

Antibody-dependent and PHA-induced cellular cytotoxicity in rheumatoid arthritis

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SUMMARY One hundred and thirty nine observations of antibody-dependent cell mediated cytotoxicity (ADCC) were made on 77 patients with rheumatoid arthritis (RA) and 17 healthy controls. There were no differences in ADCC between these 2 groups or within the RA group with regard to disease activity, duration, seropositivity, or drug treatment. Sixty observations of phytohaemagglutinin induced cytotoxicity were made on 22 patients with RA and 10 healthy controls. Again there were no differences in cytotoxicity between the 2 groups.

The immunological activity apparent in rheumatoid arthritis (RA) has led to speculation that an altered state of immune reactivity may play a role in the development of this disease. It has also led to the clinical testing of drugs such as azathioprine thought to reduce immune responsiveness. However, numerous studies of immune reactivity in RA have produced conflicting results, with no consistent evidence of altered antibody production or specific cellular immunity. Rheumatoid patients have also generally not shown any consistent alteration in the numbers or ratios of circulating immunocyte subpopulations. In addition, effective treatment of RA by 'immunosuppressive' drugs is usually not associated with any obvious reduction in immune responsiveness, thus making it difficult to monitor this type of treatment (Currey, 1978).

Two in-vitro reactions provide convenient measurements of the ability of separate subgroups of peripheral blood leucocytes to lyse target cells (Podleski, 1976). The one is antibody-dependent cell mediated cytotoxicity (ADCC), in which a subpopulation of lymphocytes (killer, or K cells) produces lysis of cells to which antibody is specifically bound (Perlmann and Perlmann, 1970); the other is phytohaemagglutinin (PHA)-induced cytotoxicity (Perlmann and Holm, 1969). Our interest in these 2 reactions arose from the observations of Campbell *et al.* (1976) that, in patients suffering from ulcerative colitis, azathioprine treatment produced a progressive

reduction in both antibody-dependent and PHA-induced cytotoxicity in the peripheral blood. This therefore appeared to offer a method of monitoring 'immunosuppressive' therapy in RA. In addition, Wisloff *et al.* (1975) found patients with RA to have reduced PHA-induced cytotoxicity compared with nonrheumatoids.

For this reason we have used these 2 tests to seek differences between rheumatoid subjects and others, and, among patients with RA, differences between those receiving and those not receiving 'slow acting' drugs such as azathioprine.

Patients

Seventy-seven patients with classical or definite RA as defined by the American Rheumatism Association (Ropes, 1959) and 17 healthy persons were tested for ADCC. Treatment groups and clinical and laboratory details are shown in Table 1. The erythrocyte sedimentation rate (ESR) (Westergren) was used as a measure of disease activity. Drug dosage and duration of treatment were recorded for each patient. Among those taking azathioprine the mean cumulative dose was 75 g (range 3 to 272 g), and the mean duration of therapy was 23.5 months (range 1 to 84 months).

Twenty-two patients with RA and 10 healthy persons were also investigated for PHA-induced cytotoxicity.

Methods

EFFECTOR CELLS

Lymphocytes were obtained from heparinised venous

Accepted for publication 1 August, 1978

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Table 1 Treatment groups: clinical and laboratory data

Group	Healthy	NSAID	AZA	D-Pen	GOLD	ALCL	Corticosteroid
No. of subjects	17	16	27	16	5	8	5
Sex (M:F)	8:9	6:10	11:16	6:10	1:4	2:6	2:3
Mean age (yr)		54.4	56.2	50.0	47.4	52.6	54.6
Range		35-67	38-70	28-62	34-68	41-78	44-73
Disease duration (yr)		11.7	9.1	8.4	9.0	12.7	18.6
Range		4-34	2-20	2-15	6-12	4-23	12-26
ESR (mm/h)		51.8	54.0	41.6	24.0	62.7	45.0
Range		20-112	3-121	6-114	10-42	24-95	43-120
Latex +ve ($\geq 1/80$)		13	20	15	5	8	5

NSAID = Nonsteroidal anti-inflammatory drugs. AZA = Azathioprine. D-Pen = D-penicillamine. ALCL = Alclofenac.

blood as previously described (Vernon-Roberts *et al.*, 1974). Medium 199, supplemented by 5% fetal calf serum of pH 7.2 was used throughout. The final cell suspension, containing >85% lymphocytes with >95% viability as assessed by trypan blue exclusion, was adjusted to 2.5×10^7 cells/ml.

TARGET CELLS

Day-old chick red blood cells (CRBC) (Tissue Culture Services Ltd.) were incubated at 37°C for 2½ hours with radioactive sodium chromate, ^{51}Cr (Amersham). The labelled CRBC were washed and resuspended with unlabelled sheep red blood cells (1×10^8 /ml) to give a target cell concentration of 1×10^6 CRBC/ml and an effector to target cell ratio of 25:1.

ANTIBODY

An antiserum to CRBC was raised in a rabbit and stored in aliquots at -20°C. This was diluted to 1×10^{-4} before use.

ADCC ASSAY

50 µl each of effector and target cell suspensions together with 50 µl antiserum were incubated at 37°C for 18 hours in the wells of Cooke Microtiter plates. All experiments were performed in triplicate, and appropriate controls were included in each run. A maximum release value was obtained by substituting 100 µl of 3% Decon 90 detergent for the effector cell suspension and the antiserum. After incubation the tray was centrifuged at 200 g for 5 minutes. The ^{51}Cr content of 100 µl of supernatant from each well was then measured for 1 minute in a gamma guard 150 counter (Tracerlab Services Ltd.). Samples were randomised before counting.

CALCULATION OF CYTOTOXICITY INDEX (CI)

The median value for each triplicate determination was used.

$$\text{CI}\% = 100 \times \frac{\text{Experimental release } ^{51}\text{Cr} - \text{Spontaneous release } ^{51}\text{Cr}}{\text{Maximal release } ^{51}\text{Cr} - \text{Spontaneous release } ^{51}\text{Cr}}$$

PHA-INDUCED CYTOTOXICITY

50 µl PHA (Wellcome Reagents Ltd.) was substituted for antiserum in the system described above. The concentrations of PHA used were 3×10^{-4} , 3×10^{-5} , and 3×10^{-6} mg/ml.

Results

ANTIBODY-DEPENDENT CELL MEDIATED CYTOTOXICITY

One hundred and thirty-nine determinations of ADCC were made on the subjects described (Table 1). The mean cytotoxicity index (CI) was similar for patients and controls whether calculated overall or for individual treatment groups (Table 2). The mean CI for patients receiving 'slow acting' drugs (azathioprine, D-penicillamine, and gold) was 38.2 which was comparable to that in healthy people or patients in the group on nonsteroidal anti-inflammatory drugs (NSAID). There were no statistically significant differences between any groups examined. Repeated observations on individual patients and healthy controls gave a consistency for CI over a 6-month period within 10%. There was no association between CI and age, disease duration, ESR, or seropositivity as determined by a titred latex test (Table 3). Moreover the CI was unrelated to disease activity as assessed by the ESR in the groups examined (Table 4).

Table 2 Mean CI in healthy and RA treatment groups

Group	Healthy	NSAID	AZA	D-Pen	Gold	ALCL	Corticosteroid
Observations	35	17	42	26	6	8	5
Mean CI%	35.5	33.7	38.3	37.1	39.3	32.3	40.4
SD	14.5	12.2	11.5	9.4	13.4	13.3	7.7

Table 3 Relationship of latex titre and CI in rheumatoid patients

Latex titre	≤ 1:40	≥ 1:320
No. of patients	11	20
Mean CI%	37.4	37.6
SD	11.1	8.3

Table 4 Relationship of ESR and CI within different treatment groups

Group	ESR mm/hour	
	≤ 49	≥ 50
AZA	37.3	39
SD	10.4	12.6
D-Pen	38.7	29.8
SD	10.6	7.7
NSAID	34.5	32.3
SD	10.1	15.0

PHA-INDUCED CELLULAR CYTOTOXICITY

Sixty determinations of PHA-induced cytotoxicity were made (Table 5). The results did not show any significant differences between the groups at the concentrations of PHA used. There was a comparable diminution in cytotoxicity in both groups for each reduction in PHA concentration.

Table 5 PHA-induced cytotoxicity in health and RA

PHA (mg/ml)	Health			RA (all drugs)		
	Observations	Mean CI%	SD	Observations	Mean CI%	SD
3 × 10 ⁻⁴	9	36.2	13.3	12	36.4	9.4
3 × 10 ⁻⁵	9	32.8	10.3	13	33.8	10.2
3 × 10 ⁻⁶	9	21.4	13.8	8	16.6	6.5

Discussion

This study does not indicate any differences in peripheral blood K cell activity between RA and health, which accords with the findings of Wisloff *et al.* (1975), Fröland *et al.* (1975), and Feldmann *et al.* (1976). These authors used a similar CRBC system. Panayi and Corrigan (1977) obtained comparable results using a Chang cell system. There are, however, reports of both increased (Sany *et al.*, 1976) and decreased ADCC in RA (Isturiz *et al.*, 1976; McGill and Twinn, 1977). It has been suggested that a reduction in ADCC might be due to a blocking effect of immune complexes in these patients. Discrepant results could also reflect differences in technique. These include the method of lymphocyte separation (Mookerjee, 1976), varying effector target cell ratios, and incubation periods.

McGill and Twinn (1977) correlated ADCC with seropositivity and disease activity. However, 14 of their 39 seropositive patients had a CI of 10% or less, of whom 7 had no demonstrable cytotoxicity. Only 1 of our patients had a CI of less than 10%, and we were unable to find any correlation between cytotoxicity and disease activity or seropositivity.

Drug treatment, in particular azathioprine, did not appear to influence ADCC in our RA patients. The fall in ADCC following azathioprine therapy reported by Campbell *et al.* (1976) occurred in patients receiving the drug for ulcerative colitis, not RA. Furthermore, these authors employed a Chang cell system and calculated CI in a manner which makes comparisons with our own and other studies difficult.

Our results do not confirm the reduction in PHA-induced cytotoxicity in RA reported by Wisloff *et al.* (1975). Differences in the method of separating lymphocytes might possibly explain this discrepancy, for mitogenic responses of lymphocytes in RA vary with the subpopulation examined (Silverman *et al.*, 1976; Rawson and Huang, 1976).

In conclusion, our studies of cellular cytotoxicity have not revealed any alterations attributable to RA or drugs, including azathioprine.

We are grateful to Mr Stephen Evans for statistical advice and comment, and to Dr Paul Zilko for help with developing the method. J. N. Rosenberg was in receipt of an Arthritis and Rheumatism Council travelling fellowship.

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