Expression of CD 21, CD 22, and the mouse erythrocyte receptor on peripheral B lymphocytes in rheumatoid arthritis

SABINE HILDEBRANDT, IRIS VON DER HEYDT, AND PETER VON WICHERT

From the University Hospital of Internal Medicine, Marburg, Federal Republic of Germany

SUMMARY The expression of the B cell antigens, CD 21, CD 22, and the mouse erythrocyte receptor (MER), on peripheral mononuclear cells (PMC) in 61 patients with rheumatoid arthritis (RA) and in 25 patients with various other forms of rheumatic disease was studied. Patients with active RA showed significantly more peripheral B cells than control patients, whereas there was no difference between patients with RA and controls in resting B cells expressing the MER on resting and activated B cells expressing CD 21. Patients with active RA had significantly less MER positive and more CD 21 positive B cells than patients with inactive disease. The relation between disease activity and expression of MER and CD 21 was independent of drug treatment or production of classical rheumatoid factor. These data may be interpreted as a sign of B cell activation in RA. In addition, patients with seronegative RA receiving gold treatment showed significantly more MER positive cells than patients receiving different drugs, whereas patients receiving non-steroidal anti-inflammatory drugs (NSAIDs) alone had significantly more CD 21 positive cells. This may be the result of different immunomodulating effects of drugs on B cell subsets.

Key words: B cell subsets, B cell activation.

In the pathogenesis of rheumatoid arthritis (RA) B lymphocyte subpopulations are thought to be partly responsible for the disturbed immunoregulation. CD 21, CD 22, and the mouse erythrocyte receptor (MER) are B cell antigens which differentiate between B cell subpopulations that may be functionally different. CD 22 (135 kilodaltons) is expressed throughout all steps of B cell activation, excluding the plasma cell, and is used as a marker for the total B cell population in the peripheral blood. MER on human B cells is an antigen of small, resting, surface immunoglobulin D positive B cells. CD 21 (former designation B 2; 140 kilodaltons) as C3d receptor and receptor of the Epstein-Barr virus is expressed on resting as well as on early activated B cells, but not on immunoglobulin D negative cells. It seems from this concept of B cell maturation that MER positive cells in the peripheral blood should decrease during B cell activation, while CD 21 positive cells should increase. Studies on the MER showed that peripheral mononuclear cells (PMC) of patients with active disease expressed significantly less MER than those of patients with inactive RA, patients with other forms of rheumatic disease, or healthy controls. The object of this study was to investigate the expression of CD 21 in regard to disease activity and the relation between CD 21 and MER. In addition, we examined the possible biasing effects of drug treatment and production of the classical rheumatoid factor on the expression of B cell antigens.

Patients and methods

The study population consisted of 61 patients (17 male, 43 female) with definite or classical RA (criteria of the American Rheumatism Association with a mean age of 59 (SD 9-4) years and a duration of disease of 7 (5-8) years. The control group...
consisted of 25 patients (11 male, 14 female) with various forms of rheumatic disease (systemic lupus erythematosus (five), psoriatic arthritis (six), Sjögren's syndrome (two), mixed connective tissue disease (three), polymyalgia rheumatica (three) and one patient for each of the following: polyarthritis, oligoarthritis, scleroderma, Reiter's disease, ankylosing spondylitis, Raynaud's disease) with a mean age of 51 (13) years and a duration of disease of 4 (2-7) years. Disease activity of patients with RA was assessed by the following criteria: erythrocyte sedimentation rate greater than 30 mm during the second hour, C reactive protein greater than 5 mg/l, more than five joints tender or swollen, or both, and general symptoms. The disease was said to be active if two or more criteria were fulfilled, moderately active with one, inactive with none.

Four of the patients with RA were treated exclusively with NSAIDs, five with azathioprine plus steroids or methotrexate plus steroids, and 23 with gold alone or gold plus NSAIDs. All other patients received various combinations of gold, steroids, chloroquine, penicillamine, sulphasalazine, NSAIDs, azathioprine, cyclophosphamide, and methotrexate.

LYMPHOCYTE ISOLATION AND DIFFERENTIATION

Peripheral mononuclear cells (PMC) were isolated from 20 ml heparinised blood over a Ficoll gradient (Seromed, 1.077) by the method of Böyum,14 washed in RPMI 1640 (Seromed) plus 10% fetal calf serum (heat inactivated; Seromed). Adherent cells were depleted by incubation in plastic tissue culture flasks at 37°C for one hour.

For the identification of the MER PMC were rosetted with mouse erythrocytes (MRBCs) by the method of Younou et al.11 Fresh MRBCs from male C 57 black mice were washed three times in RPMI. To 50 μl of 1% MRBC solution 50 μl of a lymphocyte solution (5×10⁶ cells/ml RPMI) and 30 μl of MRBCs absorbed fetal calf serum were added. This was followed by incubation at 37°C for 15 minutes, centrifugation at 200 g for five minutes at 20°C, and incubation overnight at 4°C. The cells were carefully resuspended and 3×200 cells counted with the immunofluorescence microscope after addition of 20 μl of acridine orange-ethidium bromide.

CD 21 and CD 22 were assessed by indirect immunofluorescence. Twenty microtomes of lymphocyte solution (5×10⁶ cells/ml RPMI) were dried at room temperature on a slide, fixed in acetone, and stored at −20°C. The slides were thawed by immersion in a 0-9% NaCl bath for 10 minutes and then covered with 100 μl of the first antibody for 20 minutes (monoclonal mouse antihuman antibody recognising CD 22, DAKO, M 738, 1:10 in 0-9% NaCl; or CD 21, Becton Dickinson, 7620, 1:10 in 0-9% NaCl). After an elution phase of 10 minutes in 0-9% NaCl the slides were covered with 100 μl of the second antibody for another 20 minutes (rabbit antimouse immunoglobulin, conjugated with fluorescein, DAKO, F 261, 1:50 in 0-9% NaCl). A second elution stage in 0-9% NaCl for 10 minutes followed and 200 cells were counted under the fluorescence microscope. As a control of the system we determined CD 21 and CD 22 in the same healthy donor on different days. The variability was within acceptable limits.

Enrichment of MER positive cells of a healthy person was carried out by the method previously described.10 12 PMC were rosetted with sheep red blood cells and isolated over a Ficoll gradient. The non-T cell fraction (20% B cells) was again rosetted with MRBCs and with a wide mouthed pipette carefully layered on a Ficoll gradient. After centrifugation rosettes could be found in the pellet but not in the interphase. Determination of B cell content showed B cell enrichment from 20% in the interphase to 50% in the pellet in two separate experiments.

STATISTICAL METHODS

All comparisons given were carried out with the Mann-Whitney U test. Results were considered significant if p was less than 0-05. Correlations were calculated with the Pearson correlation coefficient.

Results

RELATION BETWEEN MER AND CD 21

Table 1 shows the results of the study on CD 21 expression on enriched B cells (i.e., CD 22 positive cells of the non-T fraction) in the fraction enriched for MER positive cells and in the fraction depleted of MER positive cells (non-MER). Non-T and non-MER fractions had the same amount of CD 21/CD 22 cells, while enrichment for MER positive

<table>
<thead>
<tr>
<th>Fraction</th>
<th>CD 22+ (%)</th>
<th>CD 21+ (%)</th>
<th>CD 21/CD 22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-T cell</td>
<td>22</td>
<td>9-5</td>
<td>43</td>
</tr>
<tr>
<td>MER positive</td>
<td>53</td>
<td>4-5</td>
<td>8-5</td>
</tr>
<tr>
<td>Non-MER</td>
<td>16.5</td>
<td>7-5</td>
<td>45</td>
</tr>
</tbody>
</table>
cells was not associated with an enrichment of CD 21 positive cells. On the contrary, the ratio of CD 21/CD 22 positive cells decreased to 20% of the initial amount. Also, there was no correlation (r=0.0) between CD 21 and MER expression in any of the patients studied.

**Disease Activity**

Patients with RA had significantly more B cells (CD 22 positive cells) in the peripheral blood than control patients (p=0.039; RA:median=10%, n=57; control:median=8%, n=25). There was no difference for MER and CD 21. Because of these different B cell counts all comparisons were carried out with the ratio of MER or CD 21 count and the total B cell count (MER/CD 22, CD 21/CD 22).

Patients with inactive or moderately active disease had significantly more B cells than patients with active disease or control patients (moderately active RA:median=10.2%; inactive RA:median=10.5%; active RA:median=8.5%; control group:median =8%; Fig. 1).

Patients with inactive and moderately active RA showed significantly more MER/CD 22 than patients with active RA. In contrast with previous studies, there was no significant difference between RA and control (control:median=32%; moderately active RA:median=44%; inactive RA:median=43%; active RA:median=23%; Fig. 2). There was a significant difference within the RA group for the CD 21/CD 22 subpopulation, but not in comparison with the control. Patients with active RA showed significantly more CD 21/CD 22 cells than patients with inactive disease (active RA: median=77%; inactive RA:median=59%; Fig. 3).

**Drug Treatment**

There were three different treatment schemes with a sufficient number of patients for statistical analysis: patients receiving azathioprine plus steroids or methotrexate plus steroids (n=5), patients receiving gold alone or gold plus NSAIDs (n=23), and patients receiving NSAIDs alone (n=4). These groups showed no difference in the expression of CD 22 or the MER and were equally represented in all groups of disease activity. Patients receiving NSAIDs had significantly more CD 21/CD 22 cells in the peripheral blood (median=83%) than patients receiving gold or gold plus NSAIDs.

Fig. 2 Ratio of mouse erythrocyte receptor (MER) positive cells to CD 22 positive cells (in %) in patients with active, moderately active, and inactive RA and in the patient controls.

Fig. 3 Ratio of CD 21 positive cells to CD 22 positive cells (in %) in patients with active, moderately active, and inactive RA and in the patient controls.
(median=62%) or patients receiving methotrexate or azathioprine plus steroids (median=59%; Fig. 4).

CLASSICAL RHEUMATOID FACTOR
Patients with seronegative RA receiving gold alone or gold and NSAIDs showed significantly more MER/CD 22 cells in the peripheral blood (median=46%) than seropositive patients (median=33%) or control patients (median=32%; Fig. 5). There was no difference for patients not receiving gold. Also there was no significant relation between production of classical IgM anti-IgG rheumatoid factor and CD 21, CD 22 or between duration of disease or sex of patients and all investigated antigens.

Discussion
We found that an enrichment of cells expressing the MER does not lead to a concomitant enrichment of cells expressing CD 21. Similarly, there was no correlation between these subpopulations in the patients studied. This result implies that only a very small B cell subpopulation expresses the MER and CD 21 at the same time. These MER and CD 21 expressing cells must be responsible for the Epstein-Barr virus driven IgM anti-IgG autoantibody production found mainly in MER positive and not in MER negative cells, as a sequential expression of these antigens alone does not explain this phenomenon. Nevertheless, there seem to be larger B cell subpopulations that carry only one of each antigen, either CD 21 or MER.

In accordance with the hypothesis of a decrease in resting B cells and increase in early activated B cells in B cell activation we found in patients with active RA significantly less MER positive cells and more CD 21 positive cells than in patients with inactive RA. There was no difference in comparison with controls patients with various other forms of rheumatic disease. We did not have a group of healthy controls in this study as previous studies on the subject had shown no difference between healthy and patient controls. It could be argued that the detected differences in the expression of B cell...
Antigens in the peripheral blood may simply reflect differences in the recirculation of cells within various tissue compartments in rheumatoid disease rather than indicate B cell activation. This argument fails to explain the cause of the differential recirculation, whereas the above mentioned hypothesis on B cell activation does not exclude recirculation between compartments and does propose an explanation of the differential expression of antigens.

Anderson et al. have emphasized the importance of a CD 21/CD 20 B cell subpopulation in diseases with B cell activation. Here the CD 20 antigen (35 kilodaltons), which has a similar distribution on B cells to that of CD 22, was used as a marker for the total B cell population. They found in patients with the acquired immunodeficiency syndrome (AIDS) and the AIDS related complex, diseases with a high level of B cell activation, significantly less CD 21/CD 20 positive cells in lymph nodes than in healthy controls. Similarly, Wilson et al. showed that patients with systemic lupus erythematosus had a significantly lower expression of CD 21 in the peripheral blood than healthy controls, while the decrease in expression of CD 21 was not significant in patients with RA. The discrepancy with our own results may have various causes. In the studies cited neither disease activity nor drug treatment were taken into account. On the other hand, CD 21 is expressed only on a fraction of all activated B cells and is lost during further B cell activation. Thus we propose a model of B cell activation in which our results in RA reflect a moderate level of B cell activation, while the observations on AIDS, AIDS related complex, and systemic lupus erythematosus imply a high level of B cell activation (Fig. 6).

To exclude the possibility that the relation between B cell subsets and disease activity may be explained by biasing factors we studied the relation between B cell subpopulations and drug treatment or the presence of classical rheumatoid factor. There

---

**Fig. 6** A model of B cell antigen expression in B cell activation. ▲ = CD 22, ■ = CD 21, △ = MER.
was no relation between drug treatment and total B cell population, while the CD 21 positive cell fraction was significantly greater in patients with RA receiving NSAIDs than in patients receiving different drugs. Surprisingly we found that patients with seronegative RA had significantly more MER positive cells in the peripheral blood than patients with seropositive RA or control patients, but this only applied to patients receiving gold treatment. Nevertheless, these relations could not explain the connection between disease activity and B cell subsets as drug treatment and production of rheumatoid factor were equally distributed through all groups of disease activity.

Olsen and Jasin investigated in a retrospective study the pokeweed mitogen induced IgM and IgG rheumatoid factor production in PMC of patients with RA receiving gold treatment. Patients with definite clinical improvement showed a decrease in IgM and IgG rheumatoid factor synthesis. The authors explained this by a possible indirect B cell modulation, for instance by macrophage interaction, which leads to a decrease of activated circulating B cells. Thus the high number of MER positive cells in seronegative patients receiving gold treatment may be a sign of a good response to gold, so that previously seropositive patients have become seronegative. This explanation cannot account for seronegative patients with clinically active RA. Possibly the change in the B cell compartment begins before clinical improvement is evident, especially as Olsen and Jasin found a positive correlation between response to gold and duration of drug treatment. On the other hand, our result may be a sign of different immunoregulation in seropositive and seronegative RA.

Patients with RA receiving NSAIDs alone had more CD 21 positive cells in the peripheral blood than patients receiving a different drug. NSAIDs like indomethacin can neutralise in vitro the effects of prostaglandins like PGE₂. PGE₂ suppresses the B cell DNA production and proliferation induced by *Staphylococcus aureus* Cowan I but not pokeweed mitogen, and suppresses the production of immunoglobulin secreting cells. *Staphylococcus aureus* Cowan I induces mainly resting B cells, whereas pokeweed mitogen induces preactivated B cells. Provided that these in vitro effects apply in vivo, our results may imply that NSAIDs lead to a neutralisation of suppression by PGE₂ and thereby to an increase of activated B cells. NSAIDs may thus effect an immunomodulation of the B cell compartment, but not by an increase of resting B cells. The mechanism seems to be more complex. The study of B cell subpopulations allows insights into the state of B cell activation in patients with RA, possibly into the pathogenesis of the disease and into the effects of drug treatment in vivo.

References

19. Lorber A, Simon T, Leeb J, Peter A, Wilcox S. Chrysotherapy:

