

# Antibody-mediated cytotoxicity in rheumatoid arthritis

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**SUMMARY** Patients with active seropositive rheumatoid arthritis have reduced antibody-mediated cytotoxicity. Synovial fluid lymphocytes have very low cytotoxicity. A relationship appears to exist between disease activity and cytotoxic activity.

Antibody-mediated cytotoxicity is a process whereby nonimmune lymphocytes damage or destroy target cells in the presence of antibody to the target cell (Perlmann and Holm, 1969; MacLennan *et al.*, 1970). Reduced cytotoxic activity has been shown in systemic lupus erythematosus (Schneider *et al.*, 1975). We have measured the cytotoxic activity of peripheral blood and synovial fluid lymphocytes of patients with rheumatoid arthritis.

## Materials and methods

Seventy patients (22 males, 48 females) with classical or definite rheumatoid arthritis (RA) by the ARA criteria (Committee of the American Rheumatism Association, 1959) were studied. Their ages ranged from 18–70 years, mean 52 years. The majority were taking 2–4 g aspirin daily. 10 patients were taking up to 10 mg prednisolone daily and 17 patients 250 mg chloroquine phosphate daily. Disease activity was assessed by clinical examination and designated as very active, moderately active, or inactive. On the day of the study the erythrocyte sedimentation rate was measured (Westergren) and the serum tested for the presence of rheumatoid factor (Ball, 1950). Synovial fluid samples were obtained from 10 patients by needle aspiration. Control subjects consisted of 15 patients with osteoarthritis and 15 healthy volunteers matched approximately for age and sex with the RA patients.

## Test system

The method used was that described by Calder *et al.* (1974). Target cells were fresh sheep red blood cells (SRBC) labelled with radioactive sodium chromate

(<sup>51</sup>Cr, Amersham). Effector cells were lymphocytes obtained from peripheral blood and synovial fluid samples by centrifugation on a Triosil-Ficoll gradient. Adherent cells were removed by incubating each sample in a flat tissue culture flask for 3 hours at 37°C.

Cultures were set up in triplicate in plastic tubes (Luckham's Ltd.);  $4 \times 10^5$  lymphoid cells and  $4 \times 10^4$  target cells were added to culture medium to a total volume of 0.4 ml. In one group the target cells were sensitized by the addition of antish sheep red blood cell antibody at a final dilution of 1/10 000 (Wellcome reagents), while phosphate-buffered saline was added in place of the antibody in the other group.

Control cultures consisted of target cells incubated with an appropriate number of washed unlabelled SRBC in place of the effector lymphocytes. Maximum isotope release was estimated by adding sensitized target cells to cultures containing guinea pig serum as a source of complement. The cultures were incubated at 37°C for 18 hours in air and 5% carbon dioxide, then centrifuged at 200 g for 10 minutes; 200 µl of supernatant was then removed to another tube. The tubes were counted in a well-type scintillation counter for 1 minute (Nuclear Enterprises Ltd.).

The results are expressed as a 'cytotoxic index'

$$\frac{\text{Experimental } ^{51}\text{Cr release} - \text{spontaneous } ^{51}\text{Cr release}}{\text{Maximum } ^{51}\text{Cr release} - \text{spontaneous } ^{51}\text{Cr release}} \times 100.$$

## Results

Rheumatoid factor (RF) was detected in the serum of 39 of the 70 patients with RA. The Fig. shows that the mean cytotoxic index of the control subjects was  $31 \pm 2$  (standard error); seronegative patients  $31 \pm 1.3$ ; seropositive  $17 \pm 2$ . The seropositive patients have a significantly lower mean value than

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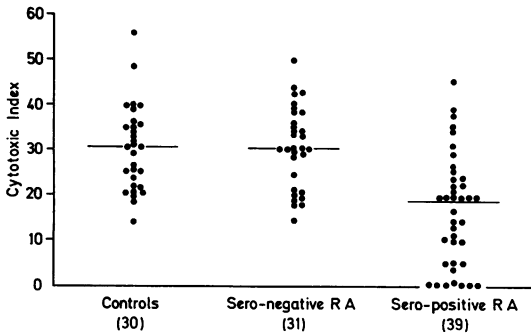


Fig. Cytotoxic index of controls and seronegative and seropositive RA patients.

the controls and the seronegative patients ( $P < 0.01$ ). There was no relationship between the degree of cytotoxicity and the titre of RF.

The relationship between disease activity and cytotoxicity is shown in Table 1. 16 patients had very active disease, of whom 14 were seropositive and 2 seronegative. The mean ( $\pm$ SE) value of the ESR was  $66 \pm 5.8$  mm/h, significantly higher than all other groups ( $P < 0.01$ ). The mean ( $\pm$ SE) value of the cytotoxic index was  $10 \pm 2.8$ , significantly lower than all other groups ( $P < 0.01$ ). Disease was inactive in 15 patients. 14 were seronegative and one was seropositive. The mean value of the ESR was  $24 \pm 3.1$  mm/h and the cytotoxic index  $31 \pm 2.2$ . 39 patients had moderately active disease, 23 seropositive and 16 seronegative. The mean value of the ESR of these 39 was  $33 \pm 5$  mm/h; seronegative patients  $29 \pm 3$  and seropositive patients  $38 \pm 4.8$ . The corresponding values of the cytotoxic index were  $26 \pm 1.9$ ,  $28 \pm 1.5$ , and  $23 \pm 2$  respectively. These values are not significantly different.

The cytotoxicity of the synovial fluid and peripheral blood lymphocytes of 10 patients is shown in Table 2. The mean ( $\pm$ SE) value of the cytotoxic index of the synovial fluid cells was  $6.5 \pm 1.2$  and the mean value of the peripheral blood cells was  $19.2 \pm 3.5$ , the difference being significant ( $P < 0.05$ ).

No significant cytotoxicity occurred when peripheral blood (or synovial fluid) lymphocytes were incubated with target cells in the absence of antibody. The mean spontaneous release of isotope was 5% in the presence of unlabelled sheep erythrocytes. Sensitized sheep cells incubated with guinea pig complement released between 55 and 65% of their label after incubation for 18 hours. Cytotoxicity did not appear to be related to treatment with corticosteroids, antimalarial drugs, or aspirin, or any

Table 1 Cytotoxic index and ESR values (mean  $\pm$ SE) of RA patients according to disease activity

Disease activity	Patients		
	n	ESR (mm/h)	Cytotoxic index
Very active	16	$66 \pm 5.8$	$10 \pm 1.8$
Inactive	15	$24 \pm 3.1$	$31 \pm 2.2$
Moderately active			
seronegative	16	$29 \pm 3$	$28 \pm 1.5$
seropositive	23	$38 \pm 4.8$	$23 \pm 2$

Table 2 Cytotoxic index of paired synovial fluid and peripheral blood lymphocytes

Case no.	Cytotoxic index	
	Peripheral blood cells	Synovial cells
1	30	9
2	20	0
3	12	10
4	50	13
5	23	9
6	0	4
7	28	3
8	14	8
9	0	11
10	13	0
Mean $\pm$ SE	$19.2 \pm 3.5$	$6.5 \pm 1.2$

combination of these drugs. The degree of cytotoxicity did not correlate with the age of patient or the duration of the disease.

## Discussion

We have found that the cytotoxic activity of peripheral blood lymphocytes from patients with seropositive RA is reduced compared with that of patients with seronegative disease and control subjects. There seems to be a relationship between the activity of the disease and the cytotoxic activity. Patients with very active disease have poor cytotoxic activity and patients with minimally active disease have normal cytotoxic activity. Synovial fluid lymphocytes are also poorly cytotoxic.

Interpretation of the cause and significance of a reduction in cytotoxic activity is limited to some extent because the effector cell has not been precisely identified and its biological role *in vivo* has not been clearly defined. Nevertheless, the following explanations of the defective cytotoxic activity observed in the study are worth considering.

In the first place it could be a manifestation of a reduction in the number of effector cells. A permanent reduction is unlikely in view of the apparent relationship between cytotoxic activity and disease activity. Such a reduction would be most likely expressed throughout all the degrees of activity of

the disease and not solely during periods of increased activity. A temporary reduction, however, might occur during periods of increased activity as a result of the sequestration of the effector cells in synovial tissue. This hypothesis, however, is not supported by the low cytotoxic activity of the synovial fluid lymphocytes.

We favour an alternative theory, namely that the cytotoxic cells are present in normal numbers but have been rendered functionally ineffective by a factor present both in the synovial fluid and in the circulation of patients with active disease. Immunglobulin complexes might be responsible. The cytotoxic reaction seems to depend on the union of immunoglobulin on the surface of the target cell with the Fc receptor of the effector cell. Aggregated IgG blocks this reaction (Calder *et al.*, 1974). Immunglobulin complexes might also block the reaction by competing with the target cell immunoglobulin for the Fc receptor of the effector cell.

Immunglobulin complexes have been shown in the synovial fluid and serum of patients with RA (Winchester *et al.*, 1971). Serum from some patients with RA has been shown to inhibit cytotoxic activity (Barnett and MacLennan, 1972). In our study the cytotoxicity of the peripheral blood lymphocytes was reduced during phases of increased disease activity, that is, at a time when circulating immunoglobulin complexes are most likely to be present.

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