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Antibody-dependent direct cytotoxicity of human lymphocytes

II. STUDIES ON PERIPHERAL BLOOD LYMPHOCYTES, SYNOVIAL FLUID CELLS AND SERA FROM PATIENTS WITH RHEUMATOID ARTHRITIS

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SUMMARY

Antibody-dependent direct cytotoxicity (ADDC) by human lymphocytes was evaluated in patients with rheumatoid arthritis and normal controls. Purified peripheral blood and synovial fluid lymphocytes mediated normal ADDC when compared to control subjects. No correlation could be obtained between the percentages of T, B and null cells in effector cell populations and the degree of ^{51}Cr released from target cells. Sera from 50% of patients with rheumatoid arthritis inhibited ADDC by normal lymphocytes; the degree of inhibition did not correlate with the titre of IgM rheumatoid factor. The pathogenic implications of these findings are discussed.

INTRODUCTION

The destruction of antibody-coated target cells by sensitized mononuclear cells was first described by Moller (1965) using sarcoma cells as targets for mouse lymphocytes. The nature of the effector cell in this system has been the subject of considerable controversy and macrophages, B cells and null cells have all been shown to mediate antibody-dependent direct cellular cytotoxicity (ADDC) (for reference see Perlmann *et al.*, 1975). Within the past few years several reports have also appeared relating this phenomenon to human diseases (Campbell *et al.*, 1972; Calder *et al.*, 1973; O'Toole *et al.*, 1974; Rachelefsky *et al.*, 1976).

In the previous paper we have shown that purified peripheral blood lymphocytes from patients with systemic lupus erythematosus have reduced cytotoxic activity against burro erythrocytes coated with specific antibody. Sera from some of these patients also inhibited target cell damage by normal lymphocytes and a correlation was found between reduced ADDC, sera with inhibitory properties and clinical activity of the disease (Scheinberg & Cathcart, 1975).

In the present report we describe our findings in patients with rheumatoid arthritis (RA). We have evaluated ADDC activity of peripheral blood and synovial fluid lymphocytes in twenty patients with seropositive rheumatoid arthritis. In addition, the ability of rheumatoid sera to interfere with ADDC by normal lymphocytes has also been investigated. Correlations between clinical features and the proportions of T, B and null cells found in effector cell populations were performed and will also be discussed.

MATERIALS AND METHODS

Patient population. Twenty patients (six female, fourteen male) with seropositive RA were selected for this study. Most of the patients had long-standing disease and severe joint destruction. All patients met the criteria of the American Rheumatism Association (Ropes *et al.*, 1958) for classical RA. Clinical and laboratory features were determined by standard methods.

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None had vasculitis. No patients in this series were receiving steroids or immunosuppressive drugs. Eighteen patients were taking daily doses of aspirin ranging from 3 to 6 g daily and two patients were receiving gold injections. Serum rheumatoid factor titres were greater than 1/320 in all patients. Synovial fluid was obtained from six patients with RA (simultaneously with peripheral blood): the mean white cell count/mm^3 and the mean percentage of lymphocytes in the group of synovial fluids studied were 9200 and 40 respectively.

Twenty normal volunteers served as a control group. Statistical analyses were performed by Student's *t*-test and analysis of variance. Comparison was made between the various study groups by Scheffe's test.

Isolation of lymphoid cells. Peripheral blood lymphocytes were separated by Ficoll–Hypaque centrifugation according to the method of Böyum (1968). After washing twice in minimal essential medium and incubating with carbonyl iron to eliminate phagocytic cells as described by Tebbi (1973), the cells were again washed, counted and adjusted to the desired concentration. Ficoll–Hypaque separation of peripheral blood yielded more than 70% of the lymphocytes in the initial preparations. Viability by trypan blue staining was greater than 95%.

The procedure for isolating lymphocytes from synovial fluids was identical to that used for peripheral blood, except that saline was used as diluent for the white cell counts, and the fluid was first treated with hyaluronidase (Wydase, Wyeth Laboratories, Philadelphia, lot no. 4732403) according to a modification of the method of Hedberg (1967).

Enumeration of T, B and null cells. Detailed description of the techniques for identifying various subpopulations of lymphocytes has been published in detail elsewhere (Scheinberg & Cathcart, 1974). In brief, T cells were enumerated by the sheep red cell rosette method: B cells were counted by detecting surface-bound immunoglobulin (SIg) using fluorescein microscopy and by recognizing cells with receptors for the third component of complement (EAC). The percentage of null cells was calculated by subtracting the percentage of T cells plus SIg-bearing cells from 100.

ADDC assay. The method used was that of Van Boxel *et al.* (1972) with minor modifications. 2×10^6 Purified lymphocytes were incubated with 1×10^5 target cells that were previously coated with specific antibody. After 18 hr incubation the tubes were centrifuged and the supernatant transferred to another tube for counting. Details regarding the ADDC assay and calculation of cytotoxicity have been described previously (Scheinberg & Cathcart, 1976).

Heat-aggregated immunoglobulin (Agg-IgG). Lyophilized Cohn-Fraction II (obtained from the State Biological Laboratories, Commonwealth of Massachusetts) was reconstituted in phosphate-buffered saline (PBS), pH 7·2, and freed of preformed aggregates by centrifuging overnight in a Sorvall Centrifuge (20,000 *g*). The upper two-thirds of the supernatant were sterilized by gentle passage through a 0·45 μm Millipore filter (Millipore Corporation, Bedford, Massachusetts). Portions of deaggregated supernatants were then heated in a water bath at 56°C for 30 min to obtain Agg-IgG and adjusted to desired concentrations in 0·1-ml volumes.

RESULTS

As noted by us previously (Brenner *et al.*, 1975) the percentages of T, B and null cells in the peripheral blood and synovial fluid of patients with RA were within normal limits. Table 1 compares the cytotoxicity and the percentages of T, B and null cells in the peripheral blood of patients with RA and normal controls. No significant differences were found between the mean levels of ADDC in RA and controls. Synovial fluid lymphocytes had cytotoxic activity comparable to that mediated by peripheral blood lymphocytes (range 25–53%) (Table 2). No correlations were obtained between the degree of ^{51}Cr release from target cells and the percentage of B cells (EAC, SIg) or null cells present in the effector cell population of peripheral blood and synovial fluid lymphocytes.

Sera from ten of twenty patients with RA inhibited cytotoxicity of lymphocytes derived from normal controls against sensitized burro erythrocytes (suppression greater than 25%) (Table 3). Normal sera or pooled AB sera showed minimal inhibitory activity in our system. In another experiment the effect of rheumatoid serum on autologous lymphocytes was tested. Four of eight sera tested significant suppressed ADDC by their own lymphocytes (Table 4). The effect of Agg-IgG on ADDC by normal human lymphocytes is shown in Table 5. Lymphocyte cytotoxicity for sensitized burro red cells was inhibited by heat aggregated IgG and the inhibition was dose-dependent.

DISCUSSION

In a previous study we have shown that sera from patients with active systemic lupus erythematosus partially inhibit the ability of normal lymphocytes to destroy antibody-coated burro erythrocytes (Scheinberg & Cathcart, 1976). We now report comparable findings in the serum of patients with rheumatoid arthritis although peripheral blood and synovial fluid lymphocytes from the same patients are able to mediate normal ADDC against sensitized xenogeneic target cells. Our failure to correlate

TABLE 1. ADDC, T, B, null cells and lymphocyte count (LC) in peripheral blood from patients with sero-positive rheumatoid arthritis and controls

Case no.	Age	Sex	Percentage ^{51}Cr release	T	B	NC	LC
R1	50	M	30	70	20	10	2640
R2	19	M	47	66	15	19	2850
R3	40	F	54	50	23	27	2420
R4	50	M	31	55	16	29	2550
R5	46	M	27	65	22	13	3100
R6	38	M	50	n.d.*	n.d.	n.d.	n.d.
R7	60	M	45	60	12	28	3000
R8	50	M	40	66	10	24	2950
R9	35	F	32	67	23	10	1750
R10	66	M	38	65	35	0	1460
R11	40	M	45	60	20	20	1525
R12	30	F	37	69	20	11	1656
R13	51	F	40	57	6	37	1600
R14	72	F	40	n.d.	n.d.	n.d.	n.d.
R15	56	M	40	72	8	20	2400
R16	43	M	45	70	n.d.	n.d.	2180
R17	70	M	38	70	20	10	1950
R18	62	M	40	n.d.	n.d.	n.d.	1900
R19	32	F	50	n.d.	n.d.	n.d.	2100
R20	26	F	44	70	15	15	1750
Mean ± s.d.			45 ± 6	64 ± 6	17 ± 7	18 ± 9	2210 ± 545
Controls†	20–55		37 ± 6	63 ± 4	18 ± 4	19 ± 4	2582 ± 687

* n.d. = Not determined.
† Fourteen male and six female.

TABLE 2. ADDC, T, B and null cells in synovial fluid lymphocytes from patients with sero-positive rheumatoid arthritis

Case no.	Age	Sex	Percentage ^{51}Cr release	T	B	NC
R1	50	M	25	73	10	17
R2	19	M	47	70	5	25
R3	32	F	53	50	16	34
R6	38	M	50	n.d.*	n.d.	n.d.
R12	30	F	37	66	15	19
R20	26	F	36	70	10	20
Mean ± s.d.			41 ± 10	65 ± 9	11 ± 4	23 ± 6

* n.d. = Not determined.

suppressed ADDC with serum latex fixation titres is not surprising since others have shown that the inhibitory activity of RA serum is dependent on the presence of 9–10S IgG–IgG aggregates rather than IgM rheumatoid factors (Barnett & MacLennan, 1972; Halberg, 1972). Indeed, our findings suggest that inhibition assays of ADDC by normal lymphocytes may provide another means of identifying soluble immune complexes since Winchester, Kunkel & Agnello (1971) have shown that 60% of patients with RA have serum components which react with monoclonal rheumatoid factor. Furthermore, the present study confirms recent observations by Halberg (1974) that aggregated IgG interferes with cytotoxicity of non-immune lymphocytes for sensitized erythrocytes and that the degree of inhibition is dose-dependent.

TABLE 3. Effect of rheumatoid sera on ADDC by normal peripheral blood lymphocytes

Case no.	Percentage suppression*	Latex titre†
R1	72	1/40960
R2	65	1/20480
R3	15	1/10240
R4	0	1/20480
R5	14	1/640
R6	25	1/10240
R7	50	1/5120
R8	20	1/640
R9	1	1/640
R10	0	1/10240
R11	48	1/10240
R12	0	1/320
R13	0	1/5120
R14	36	1/5120
R15	36	1/320
R16	0	1/40960
R17	11	1/640
R18	82	1/10240
R19	60	1/640
R20	99	1/2560

* The percentage of suppression represents the decrease in specific lysis induced by treatment of effector cells with rheumatoid sera (0·2 ml) in comparison with the decrease induced with pooled human AB sera (0·2 ml).

† Latex test was performed by a tube dilution method (Cathcart, 1975).

TABLE 4. Effect of rheumatoid sera on ADDC by autologous peripheral blood lymphocytes

Case no.	Percentage suppression*	Serum latex titre
R2	55	1/20480
R3	9	1/10240
R6	50	1/10240
R7	76	1/5120
R10	0	1/10240
R13	20	1/5120
R14	15	1/5120
R18	52	1/10240

* Percentage suppression calculated as shown in Table 3.

TABLE 5. Effect of Agg-IgG on ADDC by normal peripheral blood lymphocytes

Lymphocytes	Antibody	Agg-IgG	^{51}Cr released (ct/min)*	ADDC(%)
+	−	−	1200	—
+	+	+	2600	36
+	+	Non-Agg-IgG (2·5 mg)	2430	32
+	+	Non-Agg-IgG (0·5 mg)	2400	30
+	+	Agg-IgG (2·5 mg)	1550	7
+	+	Agg-IgG (0·5 mg)	1990	19
Repeated freeze–thawing			5000	

* Total ^{51}Cr released from target cells in the supernatant after incubation period in the presence of media, specific antibody and various concentrations of aggregated and non-aggregated human gamma-globulin.

Although a specific *in vivo* function has not been established for antibody-dependent cytolysis it is conceivable that preservation of normal ADDC in RA may be related to the degree of joint destruction by increasing tissue damage and promoting synovial inflammation. It is noteworthy that patients with Bruton's X-linked agammaglobulinaemia develop a form of polyarthritis which is clinically indistinguishable from RA (Good, Rotstein & Mazzitello, 1957) despite the fact that bone marrow-derived lymphocytes appear to play a key role in human ADDC (Perlmann & Perlmann, 1970). It is also not possible to

determine at the present time whether inhibitions of ADDC by certain RA sera is helpful or harmful to the host. Since we have shown in the present study that lymphocytes from patients with RA kill less efficiently in the presence of autologous serum it is possible that this phenomenon also occurs *in vivo* and therefore reduces the degree of synovitis. On the other hand, if RA is caused by an occult virus infection as suggested by Smith (1971) ADDC might aggravate disease activity since immune lysis of virus-infected cells appears to be blocked by rheumatoid factors (Gipson & Daniels, 1975). Although these and many other questions concerning the pathogenesis of RA remain to be answered we have concluded that assessment of ADDC is clinically useful in differentiating patients with RA from those with SLE and that the marked suppression of ADDC by RA serum may be helpful in detecting circulating immune complexes in patients with both disorders.

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