Uptake and degradation of soluble aggregates of IgG by monocytes of patients with rheumatoid arthritis: relation to disease activity

A H M Heurkens, M L Westedt, F C Breedveld, E Jonges, A Cats, Th Stijnen, M R Daha

Abstract
Monocytes from patients with rheumatoid arthritis (RA) and rheumatoid vasculitis have a diminished ability to degrade soluble complexes of aggregated IgG in the absence (mediated by Fc receptors) as well as in the presence of complement (C) (mediated by (Fc+C) receptors). To investigate whether a relation exists between the receptor mediated degradation of aggregated IgG by adherent monocytes and disease activity a longitudinal study was performed in 79 patients with RA and rheumatoid vasculitis over a period of 16 months. Adherent monocytes were incubated in vitro with 125I labelled IgG aggregates of restricted size in the absence or presence of fresh serum and the percentage of catabolised IgG aggregates was measured. Cross sectionally the degradation of aggregated IgG by monocytes, mediated by Fc and (Fc+C) receptors, correlated significantly with disease activity as scored by the Ritchie articular index, the presence of extra-articular features, and circulating immune complexes. A high number of Fc receptors on monocytes correlated with diminished degradation, whereas high numbers of complement receptors 1 and 3 correlated with enhanced degradation of aggregated IgG mediated by both Fc and (Fc+C) receptors.

The degradation of aggregated IgG by monocytes did not correlate with disease activity in individual patients followed up longitudinally. When patient groups were formed according to the results of longitudinal studies, however, degradation of aggregated IgG mediated by Fc and (Fc+C) receptors was significantly decreased in patients with rheumatoid vasculitis and in patients with active RA in comparison with patients with inactive RA and healthy controls. Patients with active RA and rheumatoid vasculitis also expressed significantly more Fc receptors and less complement receptors on the monocytes than patients with inactive RA. Drug treatment did not correlate with receptor expression or the degradation of aggregated IgG by monocytes either in cross sectional or longitudinal studies.

It is concluded that in RA disease activity is related to receptor expression and the degradation of soluble immune aggregates by monocytes.

Circulating immune complexes are considered to play a part in the pathogenesis of several disease manifestations of rheumatoid arthritis (RA), including rheumatoid vasculitis.1-3 The serum concentration of immune complexes is the result of either their increased production or diminished degradation, or both. The cells responsible for the clearance of immune complexes belong to the mononuclear phagocyte system,4 of which monocytes are the immediate precursors.5 The function of the mononuclear phagocyte system is decreased in several connective tissue diseases, including systemic lupus erythematosus6 7 and RA.8 9 Recently, it was shown that patients with RA have significantly slower elimination rates of 125I labelled aggregated IgG from their circulation than normal controls.10 In a previous in vitro study Daha et al found that adherent monocytes from patients with RA have a diminished ability to degrade soluble IgG aggregates than normal controls.11 In this study both Fc receptor and complement receptor dysfunction were considered responsible for this decreased degradation of immune complexes.

To explore the relation between the degradation of immune complexes by adherent monocytes of patients with RA and clinical and laboratory indices of disease activity we performed a longitudinal study in various groups of patients with RA over a period of 16 months.

Patients and methods

PATIENTS
Seventy nine of an initial 88 seropositive patients with definite or classical RA were followed up for 16 months at four monthly intervals. Of the nine patients who were lost to follow up, five refused further evaluation and four died during the course of the study. The mean age of the 79 patients (44 women, 35 men) was 55 (SD 14) years (range 22–81) and the mean disease duration was 8 (6) years (range 0–26). These data were the same for the complete group of 88 patients.

The activity of joint disease was assessed by one observer (MLW) using Ritchie's articular index: a score of 16 or more was considered indicative of active RA. Twenty patients had active RA during the whole period of the study. Rheumatoid vasculitis was diagnosed in 16 patients because of the presence of one or more of the following features: deep ulcers, nailfold lesions, digital gangrene, purpuric eruptions, or neuropathy. In 14 of these 16 patients rheumatoid vasculitis was proved histologically by biopsy of skin or muscle. The remaining two patients had nailfold thrombi and several extra-articular features. Patients were considered to have extra-articular features when nodules,
pleuritis, pericarditis, episcleritis, neuropathy, or Felty's syndrome were present. Thirty of 79 patients evaluated had extra-articular features: 11 of 16 (69%) with rheumatoid vasculitis, six of 20 (30%) with active RA, and 13 of 43 (30%) with inactive RA.

At the beginning of the study disease modifying drugs (antimalarial drugs, gold, D-penicillamine, azathioprine, cyclophosphamide) were used by four out of 16 (25%) patients with rheumatoid vasculitis, 11 out of 20 (55%) patients with active RA, and 23 out of 43 (53%) patients with inactive RA. Prednisone was used by five patients with rheumatoid vasculitis, two with active RA, and three with inactive RA. All patients were treated with non-steroidal anti-inflammatory drugs. A control group comprised 20 normal volunteers (10 women, 10 men) with a mean age of 36 (5) years (range 21–54).

MONOCYTES
Isolation of monocytes from peripheral blood of the patients was carried out as described previously. Briefly, peripheral blood was subjected to Ficoll-Isopaque density gradient centrifugation at 4°C. The interface layer was washed twice with RPMI 1640 medium containing 0.5% bovine serum albumin (BSA) and finally, resuspended in the same medium to a concentration of 10^6 cells/ml. Volumes containing a known number of cells per ml were allowed to adhere to lightly silicised glass tubes for two hours at 37°C. The percentage of adherent cells was calculated by counting the number of non-adherent cells.

IMMUNOGLOBULIN AGGREGATES
Human IgG was isolated, as described previously, from the pooled serum samples of 10 healthy subjects by anion exchange chromatography on DE-52 cellulose (Whatman, Kent, Great Britain) followed by carboxymethyl CM-C50 Sephadex and gel filtration on Sephacryl S300 (Pharmacia Fine Chemicals, Uppsala, Sweden). The IgG pool was concentrated to 20 mg/ml in 0.01 M borate buffered saline, and 1 ml of the IgG preparation was radiolabelled with 125I to a specific activity of 7.5 GBq/g IgG by the lactoperoxidase method.

After aggregation for 20 minutes at 63°C stable and soluble 125I labelled IgG aggregates of various sizes were isolated by gel filtration on a Sepharose-4B column. Fractions containing polymeric IgG were subjected to sucrose density ultracentrifugation, and the molecular weight was calculated as described before. No correlation was found between age and the uptake and degradation of 125I labelled IgG aggregates by adherent monocytes within a group of 20 healthy donors (ages ranging from 20 to 55 years).

Fc RECEPTOR MEDIATED UPTAKE AND DEGRADATION OF 125I LABELLED IgG AGGREGATES
The uptake and degradation of 125I labelled IgG aggregates by adherent peripheral blood mono-
cytes were measured as described previously. Briefly, tubes containing the adherent cells were placed in ice, and then 100 μl 125I labelled IgG aggregates (10 fmol) in RPMI 1640/0.5% BSA were added at 0°C to the tubes or to the control tube without cells. The reaction mixtures were then transferred to a 37°C incubator and incubated with continuous agitation for 16 hours. The supernatants were collected after centrifugation of the tubes for 10 minutes at 1500 g. To determine the amount of degradation the supernatants were added to 1 ml 20% trichloroacetic acid and the radioactivity in the pellet and supernatant measured after centrifugation for 10 minutes at 1500 g. From the radioactivity measured in the supernatant divided by total radioactivity (supernatant+precipitate) the percentage of degradation was calculated.

DETERMINATION OF THE NUMBER OF Fc RECEPTORS ON ADHERENT MONOCYTES
The number of binding sites for 125I labelled IgG aggregates was used as a measure for the amount of functional Fc receptors on monocytes. Briefly, adherent monocytes (10^6 cells) in lightly silicised glass tubes were incubated with 125I labelled IgG aggregates in a final volume of 50 μl RPMI/BSA for 16 hours at 4°C. After incubation 200 μl of ice cold RPMI/BSA were added and the tubes were centrifuged for 10 minutes at 3000 rpm at 4°C. Supernatants were discarded and the washing procedure repeated three more times. The radioactivity associated with the cells was calculated using tubes without cells and incubated with 125I labelled IgG aggregates as controls. Assays were performed in triplicate.

DETERMINATION OF THE NUMBERS OF COMPLEMENT RECEPTORS 1 AND 3 ON ADHERENT MONOCYTES
125I labelled Fab' antibodies against complement receptors 1 and 3 were used to determine the numbers of these receptors on adherent monocytes. It was assumed that one Fab' molecule binds to one molecule of complement receptor 1 or 3. As controls Fab' fragments of a non-relevant monoclonal antibody were included. One million monocytes were washed and interacted with an excess of 50 ng of 125I labelled Fab' during two hours at 4°C in RPMI/BSA. After washing three times with phosphate buffered saline/BSA the amount of bound radioactivity was determined, and from this the numbers of complement receptors 1 and 3 were calculated.
DETECTION OF IMMUNE COMPLEX CONCENTRATIONS IN SERUM SAMPLES

Immune complex concentrations in serum samples were detected by a Clq binding assay.13

STATISTICAL METHODS

Spearman’s rank correlation coefficient was used to detect associations between uptake and degradation of aggregated IgG by adherent monocytes and clinical or laboratory indices of disease activity at each of the five visits of the patients to the outpatient clinic.

The patients were divided into three groups: active RA, inactive RA, and rheumatoid vasculitis. The differences in the numbers of Fc receptors and complement receptors 1 and 3 on monocytes and the degradation of aggregated IgG by monocytes were assessed by Student’s two sample t test. Differences in the number of receptors and the degradation of aggregated IgG between normal controls and patient groups were assessed by the Wilcoxon’s two sample test.

### Table 1 Correlation between degradation of aggregated IgG via Fc and (Fc+C) receptors by adherent monocytes and Ritchie's articular index (RI), extra-articular features (EAF), and immune complexes (IC)

<table>
<thead>
<tr>
<th>Degradation of aggregated IgG by monocytes via receptors</th>
<th>Time of assessment after entry (months)</th>
<th>Correlation coefficient</th>
<th>RI</th>
<th>EAF</th>
<th>IC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fc</td>
<td>0</td>
<td>-0.69**</td>
<td>-0.32*</td>
<td>-0.45**</td>
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</tr>
<tr>
<td>(Fc+C)</td>
<td>4</td>
<td>-0.55**</td>
<td>-0.19</td>
<td>-0.30</td>
<td></td>
</tr>
<tr>
<td>Fc</td>
<td>8</td>
<td>-0.46**</td>
<td>-0.26</td>
<td>-0.14</td>
<td></td>
</tr>
<tr>
<td>(Fc+C)</td>
<td>8</td>
<td>-0.49**</td>
<td>-0.26</td>
<td>-0.30</td>
<td></td>
</tr>
<tr>
<td>Fc</td>
<td>12</td>
<td>-0.49**</td>
<td>-0.29</td>
<td>-0.34</td>
<td></td>
</tr>
<tr>
<td>(Fc+C)</td>
<td>12</td>
<td>-0.51**</td>
<td>-0.26</td>
<td>-0.38</td>
<td></td>
</tr>
<tr>
<td>Fc</td>
<td>16</td>
<td>-0.57**</td>
<td>-0.34</td>
<td>-0.42**</td>
<td></td>
</tr>
<tr>
<td>(Fc+C)</td>
<td>16</td>
<td>-0.53**</td>
<td>-0.28</td>
<td>-0.47**</td>
<td></td>
</tr>
<tr>
<td>Fc</td>
<td>16</td>
<td>-0.47**</td>
<td>-0.11</td>
<td>-0.28</td>
<td></td>
</tr>
<tr>
<td>(Fc+C)</td>
<td>16</td>
<td>-0.45**</td>
<td>-0.25</td>
<td>-0.45**</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.01; **p<0.001.

Immune complexes were measured by a Clq binding assay.11

### Table 2 Correlation between degradation of aggregated IgG via Fc and (Fc+C) receptors by adherent monocytes and the number of Fc receptors (FcR) and complement (C) receptors 1 and 3 (CR1, CR3) on monocytes

<table>
<thead>
<tr>
<th>Degradation of aggregated IgG by monocytes via receptors</th>
<th>Time of assessment after entry (months)</th>
<th>Correlation coefficient</th>
<th>FcR</th>
<th>CR1</th>
<th>CR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fc</td>
<td>0</td>
<td>-0.44**</td>
<td>-0.52**</td>
<td>-0.42**</td>
<td></td>
</tr>
<tr>
<td>(Fc+C)</td>
<td>4</td>
<td>-0.64**</td>
<td>-0.56**</td>
<td>-0.43**</td>
<td></td>
</tr>
<tr>
<td>Fc</td>
<td>8</td>
<td>-0.40*</td>
<td>-0.29</td>
<td>-0.35</td>
<td></td>
</tr>
<tr>
<td>(Fc+C)</td>
<td>8</td>
<td>-0.47**</td>
<td>-0.37**</td>
<td>-0.45**</td>
<td></td>
</tr>
<tr>
<td>Fc</td>
<td>12</td>
<td>-0.38*</td>
<td>-0.34</td>
<td>-0.27</td>
<td></td>
</tr>
<tr>
<td>(Fc+C)</td>
<td>12</td>
<td>-0.50*</td>
<td>-0.47**</td>
<td>-0.55**</td>
<td></td>
</tr>
<tr>
<td>Fc</td>
<td>16</td>
<td>-0.50*</td>
<td>-0.48**</td>
<td>-0.37</td>
<td></td>
</tr>
<tr>
<td>(Fc+C)</td>
<td>16</td>
<td>-0.53*</td>
<td>-0.51**</td>
<td>-0.45**</td>
<td></td>
</tr>
<tr>
<td>Fc</td>
<td>16</td>
<td>-0.41**</td>
<td>-0.41**</td>
<td>-0.23</td>
<td></td>
</tr>
<tr>
<td>(Fc+C)</td>
<td>16</td>
<td>-0.41**</td>
<td>-0.41**</td>
<td>-0.26</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.01; **p<0.001.

### Table 3 Correlation of the number of Fc receptors (FcR) and complement receptors 1 and 3 (CR1, CR3) on monocytes and the degradation of aggregated IgG via Fc and (Fc+C) receptors by adherent monocytes during the course of the study in the individual patients

<table>
<thead>
<tr>
<th>Degradation of aggregated IgG by monocytes via receptors</th>
<th>Mean (SEM) of Spearman’s rank correlation coefficients for:</th>
<th>FcR</th>
<th>CR1</th>
<th>CR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fc</td>
<td>-0.198 (0.070)*</td>
<td>0.150 (0.069)</td>
<td>0.095 (0.072)</td>
<td></td>
</tr>
<tr>
<td>(Fc+C)</td>
<td>-0.222 (0.071)*</td>
<td>0.087 (0.070)</td>
<td>0.160 (0.071)</td>
<td></td>
</tr>
</tbody>
</table>

Significance of deviation of mean from zero assessed by Student’s one sample t test.

*p<0.01.

A possible relation within patients between the degradation of aggregated IgG by monocytes and clinical and laboratory indices of disease activity were assessed by computing firstly, Spearman’s rank correlation coefficient for each patient separately. Next, Student’s one sample t test was used to test the hypothesis that the (weighted) mean of the correlation coefficient was equal to zero.16

### Results

Cross sectional comparison of patients’ data obtained at all points of assessment showed that active joint inflammation and high concentrations of immune complexes correlated significantly with a diminished degradation of aggregated IgG via Fc and (Fc+C) receptors by adherent monocytes (table 1). The presence of extra-articular features correlated only weakly with the degradation of aggregated IgG via Fc and (Fc+C) receptors by adherent monocytes (table 1). No correlation was found between the use of disease modifying drugs or prednisone and the degradation of aggregated IgG via Fc and (Fc+C) receptors by adherent monocytes.

Cross sectional studies in all patients showed that high numbers of Fc receptors on monocytes correlated with a diminished degradation of aggregated IgG via Fc and (Fc+C) receptors by these monocytes (table 2). High numbers of complement receptors 1 and 3 on monocytes correlated with an enhanced degradation of aggregated IgG via Fc and (Fc+C) receptors (table 2).

To investigate a possible relation between clinical and laboratory data within individual patients in a longitudinal study correlation coefficients were computed for each patient separately. Each analysis was based on five observations for each patient. The fluctuations in degradation of aggregated IgG via Fc and (Fc+C) receptors by monocytes correlated only weakly with Ritchie’s articular index (r=0.12; p=0.07 and r=0.16; p=0.02, respectively), the presence of extra-articular features (r=0.41; p=0.07 and r=0.37; p=0.03, respectively) and the concentration of circulating immune complexes (r=0.05; p=0.05 and r=0.16; respectively). A low, but significant, negative correlation was found between fluctuations in the number of Fc receptors on monocytes and the degradation of aggregated IgG via Fc and (Fc+C) receptors by these monocytes (table 3). No correlation was found between the fluctuation in the number of complement receptors 1 and 3 on monocytes and the degradation of aggregated IgG by these monocytes. Finally, no correlation could be established between the use of disease modifying drugs or prednisone and the degradation of aggregated IgG by monocytes in the longitudinal study.

To investigate further the relation between disease activity, the number of Fc receptors and complement receptors 1 and 3, and the degradation of aggregated IgG via these receptors patients were subdivided into three groups: rheumatoid vasculitis, active RA, and inactive RA. Patients were considered to have active disease when Ritchie’s articular index was...
For each (SD) are monocytes (fig 2). The binding of aggregated IgG reflects the number of Fc receptors on monocytes which are significantly increased in patients with rheumatoid vasculitis or active RA compared with patients with inactive RA (fig 1). Patients with rheumatoid vasculitis and active RA had significantly fewer complement receptors 1 and 3 on their monocytes than patients with inactive RA (fig 2). No significant differences in the number of Fc receptors and complement receptors 1 and 3 were found between patients with active RA and those with rheumatoid vasculitis (fig 2).

The degradation of aggregated IgG via Fc and (Fc+C) receptors by monocytes was significantly decreased in patients with active RA and rheumatoid vasculitis (figs 1 and 3) compared with patients with inactive RA and normal controls.

Discussion
In a previous cross sectional study it was shown that the degradation of aggregated IgG by monocytes via Fc and (Fc+C) receptors was significantly decreased in patients with RA compared with normal controls. The number of Fc receptors on monocytes of patients with RA was reported to be increased and the number of complement receptors 1 decreased. The results of our study confirm these findings and, additionally, show that complement receptors 3 are also decreased on monocytes of patients with RA. Comparison of data for individual patients followed up longitudinally showed no significant relation between disease activity and the degradation of aggregated IgG, however. This discrepancy between the results for the cross sectional and longitudinal studies might be due to the short duration of the study. The fluctuations found in Ritchie's articular index, extra-articular features, serum immune complex titres, and degradation of aggregated IgG within the individual patient during the time of the study were small.

An increased number of Fc receptors on monocytes from patients with RA has previously been reported by other authors. In our study we found that monocytes from patients with RA bound significantly more IgG at 4°C, which reflects the number of Fc receptors. The increased binding of 125I-labelled IgG aggregates to monocytes of patients with RA compared with that to monocytes might also be due to the presence of cell bound...
rheumatoid factor. Measurement of Fc receptor expression, however, using F(ab)’2 fragments of a monoclonal antibody against the Fc receptors on monocytes, also showed a significantly higher expression of Fc receptors on monocytes from a selected number of patients with RA than on monocytes from normal controls (data not shown). The diminished ability of monocytes from patients with active RA or rheumatoid vasculitis to degrade aggregated IgG via Fc receptors is surprising, therefore, and suggests a defect in the intracellular processing of aggregated IgG. This is supported by previous observations that binding is the rate determining step in the degradation of immune complexes by macrophages. Fc receptor mediated degradation of aggregated IgG by monocytes was impaired in all patients when compared with normal controls. Processing of aggregated IgG by monocytes in the presence of complement was even more impaired in patients with active RA and rheumatoid vasculitis than in patients with inactive RA. Complement receptors 1 and 3 both contribute to the complement mediated binding and degradation of soluble immune complexes by monocytes. Whether the impaired (Fc+C) receptor mediated degradation of aggregated IgG by monocytes in RA has to be attributed to decreased complement receptor expression or to disturbed intracellular processing of aggregated IgG is unknown at present.

The results of this in vivo study are in line with recent in vivo studies on mononuclear phagocyte function in patients with RA. Lobbato et al. used radiolabelling to show decreased clearance of soluble immune aggregates from the circulation. Similar results were previously reported with IgG coated erythrocytes as a probe. The in vitro degradation of aggregated IgG by monocytes may be regarded as a model for the function of the mononuclear phagocyte system in vivo. Impaired degradation of aggregated IgG reflects impaired mononuclear phagocyte function, which can result in high concentrations of circulating immune complexes. There is considerable evidence that immune complexes are important in the pathogenesis of rheumatoid vasculitis. Experimental studies showed that similar vasculitic lesions were reproduced by the intravenous administration of immune complexes. Clinical studies showed that patients with rheumatoid vasculitis have titres of circulating immune complexes higher than those of patients with uncomplicated RA.22 23 and a positive correlation has been reported between the titre of immune complexes and the occurrence of clinical manifestations of rheumatoid vasculitis.24

In our study we found no significant differences in the receptor expression and the processing of aggregated IgG by monocytes between patients with active RA and those with rheumatoid vasculitis. Apparently there is no direct relation between impaired processing of aggregated IgG by monocytes and the occurrence of vasculitis. Corticosteroids have been reported to inhibit the expression of Fc receptors25 and probably also the expression of complement receptors on monocytes.26 In this study no relation was found between the use of prednisone or disease modifying drugs and the expression of receptors on monocytes nor with the degradation of aggregated IgG.

Fc receptor and complement receptor expression of macrophages can be augmented by lymphokine stimulation. In this study we noted an increase in Fc receptor expression but a decrease in expression of complement receptors 1 and 3 on monocytes of patients with active RA and rheumatoid vasculitis. It is, at present, difficult to find a suitable explanation for this observation.

In summary, monocytes of patients with RA express more Fc receptors, less complement receptors 1 and 3, and have a diminished ability to degrade aggregated IgG than do monocytes of healthy donors. The results of this suggest that these changes are related to disease activity.
Degradation of aggregated IgG by monocytes


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