunn’s post-test. Correlation coefficients were calculated by Spearman’s test. For calculation of relapse free survival, survival curves were calculated using Kaplan-Meier estimates for survival distribution. Differences between groups in survival were analysed with log-rank test with disease free survival as dependent variable. Endpoint for survival analysis was the occurrence of relapse. A two-sided p-value <0.05 was considered statistically significant.

**RESULTS**

**Clinical characteristics**

Fifty-two out of the 87 included patients (60%) experienced one or more relapses of disease, 26 patients (63%) in the group switched to azathioprine maintenance (n=41) and 26 patients (57%) in the group on cyclophosphamide (n=46). Clinical characteristics at diagnosis of the included patients are given in table 1. Overall actuarial relapse free survival was 72% at 24 months and 34% at 60 months after diagnosis. Baseline characteristics and overall relapse free survival did not differ between the cyclophosphamide maintenance group and the azathioprine maintenance group. Also, clinical characteristics at diagnosis did not differ between patients with (n=52) and without relapse (n=35) during follow-up. Clinical characteristics at relapse are shown in table 2.
Table 2. Clinical Characteristics at relapse

<table>
<thead>
<tr>
<th>At relapse</th>
<th>Patients experiencing relapse (n=52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-ANCA titer (IIF)#</td>
<td>1:320</td>
</tr>
<tr>
<td>CRP (mg/l)*</td>
<td>50.8 ± 53.0</td>
</tr>
<tr>
<td>Creatinine (mmol/l)#</td>
<td>118 (71-1243)</td>
</tr>
<tr>
<td>BVAS#</td>
<td>12.5 (3-24)</td>
</tr>
<tr>
<td>Organ Involvement</td>
<td></td>
</tr>
<tr>
<td>Ear, nose, throat: n (%)</td>
<td>34 (65%)</td>
</tr>
<tr>
<td>Lung: n (%)</td>
<td>5 (10%)</td>
</tr>
<tr>
<td>Kidney: n (%)</td>
<td>33 (63%)</td>
</tr>
</tbody>
</table>

* mean ± SD, # median (range)

After starting immunosuppressive therapy many patients became negative for ANCA: at 6 months, 49% was negative for ANCA by ELISA and 69% negative for ANCA by IIF (Figure 1). Subsequently, an increasing number became again positive for ANCA during follow-up (Figure 1).

Figure 1. ANCA-status during follow-up. Percentage of patients at risk for relapse who are positive for C-ANCA by IIF (≥1:40) (dark gray squares) or for PR3-ANCA by direct ELISA (≥10 U/ml) (light gray squares) during follow up, at 3, 6, 12, 18, and 24 months after diagnosis.
Soluble IL-2R

We measured levels of the T cell activation marker sIL-2R during follow-up in PR3-ANCA-associated vasculitis. Plasma sIL-2R levels in patients with PR3-ANCA-associated vasculitis at diagnosis and during follow-up were significantly higher than in healthy controls (p<0.01), except at 6 months after diagnosis (Figure 2a). At diagnosis and at relapse, plasma sIL-2R levels correlated significantly with BVAS (r=0.46, p < 0.001; r=0.40, p<0.01, respectively) (table 3). Plasma sIL-2R levels at diagnosis were significantly higher than at all time points during follow-up (p<0.05), apart from 24 months after diagnosis when levels did not significantly differ from sIL-2R levels at diagnosis (p=0.31) (Figure 2a). Levels of sIL-2R fell after starting immunosuppressive therapy, but, subsequently, rose again. Accordingly, at 12, 18, and 24 months after diagnosis sIL-2R levels were significantly higher than at 6 months after diagnosis (p=0.03; p=0.006; p=0.007, respectively).

Soluble IL-2R levels as measured at several time points during the first 24 months of follow-up did not differ between patients with a relapse within 5 years after diagnosis and those who did not relapse.

We analysed whether sIL-2R levels differed between patients positive for ANCA and patients negative for ANCA during follow-up. Only at 18 months after diagnosis, sIL-2R levels were significantly higher in patients positive for PR3-ANCA by ELISA as compared to PR3-ANCA negative patients (p=0.022) (Figure 3a). At all other time-points during follow-up, that is at 3, 6, 12, and 24 months after diagnosis, patients positive for ANCA did not have significantly higher levels of sIL-2R as compared to patients negative for ANCA.

Plasma levels of sIL-2R correlated significantly (p<0.01) with levels of sCD30 but not with CRP levels at diagnosis and at all time points during follow up.

Soluble CD30

Like sIL-2R, sCD30 is a marker of T cell activation. We evaluated sCD30 levels during follow-up in patients with PR3-ANCA-associated vasculitis. Plasma levels of sCD30 were significantly higher in patients with PR3-ANCA associated vasculitis, both at diagnosis and during follow-up, than in controls (p<0.001) (Figure 2b). Soluble CD30 could be detected in only 3 out of 31 control samples. Patients with PR3-ANCA associated vasculitis had higher sCD30 levels at diagnosis than at all time points during follow-up (p<0.05), apart from 24 months after diagnosis when this difference was not statistically significant (p=0.054). At diagnosis, sCD30 levels correlated significantly with BVAS (r=0.40, p = 0.003) (table 3). In contrast, sCD30 levels were not significantly higher at relapse than during follow-up, and did not correlate with BVAS (r=0.09, p = 0.56). sCD30 levels during follow-up did not differ
between patients with a relapse and those who did not relapse. sCD30 levels at diagnosis were, however, significantly higher in patients who later experienced a relapse (median 56.6 U/ml, range 9.5-310.7) compared to patients who did not relapse (median 32.0 U/ml, range 7.4-106.0). Consequently, patients with a sCD30 level higher than median (39.3 U/ml) had a significantly higher risk to experience relapse than patients with sCD30 levels lower than median (RR 3.3, 95% CI 1.1-4.6; p=0.01). Additionally, we analysed whether sCD30 levels were associated with ANCA status. Plasma levels of sCD30 did not differ significantly between patients positive for C-ANCA by IIF or PR3-ANCA by ELISA and patients who were ANCA negative at 3, 6, and 12 months after diagnosis (Figure 3b). Patients who were positive for PR3-ANCA at 18 or 24 months had significantly elevated sCD30 plasma levels compared to patients negative for PR3-ANCA (p<0.001; p=0.031) (Figure 3b). Patients who were positive for PR3-ANCA by ELISA at 18 months, already had significantly higher sCD30 levels at 12 months after diagnosis (p = 0.034). At 24 months after diagnosis patients positive for C-ANCA (IIF) had significantly higher sCD30 plasma levels compared to C-ANCA negative patients (p=0.025).

Levels of sCD30 during follow-up correlated significantly with sIL-2R and CRP. At diagnosis and relapse sCD30 also correlated significantly with sIL-2R but not with CRP.

**IL-10**

IL-10 is a Th2-cytokine with immunosuppressive qualities. We studied IL-10 levels longitudinally, and evaluated to what extent increased levels during follow-up might protect against disease relapse in PR3-ANCA-associated vasculitis.

At diagnosis, patients’ IL-10 levels were significantly higher than at 3 and 6 months after diagnosis (both p < 0.01). In patients with PR3-ANCA-associated vasculitis IL-10 levels were significantly lower 6 months after diagnosis than in healthy controls (p<0.05) (Figure 2c). IL-10 levels at relapse were significantly higher than at all time points during follow-up (p<0.01). Plasma IL-10 levels increased during follow-up; at 24 months after diagnosis, when immunosuppressive treatment had been stopped, IL-10 levels were significantly higher than at 3, 6, 12 and 18 months after diagnosis (p<0.05) (Figure 2c).

Patients experiencing a relapse within a period of 60 months of follow-up had significantly lower IL-10 levels at 3 months after diagnosis than patients who did not relapse. At 3 months after diagnosis IL-10 levels higher than median (61 pg/ml) were associated with a significantly reduced risk to experience relapse compared to IL-10 levels lower than median (RR 0.56, 95% CI 0.31-0.98; p=0.044).
Figure 2. Plasma levels of sIL-2R, sCD30, IL-10, and BAFF. Box plots indicating 5-95% range (error bars), 25-75% range (boxes), and median value (horizontal lines) of plasma levels of soluble IL-2R (A), soluble CD30 (B), IL-10 (C) and BAFF (D) in 87 patients with PR3-ANCA-associated vasculitis at diagnosis (d), and during follow-up at 3, 6, 12, 18, and 24 months after diagnosis for patients who had not yet experienced a relapse. In addition, levels are depicted at relapse (r) (n=52) and in 31 healthy controls (c). * p<0.05 as compared to healthy controls (Kruskal Wallis, Dunn’s post-test).
Figure 3. Plasma levels of sIL-2R, sCD30, IL-10, and BAFF in patients positive and negative for C-ANCA and PR3-ANCA at 18 and 24 months of follow-up. Box plots indicating 5-95% range (error bars), 25-75% range (boxes) and median value (horizontal lines) of plasma levels of soluble IL-2R (A), soluble CD30 (B), IL-10 (C), and BAFF (D) in patients positive for C-ANCA by IIF and PR3-ANCA by ELISA as compared to ANCA-negative patients at 18 and 24 months after diagnosis. * p<0.05 (Mann-Whitney U test).

Plasma IL-10 levels at all time points during follow-up did not differ between patients who were either positive or negative for ANCA as determined by ELISA and IIF.

BAFF
The polyclonal B cell stimulator BAFF has been associated with autoimmunity. We measured plasma levels of BAFF in PR3-ANCA-associated vasculitis and assessed the relation between BAFF and disease-activity, as well as between BAFF and ANCA levels.
Table 3. Correlations between plasma levels of immune markers and disease-activity (BVAS) at diagnosis and relapse.

<table>
<thead>
<tr>
<th></th>
<th>diagnosis (n=87)</th>
<th>relapse (n=52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sIL-2R</td>
<td>0.46***</td>
<td>0.40**</td>
</tr>
<tr>
<td>sCD30</td>
<td>0.40**</td>
<td>0.09</td>
</tr>
<tr>
<td>IL-10</td>
<td>-0.20</td>
<td>0.12</td>
</tr>
<tr>
<td>BAFF</td>
<td>-0.09</td>
<td>0.11</td>
</tr>
<tr>
<td>CRP</td>
<td>0.36**</td>
<td>0.46**</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001

Plasma levels of BAFF were significantly higher at all time points in patients than in controls (p<0.001) (Figure 2d). In patients with ANCA-associated vasculitis BAFF levels tended to be higher at diagnosis than at 12 months after diagnosis (p=0.051). BAFF levels were significantly higher at 18 months than at 12 months after diagnosis (p=0.030) (Figure 2d). BAFF levels of patients with a relapse did not differ significantly from levels in patients without a relapse. Plasma levels of BAFF did not differ between patients positive and negative for ANCA, as determined by ELISA or IIF.

DISCUSSION

Several observations suggest T cell involvement in ANCA-associated vasculitis. Activated T cells are present in lesions and T cell help is necessary to produce high affinity ANCA. Additionally, elevated plasma levels of the T cell activation markers sIL-2R and sCD30 have been reported in ANCA-associated vasculitis, and levels correlated with disease activity [6, 8, 9]. Both sIL-2R and sCD30 plasma levels differed between limited and generalized disease, as well as between active and inactive disease [6, 8, 9]. Also in this study, sIL-2R- and sCD30 levels at diagnosis correlated with disease activity. Plasma levels of sIL-2R and sCD30 were higher at diagnosis than during follow-up. Although immunosuppressive therapy thus resulted in a decrease, plasma levels remained elevated as compared to healthy controls. Both sIL-2R and sCD30 levels subsequently increased while tapering and eventually stopping immunosuppressive treatment, reflecting increased T cell activity in patients in remission that is not completely suppressed or abrogated by treatment. These findings are in line with the observation that increased percentages of activated T cells are present in patients with
AAV in remission [10]. Levels of sIL-2R and sCD30, but not of the B cell activator BAFF, were associated with positive ANCA serology during follow-up. At 18 and at 24 months after diagnosis, both patients positive for C-ANCA (IIF) and PR3-ANCA (ELISA) had higher levels of sIL-2R and sCD30 than ANCA negative patients. Therefore, ANCA-positivity seems part of a broader, possibly T cell driven, immune-activation present in patients with AAV.

Obviously, clinical interpretation of levels of immune markers can be complicated by intercurrent infections. Additionally, our study is limited by its retrospective nature. Nevertheless, the association of T cell activation with the PR3-ANCA autoantibody response suggests an initiating role for T cell activation in PR3-ANCA-associated vasculitis. However, levels of sIL-2R and sCD30 did not differ between patients with PR3-ANCA-associated vasculitis who experienced a relapse and those who did not relapse during follow-up. In contrast, Schmitt et al found that patients with WG who relapsed had significantly higher sIL-2R levels before disease exacerbation than patients who did not relapse. [6]

As current immunosuppressive regimens are insufficient to maintain long lasting remission in a major part of patients with PR3-ANCA-associated vasculitis, identification of patients at risk for relapse is important. Our data suggest that the persistent presence of elevated levels of markers for T cell activation, although related with ANCA status, does not identify patients at risk for relapse.

In contrast to sIL-2R and sCD30, IL-10 is a cytokine with immunosuppressive and anti-inflammatory properties. Regulatory T cell subsets among other cells produce IL-10 [19]. Additionally, IL-10 is associated with type 2 T helper cells (Th2) and can promote polyclonal hypergammaglobulinemia [20]. In Systemic Lupus Erythematosus (SLE), IL-10 levels reflect disease activity [21]. We found that IL-10 levels in patients with ANCA-associated vasculitis were elevated at diagnosis and subsequently decreased. At 6 months after diagnosis IL-10 levels were significantly lower as compared to healthy controls. Additionally, low levels of IL-10 3 months after diagnosis were associated with increased relapse rate during 60 months of follow-up. Recently, Ohlsson et al reported on the same association between low IL-10 levels in patients with AAV 3-months prior to relapse [11]. In addition, both in WG and MPA, a shift towards the homozygous AA genotype has been found in the IL-10 (-1082) polymorphism [22]. In vitro this genotype correlates with low IL-10 secretion by ConA-stimulated peripheral blood mononuclear cells of healthy individuals [23]. These data and our clinical findings suggest that low plasma levels of IL-10 influence disease development and exacerbation.
Whereas T cells are operative in production of ANCA, other factors, e.g. BAFF, may be involved as well. BAFF is a member of the tumor necrosis factor (TNF) ligand superfamily and is expressed by monocytes, macrophages, dendritic cells, and, possibly, activated T cells [24-26]. Since BAFF stimulates all B cells and thereby promotes their survival [27], it is thought to contribute to autoimmunity by stimulating autoreactive B cells. In patients with SLE, Rheumatoid Arthritis (RA) and Sjögren syndrome, elevated serum levels of BAFF have been found. Levels correlated with serum IgG levels and anti-double stranded DNA antibody titers among SLE patients and with rheumatoid factor (RF) titers among seropositive RA patients [12, 28]. Moreover, in Sjögren syndrome, levels of BAFF correlated with both anti-SSA and anti-SSB antibodies and RF. Recently, two groups reported, on elevated levels of BAFF in Wegener’s Granulomatosis [29, 30]. We found that levels of BAFF were elevated in patients with PR3-ANCA-associated vasculitis as compared to healthy controls. However, BAFF levels did not correlate with ANCA titers, and ANCA positive patients did not have higher BAFF levels than ANCA negative patients. Also in previous studies, no correlation was found in patients with WG between B cell activation and serum levels of ANCA, RF, IgM and IgG, the latter being markers of polyclonal B cell activation [10]. The data, therefore, suggest that elevated BAFF levels are rather a result of a stimulated immune-system than a factor related to the PR3-ANCA response or to disease activity.

In summary, in PR3-ANCA-associated vasculitis, markers of T cell activation are increased at diagnosis and correlate with disease activity. Although immunosuppressive therapy results in a decrease, T cell activity does not normalize. Upon tapering and stopping immunosuppression levels of T cell activation markers rise again, reflecting increased T cell activity. This increased T cell activity is associated with ANCA positivity, and should be viewed as a result of a T cell driven immune-response which could be at the basis of this disease.

REFERENCES


