Subnormal sensitivity of neutrophils to complement split product C5a in rheumatoid arthritis: relation to complement catabolism and disease extent

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SUMMARY The capacity of circulating neutrophils for activation by complement was studied in outpatients with classical or definite rheumatoid arthritis during treatment with dextropropoxyphene only. Analysis of dose-response in the Boyden chamber assay of chemotaxis showed that sensitivity to the potent, complement derived anaphylatoxin, C5a, was markedly decreased, especially in those patients with few joints involved. In contrast, peak response to C5a was within the normal range. Increased complement 3c split products in plasma of the patients suggested involvement of complement cascade reactions. Subnormal sensitivity of neutrophils to phlogistic mediators released by complement may tend to limit their recruitment and potentially tissue destroying secretion locally in rheumatoid arthritis.

Key words: chemotaxis, complement 3, complement 5, inflammation.

Neutrophils are abundant locally in rheumatoid arthritis, both in the synovial fluid\(^1\) and at the interphase of cartilage with pannus.\(^2\)\(^-\)\(^3\) Lysosomal enzymes and other mediators of inflammation released by neutrophils after activation with immune complexes or complement split products may account, at least in part, for rheumatoid inflammation and tissue destruction.\(^4\)

Cellular factors may be involved in limiting neutrophil recruitment from peripheral blood. Recently demonstrated, specific receptors\(^5\) for the major, complement derived chemotactic factor, C5a\(^6\) may be selectively desensitised at least during in-vitro conditions.\(^7\)\(^-\)\(^8\) In experimental animals complement appears to be essential for accumulation of neutrophils in diverse acute inflammatory reactions.\(^9\)\(^-\)\(^11\) Raised circulating levels of C3 split products in rheumatoid arthritis and a positive concentration gradient of the split product between synovial fluid and plasma\(^12\) suggest involvement of complement with release of phagocyte activating split products.

The objective of the present study in rheumatoid arthritis was to assess the capacity of circulating neutrophils for activation of chemotaxis by the major, complement derived chemotactic factor, C5a. Plasma c split product of C3 (C3c) was quantified as a parameter of complement catabolism. C3c is released by activation of both the classical and alternative pathways of complement.\(^13\)

Materials and methods

Patient population

Fifteen consecutive outpatients with classical or definite rheumatoid arthritis according to the criteria of the American Rheumatism Association were studied. Exclusion criteria were: (1) treatment with gold compounds, penicillamine, or steroids within six preceding weeks; and (2) complicating infections or conditions, such as atopy, affecting the immune system. Aspirin and non-steroidal anti-inflammatory drugs were withdrawn for a median of 10 days, range 7–18 days, and during this washout period pain was controlled with dextropropoxyphene only. All patients were interviewed and examined by the same physician before drawing blood samples for investigation on the same day. The patients, 13 females and two males, had a
Complement and neutrophil function in rheumatoid arthritis

median age of 63 years, range 19–73 years. Clinical
data and routine laboratory values of the patients
are given in Table 1.

Fifteen healthy volunteers including hospital or
laboratory personnel on no medication acted as
controls. They were 11 females, and four males,
aged 23–67 years, median 46 years. All patients and
controls. They were 11 females and four males,
the protocol had been accepted by the region’s
scientific-ethical committee for studies in humans.

**REAGENTS**

Casein (alkaliloschich, Merck, Darmstadt, FRG), ε-
aminocaproic acid (Meco Benzon, Copenhagen,
Denmark), ethylene diaminetetra-acetate (EDTA)
(Merck), 3 μm pore size Sartorius filters with
a thickness of 200 μm (Sartorius, Göttingen,
FRG), purified human albumin (Behringwerke,
Marburg, FRG), Lymphoprep (Nyggaard, Oslo,
Norway), methylcellulose (Apodan, Copenhagen,
Denmark), Sephadex G75 gel (Pharmacia Fine
Chemicals, Uppsala, Sweden), standard human
serum (Behringwerke), and zymosan (Sigma, St
Louis, USA) were employed. Rabbit antibodies
to human C3c, C3d, and C5 and rabbit immuno-
globulins from non-immunised rabbits were from
DAKO immunoglobulins, Copenhagen, Denmark.
Gey's solution was prepared according to the
method of Wilkinson.14

**CHEMOTACTANTS**

Complement anaphylatoxin C5a was prepared from
complement activated plasma according to proved
principles13,16 by: (1) preincubation of heparin
plasma (2 IU/ml) overnight with 1 M ε-aminocaproic
acid at 4°C; (2) activation with zymosan (20 g/l) for
75 minutes at 37°C; and (3) chromatography on
Sephadex G75.17 Chemotactic fractions eluted in
the molecular weight region of 15 000 were pooled,
freeze dried, dialysed against Gey's solution, frozen
in liquid nitrogen, and kept at −80°C until assess-
ment.

Idenically prepared, low molecular weight frac-
tions of non-activated plasma did not show chemo-
tactic activity. Moreover, activity could be abolised
by incubation with antibody to C5 but not with
antibody to C3 or antibody from non-immunised
rabbits.17 Complete separation of this chemotactic
preparation from native complement components
C3 and C5 of the plasma was shown by fused rocket
immunoelectrophoresis18 of individual fractions
against specific antibody. Casein (5 g/l) was dis-
solved in modified Gey's solution at pH 12 accord-
ing to the method of Wilkinson.14

**NEUTROPHIL CHEMOTAXIS**

Neutrophils were purified from peripheral blood
drawn in EDTA (10 mM). After a methylcellulose
sedimentation of the red cells, buffy coat leucocytes
were further fractionated by gradient centrifugation
on Lymphoprep, washed twice, and adjusted to 2×10^9
cells/l in Gey's solution with albumin (2%).

Activation of neutrophil chemotaxis was assessed in
Sartorius 3 μm pore size filters placed in modified
Boyden chambers.14 Experiments were run for 45
minutes at 37°C and the filters were fixed, stained,
and read by the leading front technique.19

A panel of C5a dilutions in Gey's solution, and
casein or Gey's solution alone were placed in the
lower compartment of the Boyden chambers. Sensi-
tivity to C5a was expressed in titres of the activator,
corresponding to a half peak response. Values of
non-activated, spontaneous migration towards
Gey's solution were subtracted before calculation of
the titres by linear intrapolation in the ascending
part of the dose-response curves. All results are
based on medians of nine determinations on two
filters.

**CIRCULATING C3c**

Split products of C3 were quantified in EDTA
plasma (10 mM), frozen in liquid nitrogen, and kept
at −80°C for less than three months before quanti-
fication by intermediate gel technique.20 Precipita-
tion of native C3 and C3 split products with d
specificity was accomplished in the intermediate gel
by antibody specific for C3d, permitting C3c rockets
to be formed in the upper gel by C3c antibody. The
specificity of this method has previously been
demonstrated.20 Standard human serum from
Behringwerke containing C3 in the form of C3c only
(0.92 g/l) served as antigen standard.

**Table 1 Clinical data and routine laboratory values of 15 patients with rheumatoid arthritis**

<table>
<thead>
<tr>
<th>Affected joints (number)</th>
<th>Morning stiffness (min)</th>
<th>Disease duration (years)</th>
<th>Sedimentation rate (mm/h)</th>
<th>C-reactive protein* (mg/l)</th>
<th>Rose-Waaler titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 (0–54)</td>
<td>45 (0–300)</td>
<td>14 (3–36)</td>
<td>31 (2–110)</td>
<td>32 (3–106)</td>
<td>160 (40–&gt;5120)</td>
</tr>
</tbody>
</table>

Values are median with range in parentheses.
*Reference interval: ≤10 mg/l.
STATISTICS
The Mann-Whitney rank sum test and the Spearman rank correlation test were applied.

Results
Individual dose-response curves for C5a activation of neutrophil chemotaxis all increased to reach a peak value within the panel of titres tested. Further increases of activator concentration led to declining responses. Representative dose-response curves for rheumatoid arthritis patients and normal controls are given in Fig. 1. Table 2 shows neutrophil responses to C5a in total patient and control groups.

Sensitivity of peripheral neutrophils to C5a was expressed in titres corresponding to a half maximal response in individual tests. Medians were 40 in rheumatoid arthritis patients and 82 in healthy volunteers. This twofold decrease of sensitivity to specific stimulation was significant at the p<0.005 level, with subnormal values in 10 to 15 rheumatoid arthritis tests (Fig. 2). In contrast, peak response of neutrophils to C5a was normal in the patients (p>0.10, Table 3).

Non-specific activation with casein and the spontaneous motility of the cells tested with Gey's

Fig. 1 Representative dose-response curves for neutrophil chemotaxis to C5a in one patient with rheumatoid arthritis (RA) and in one normal control (N). Migration was activated by twofold dilutions of C5a or was assessed in the absence of attractant, control (C).

Fig. 2 Decreased sensitivity to C5a in patients with rheumatoid arthritis (RA) compared with normal controls (N) (p<0.005). Individual titres of C5a corresponding to half maximal response are given.

<table>
<thead>
<tr>
<th>Neutrophils</th>
<th>C5a titres</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Rheumatoid arthritis (n=15)</td>
<td>100 (73-146)</td>
</tr>
<tr>
<td>Control (n=15)</td>
<td>142 (29-157)</td>
</tr>
</tbody>
</table>

Values are medians with ranges in parentheses expressed in μm/45 min.
Table 3  Neutrophil chemotaxis in rheumatoid arthritis (RA)

<table>
<thead>
<tr>
<th>Neutrophils</th>
<th>Chemotaxis*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Casein</td>
</tr>
<tr>
<td>Rheumatoid arthritis (n=15)</td>
<td>96 (82-122)</td>
</tr>
<tr>
<td>Control (n=15)</td>
<td>106 (75-131)</td>
</tr>
</tbody>
</table>

*Values are median with range in parentheses expressed in um/45 min. Casein concentration 5 g/l; C5a titre yielding maximal response in individual tests.

solution in both chamber compartments did not differ between groups of rheumatoid arthritis patients and normal controls (p>0.10) (Table 3). Values of sensitivity to C5a correlated negatively with numbers of affected joints (r = -0.66, p<0.01, n=15) (Fig. 3). Patients with a large number of swollen and/or painful joints all reacted normally in the sensitivity test (Fig. 3). No significant correlation could be established between other clinical parameters such as duration of the disease or duration of morning stiffness and any neutrophil function test (p>0.10).

Levels of the c split product of C3 (C3c) in the circulation were slightly but significantly raised in rheumatoid arthritis patients (p<0.025) (Fig. 4). This indicator of C3 catabolism did not correlate with clinical parameters recorded.

Cell suspensions ready for chemotaxis experi-

![Fig. 3](image)

Fig. 3 Negative correlation of C5a sensitivity with numbers of affected joints in rheumatoid arthritis patients (r = -0.66, p<0.01, n=15). C5a titres correspond with a half maximal response. Hatched area indicates reference interval.

![Fig. 4](image)

Fig. 4 Raised levels of c split products of C3 (C3c) in the circulation of rheumatoid arthritis patients (RA) compared with normal controls (N) (p<0.025).

Discussion

The present study shows a significant abnormality of circulating neutrophils in well established rheumatoid arthritis. Cellular sensitivity to complement 5a was decreased by a factor of two as found from studies of chemotaxis in the proved Boyden chamber assay. Normal peak response by C5a suggested an unaffected basic cell function. The findings are compatible with a competitive inhibition of complement 5a receptors on neutrophils. Normal chemotaxis to casein seems to exclude the possibility of major, intrinsic defects of cellular locomotion.

A selective refractoriness of circulating neutrophils to C5a has previously been reported during haemodialysis leading to intravascular activation of complement. Moreover, incubation of neutrophils with purified C5a in vitro induced a selective desensitisation of specific receptors, whereas casein responses remained unaffected. In rheumatoid arthritis, similarly, intravascular activation of complement may be primary to the cellular dysfunction.
phenomenon. Hypercatabolism of complement C3, that is, activation of complement by either the classical or alternative pathway, was suggested in the present study by the demonstration of an increase in the level of C3c split product in the circulation. This finding confirmed previous observations of increased C3d, another C3 split product, assessed by an entirely different technique.12

Based on the studies of normal leucocytes incubated in vitro with C5a the possibility of a role for specific desensitisation of C5a receptors in limiting potentially harmful neutrophil functions in inflammation has been discussed.7 8 The present study of patients with rheumatoid arthritis suggests a modulating influence of C5a dysfunction for the extent of rheumatic inflammation. Values of dysfunction correlated negatively with numbers of affected joints, the cell function being normal in all patients with many joints involved. Neutrophil dysfunction in rheumatoid arthritis may act in concert with humoral inhibitors23-25 to produce the decreased migration during simulated in-vivo conditions in skin window chambers.26

In Crohn's disease a similar association of defective neutrophil accumulation in skin window chambers, hypercatabolism of complement C3, and decreased sensitivity to C5a suggest that a selective 'down regulation' of neutrophil function may be operative in diverse chronic inflammatory conditions. Intravascular activation of complement may be the common denominator.

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References

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