Behaviour of effector cells, synovial fluids, and sera from rheumatoid arthritis patients in antibody-dependent cell-mediated cytotoxicity

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SUMMARY Antibody-dependent cell-mediated cytotoxicity (ADCC) was examined in patients with rheumatoid arthritis (RA). The cytotoxicity of peripheral blood leucocytes from patients with RA was similar to that found in normal persons, whereas ADCC was less effective in RA synovial fluid cells. It is possible that the activity in these cells is lower because of immune complexes and other factors being absorbed from the synovial fluid itself. Although patients' sera had little effect on normal peripheral blood leucocytes, synovial fluid from RA patients was markedly inhibitory in ADCC. The degree of inhibition correlated significantly with the clinical status of the patients.

Antibody-dependent cell-mediated cytotoxicity (ADCC) involves the cytolysis of antibody-coated target cells, which are recognised through Fc receptors on the effector cells. Since this phenomenon was first described (Perlmann and Holm, 1969; MacLennan *et al.*, 1970) many workers have undertaken investigations using mammalian erythrocytes or nucleated cells as targets, and implicating as effectors adherent mononuclear cells, nonphagocytic nonadherent Fc-receptor-bearing lymphocytes, and polymorphonuclear leucocytes (MacLennan *et al.*, 1972; Perlmann *et al.*, 1972; Gale and Zigelboim, 1974; MacDonald *et al.*, 1975; Trinchieri *et al.*, 1975; Penfold *et al.*, 1976; Fink *et al.*, 1977).

The importance of ADCC as a mechanism in which extracellular killing of antibody-coated targets may provide a defence against infection and the possible role of ADCC in tumour immunity and allograft rejection have been strongly inferred from *in vitro* studies. In rheumatoid arthritis (RA) it is conceivable that ADCC may be associated with the formation of rheumatoid pannus tissue and could be a significant factor in the chronicity of the inflammation. Therefore we have compared ADCC of patients' cells to normal cells, and investigated the interaction of RA sera and synovial fluids with normal ADCC.

Patients and methods

A total of 57 patients with RA were studied (46 female, 13 male). Their average age was 57.5 years (range 27 to 81 years), and duration of disease was 13.0 years (range 0.5 to 40 years). All patients were classified according to American Rheumatism Association criteria as classical, definite, or probable. Activity of disease was assessed clinically and designated as active (or very active), moderately active, and inactive. The clinical criteria used were duration of morning stiffness, synovial swelling and presence or absence of effusions, and tenderness of affected joints. Patients who were designated very active or active had morning stiffness of over 3 hours, effusions of 1 or more joints, and a marked synovial swelling and tenderness of the affected joints. Moderately active patients had morning stiffness lasting for more than 1 hour but less than 3 hours, moderate synovial swelling and minimal tenderness of the affected joints, and no effusions. The inactive category of patients had morning stiffness of less than 1 hour, minimal or no synovial swelling, and no tenderness of the affected joints.

Synovial fluid samples were obtained by needle aspiration. The synovial fluid cells (SFC) and peripheral blood leucocytes (PBL) were examined in ADCC on the day of collection; cell-free synovial fluids and sera were stored at -20°C. PBL and sera from 13 healthy volunteers working in the immunology laboratory served as controls, and synovial

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fluids from 4 osteoarthritis (OA) patients were used as control effusions. Since it was not possible to obtain normal SFC, PBL were compared. In addition, synovial fluids and sera from 22 patients with other connective tissue diseases including ankylosing spondylitis, psoriatic arthritis, systemic lupus erythematosus, Reiter's disease, and Sjögren's syndrome were studied.

ASSAY

The method of assay was essentially that of Perlmann and Perlmann (1970). A microtitre assay was adapted from Zeijlmaaker *et al.* (1975). Eagle's minimum essential medium (MEM, Flow Laboratories, Irvine, Scotland) was used throughout. For incubations and culture the MEM was supplemented with fetal calf serum (FCS, Flow Laboratories) and heat-inactivated at 56°C for 30 minutes. The targets were fresh cells taken from the wing vein of a white Leghorn chicken (CRBC) and labelled with $150 \mu\text{CiNa}_2^{51}\text{CrO}_4$ (CJS, 1P Radiochemical Centre, Amersham). After incubation for 1 hour with 5% CO_2 and air at 37°C in MEM supplemented with 10% FCS the CRBC were washed 4 times in cold MEM with 5% FCS, then diluted to a concentration of 1×10^5 cells per ml. 10^7 sheep red blood cells (SRBC) were added to each ml of CRBC suspension to ensure a consistently low spontaneous release of isotope from the CRBC. Effector cells were obtained from peripheral blood by sedimentation at 37°C for 30 minutes, 4 parts blood to 1 part plasmagel being used. The supernatant was diluted with MEM at 37°C and washed 4 times. The effector cells from fresh synovial fluids were washed 4 times with MEM at 37°C and constituted the whole white cell population. Finally the effectors were resuspended in MEM supplemented with 5% FCS. Cyto-centrifuge preparations of some normal and RA samples were prepared.

Cultures were set up in triplicate in Cooke round-bottomed microtitre plates. 5×10^3 target cells were introduced into the wells in 50 μl aliquots. Effector cells were added to produce effector-to-target ratios of 5:1, 1:1, and 1:5. In the experimental wells (EXP) 50 μl of rabbit anti-CRBC antibody in MEM with 5% FCS were added to give a final dilution of $\frac{1}{30\,000}$. In one control (CONT), MEM with 5% FCS replaced antibody. In addition 2 more controls were included: to estimate the spontaneous release of the CRBC targets were incubated in medium alone, and to ensure that antibody did not lyse the CRBC this was incubated with targets. The maximum ^{51}Cr release (MAX) was estimated by adding 100 μl of 3% Decon to target cells. The total radioactivity was measured by counting labelled target cells alone.

The total volume in the microtitre wells was 150 μl , and the plates were incubated at 37°C for 18 hours in air and 5% CO_2 .

After incubation 100 μl of supernatant were removed from each well; isotope release was measured in a Wallac gamma counter. The maximum release, experimental release, and control release were expressed as a percentage of the total release, and cytotoxicity was calculated as follows:

$$\frac{\text{EXP release} - \text{CONT release}}{\text{MAX release} - \text{CONT release}} \times 100$$

EFFECT OF SERA AND SYNOVIAL FLUIDS ON NORMAL ADCC

Prior to use synovial fluids were treated with hyalase (Fisons UK Eire) at 75 units per ml synovial fluid to reduce viscosity (Hannestad, 1967). All sera and synovial fluids were heat-inactivated at 56°C for 30 minutes. 5 μl amounts of serum or synovial fluid were included in the assay and normal PBL used as effectors at a 5:1 effector-to-target ratio.

Results

PATIENTS' CELLS IN ADCC

No significant difference was seen between PBL from 11 normal individuals and 9 patients with classical RA ($P > 0.3$). Three patients were clinically active, 3 moderately active, and 3 inactive (Fig. 1). However, when SFC from 10 patients were assayed in the same way, a significant difference in cytotoxicity was observed ($P < 0.005$) (Fig. 2). Eight patients had classical RA, 1 definite, and 1 possible

Fig. 1 ADCC of normal ($\blacktriangle$) and rheumatoid ($\triangle$) peripheral blood leucocytes at the 1:5, 1:1, and 5:1 effector-to-target cell (E/T) ratio.

Fig. 2 ADCC of normal peripheral blood leucocytes (▲) compared with rheumatoid synovial fluid cells (○).

RA. No difference could be seen between patients with clinically active disease and those with moderately active or inactive RA ($P > 0.95$).

CYTOCENTRIFUGE PREPARATIONS

To test whether differences in effector cell activity of RA PBL and SFC were due to variations in the numbers of effector cells cytocentrifuge preparations of representative samples were made. The proportions of each cell population, as distinguished by staining the slides with May-Grünwald-Giesma stain, were similar (Fig. 3).

Fig. 3 Cytocentrifuge preparations of normal and rheumatoid samples showing the distribution of cell populations.

EFFECTS OF SERA AND SYNOVIAL FLUIDS IN NORMAL ADCC

When 5 μ l aliquots of sera were included in the assay using normal PBL effectors, no significant difference was observed between 8 normal human sera (NHS) and 17 sera from RA patients ($P < 0.1$). There was a significant difference ($P < 0.025$) between NHS and 8 sera from patients with other connective tissue diseases (Fig. 4). However, in relation to NHS there was a significant inhibition of cytotoxicity on addition of 5 μ l samples of synovial fluid from RA patients ($P < 0.005$) and patients with other inflammatory connective tissue disorders ($P < 0.001$) (Fig. 4). Compared to NHS, fluids from patients with OA were also significantly inhibitory ($P < 0.02$) but less so than the other fluids tested. This might be a reflection of the difficulty in selecting patients with degenerative joint disease with no other concurrent disease. Since normal synovial fluid is not available for ethical and other reasons, it was not possible to determine whether there were factors in synovial fluid which are naturally inhibitory in ADCC.

30 RA synovial fluids were examined; 23 patients had classical RA, 2 definite RA, and 5 probable RA. Synovial fluids from RA patients with active disease showed significantly more inhibition than those from patients with moderately active and inactive disease ($P < 0.005$) (Fig. 5). This was not so when patients' SFC were examined.

However, there was no difference in inhibitory capacity of synovial fluids of RA patients with or without radiological erosions, or positive and negative serology. Similarly, the drug regimen did not seem to influence the degree of inhibition of cytotoxicity by the synovial fluids. Anti-inflammatory

Fig. 4 Inhibition of ADCC by sera and synovial fluids.

Fig. 5 The relationship between rheumatoid disease activity and inhibition of normal ADCC by synovial fluids.

drugs, immunosuppressive drugs, and steroid therapy, whether taken singly or in combination, did not affect the inhibitory capacity of the synovial fluids.

Discussion

Several different effector cell types have been implicated in ADCC, and it would appear that the choice of target cell could be a determining factor. Since at this stage we are not sure which cells might play an active role in RA, it was decided to combine all effector populations and include whole white cell populations when comparing normal individuals and patients with RA.

This study showed that RA SFC were significantly less cytotoxic than with normal PBL. Diaz-Jouanen *et al.* (1976a) reported a decrease in cytotoxicity with RA SFC (using polymorphs or lymphocytes as effectors) compared with peripheral blood lymphocytes from RA patients and normal controls. Cytotoxicity was assessed by isotope released from sensitised CRBC targets. Conversely, using burro erythrocyte targets, Scheinberg and Cathcart (1976) found that purified rheumatoid peripheral blood lymphocytes and SFC mediated ADCC normally, and that no correlation could be obtained between the percentages of T, B, and null cells in the effector populations and the amount of cytotoxicity.

In their experiments on the inhibition of cytotoxicity using RA sera and synovial fluids Diaz-Jouanen *et al.* (1976b) found that most sera and

synovial fluids were inhibitory, whereas Scheinberg and Cathcart (1976) showed that sera from 50% of their patients with RA inhibited ADCC by normal lymphocytes. Our studies have shown clearly that RA synovial fluids show more inhibition of ADCC than NHS, and it seems most likely that this can be attributed to the higher concentration of C1q binding complexes in RA joint effusions in relation to serum (Hay *et al.*, 1978), and, further, that ADCC activity is lower in RA SFC as a result of blocking of their Fc receptors by these complexes. It is not surprising, therefore that the cytotoxicity of normal and RA PBL is similar in view of the comparable effects of NHS and RA sera on normal effector cells. However, we were unable to produce further evidence of immune complex involvement in reduced cytotoxicity using the methods described by Feldman *et al.*, (1976), who demonstrated a partial regeneration of ADCC by treatment of peripheral blood lymphocytes of SLE patients with Pronase and DNase, followed by incubation overnight (Cooke, unpublished data).

The greatest inhibition of normal cells was by synovial fluids from patients with the most active disease. On this basis, we would expect there to be a similar relationship between disease activity and reduced cytotoxicity of SFC, but insufficient numbers were studied to establish this point.

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