

ORIGINAL PAPER

SUPPRESSION AND STIMULATION OF
IMMUNOGLOBULIN SYNTHESIS BY AGENTS PRESENT
IN RHEUMATOID SYNOVIAL EFFUSIONS

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SUMMARY

Synovial effusions from 14 patients with rheumatoid arthritis contain factors capable of enhancing and suppressing the production of immunoglobulin when added to normal peripheral lymphocyte cultures *in vitro*. Utilizing a radioactive immune coprecipitation technique it could be demonstrated that enhancement was observed upon addition of relatively small amounts of synovial fluid, while addition of higher amounts resulted in suppression of immunoglobulin synthesis. Nine of ten non-rheumatoid joint effusions showed neither enhancing nor suppressing activities. The possible relationship of these events to lymphokine production in the joint is discussed.

THE participation of immune mechanisms in the pathogenesis of rheumatoid arthritis (RA) is well established (Pekin *et al.*, 1964; Smiley *et al.*, 1968; Winchester *et al.*, 1969; Rodman *et al.*, 1967). Recently, we described the presence in rheumatoid synovial effusions of enhancing factors capable of stimulating immunoglobulin synthesis in peripheral-blood lymphocyte cultures *in vitro* (Rosenthal *et al.*, 1976). The factors described are a low-molecular factor which elutes from an Agarose A 5m column with serum albumin, presumably a lymphokine, and a high-molecular factor, which elutes from such a column with an IgM marker, presumably an immune complex. The low-molecular factor is non-specific and stimulates immunoglobulin synthesis in both rheumatoid and normal lymphocyte cultures. The high-molecular factor showed specificity in its exclusive stimulation of rheumatoid cells. Recently, evidence has accumulated that in the collaboration between T-B cells and macrophages, T cells not only have an enhancing effect on antibody response, but also have a suppressive effect on antibody formation (Baker *et al.*, 1973; Hardin *et al.*, 1973; Dutton, 1973; Herzenberg *et al.*, 1973; Sjöberg, 1972). The experiments presented here were designed to investigate the possibility that not only a B-cell enhancing function, but also B-cell suppression function is mediated by soluble factors. Such B-cell inhibitory factors may be produced *in vivo* by the inflamed synovium in rheumatoid arthritis and be present in the synovial effusion.

MATERIALS AND METHODS

Knee-joint effusions from 14 patients with classical or definite rheumatoid arthritis (RA) as defined by the A.R.A. criteria (Ropes *et al.*, 1959) and from 10 non-rheumatoid patients were obtained. The non-rheumatoid patients included six with osteoarthritis, two with ankylosing spondylitis, one with Reiter's syndrome and one with post-traumatic effusion.

The effusions were immediately centrifuged at 2500 rev./min for 20 minutes at room temperature to remove cellular elements and the cell-free effusions treated with 0.02 mg of hyaluronidase/ml of joint fluid (bovine testes hyaluronidase, type I, Sigma Chem.

Accepted for publication August 1975.

Corp., St. Louis, Mo.) for 2 hours at 37°C. The treated synovial effusions were centrifuged again and then either tested immediately or frozen at -70°C until tested.

Lymphocyte cultures were prepared from heparinized peripheral blood obtained from eight different normal donors utilizing the Ficoll-Hypaque gradient separation procedure (Böyum, 1968). Five to 15×10^6 lymphocytes were cultured in 2 ml of RPMI 1640 medium buffered with Hepes buffer (Gibco, Grand Island, N.Y.) and supplemented with 10% heat-inactivated fetal calf serum (FCS) and antibiotics (Penicillin 100 u/ml, Streptomycin 100 µg/ml). Upon addition of prepared synovial fluid, lymphocyte cultures were incubated at 37°C in 95% air-5% CO₂. After 48 hours, cultures were centrifuged at 800 rev./min for 10 minutes and the medium containing all added materials removed and replaced by 2 ml of special minimal essential medium (MEM) (Gibco, Grand Island, N.Y.) lacking four essential amino acids and containing 5% pooled, heat-inactivated human AB serum and antibiotics. To each culture, 5 µCi of ¹⁴C-labelled amino acid mixture (Schwarz-Mann, Orangeburg, N.Y.) was added containing the four amino acids L-arginine (specific activity 312 mCi/mmol), L-leucine (s.a. 312 mCi/mmol), L-lysine (s.a. 310 mCi/mmol) and L-valine (s.a. 258 mCi/mmol) missing in the special MEM, each at a concentration of 250 µCi/ml. The cultures were then incubated under the same conditions for another 24-hour period. On termination of culture, all cellular elements were removed by ultracentrifugation at 35 000 rev./min for 1 hour and the supernatants then dialysed against four changes of 0.01 M-phosphate-buffered saline (PBS), pH 7.2.

The determination of the incorporation of ¹⁴C-amino acids into new synthesized immunoglobulin was performed according to the method of Jasin and Ziff (1969) as modified by Jasin and Ziff (1975). In short, aliquots of 0.1 ml of the supernatants were taken for the determinations of total radioactivity, the TCA-precipitable radioactivity, the radioactivity present in the immune precipitate formed in slight antibody excess with polyvalent goat anti-human immunoglobulin antiserum (Meloy Labs., Springfield, Va.) and in a nonspecific precipitate formed in equivalence by egg albumin-anti-egg albumin. The counts obtained for the nonspecific precipitation represented about 2-5% of the total counts and were subtracted from the counts obtained for the immune precipitate. The precipitates were counted upon addition of 1 ml of 0.1M-NaOH, 2 ml of saline and 10 ml of Aquasol (New England Nuclear, Boston, Mass.) in a Beckman fluid scintillation counter. Cell viability counts performed at the beginning and in some experiments at the end of the cultures were done utilizing the trypan blue dye exclusion technique.

RESULTS

The effect of synovial effusions on cell viability counts and protein synthesis of normal lymphocyte cultures

Different peripheral lymphocyte cultures from different normal donors were incubated in the presence of three different concentrations of six rheumatoid and four non-rheumatoid joint effusions. After 72 hours of incubation, cell viability counts using the trypan blue exclusion technique were performed and are given in Fig. 1. It can be seen that three different amounts of either rheumatoid or non-rheumatoid synovial effusions present in the lymphocyte culture did not change the cell viability counts significantly.

Similar findings are observed in the estimation of protein synthesis as measured by the counts of ¹⁴C precipitated with 5% TCA in 0.1 ml of culture supernatant following an extensive dialysis (Fig. 2). The estimated protein synthesis shows no significant change for the different concentration of effusions added, suggesting that not only is viability not jeopardized, but also no toxic effect is exerted upon the lymphocyte cultures. The

apparent trend toward a decreased incorporation of the ^{14}C -label in the TCA precipitate was not present upon examination of the individual lymphocyte cultures.

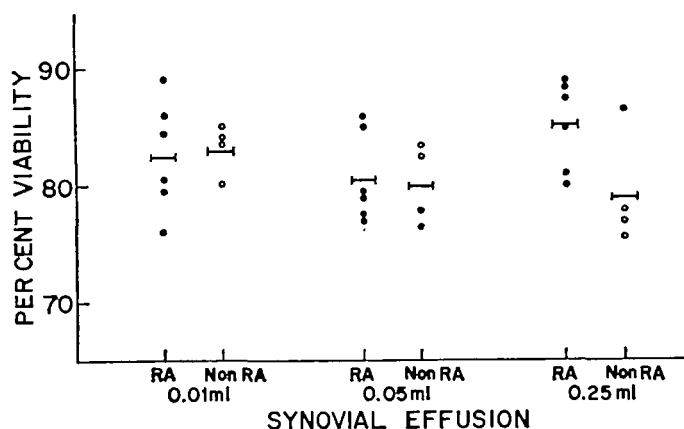


FIG. 1.—Percentage viability at termination of cultures as determined by the trypan blue dye exclusion technique. All determinations were performed in duplicate. There is no significant statistical difference between the values obtained for RA and non-RA synovial effusions containing cultures.

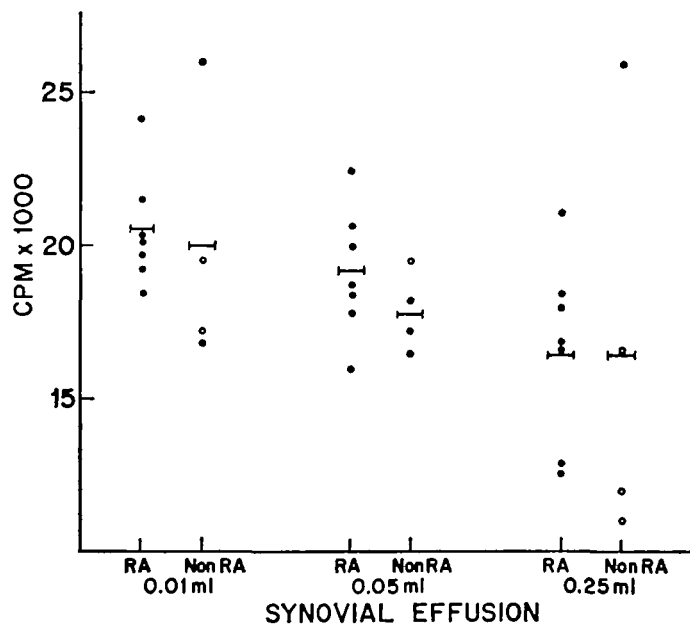


FIG. 2.—Counts per minute obtained for the TCA-precipitable material in 0.1 ml of culture supernatant. All determinations were performed in duplicate. There is no significant statistical difference between the values obtained for RA and non-RA synovial effusions containing cultures.

The effect of different amounts of synovial effusions on immunoglobulin synthesis of normal lymphocyte cultures

Utilizing a radioactive immune coprecipitation assay, immunoglobulin determinations of normal lymphocyte cultures in the presence of different amounts of joint effusions were performed (Table). Immunoglobulin synthesis in the presence of varying amounts

TABLE

THE EFFECT OF DIFFERENT AMOUNTS OF RHEUMATOID AND NON-RHEUMATOID SYNOVIAL EFFUSIONS ON IMMUNOGLOBULIN SYNTHESIS IN NORMAL PERIPHERAL BLOOD LYMPHOCYTES

Amount of effusion added	Rheumatoid arthritis ¹		Non-rheumatoid arthritis ¹	
	ct./min \pm s.d. ²	S.I. \pm s.d. ³	ct./min \pm s.d. ²	S.I. \pm s.d. ³
0.25 ml	597 \pm 97 (<i>P</i> < 0.01)	0.75 \pm 0.13 (<i>P</i> = 0.05)	820 \pm 118 (N.S.)	0.88 \pm 0.11
0.20 ml	1190 \pm 489 (N.S.)	1.14 \pm 0.30 (N.S.)	920 \pm 55 (N.S.)	1.06 \pm 0.17
0.10 ml	1783 \pm 330 (<i>P</i> < 0.01)	1.77 \pm 0.17 (<i>P</i> < 0.001)	827 \pm 147 (N.S.)	0.93 \pm 0.15
0.05 ml	2027 \pm 880 (<i>P</i> < 0.01)	2.14 \pm 0.88 (<i>P</i> < 0.001)	852 \pm 79 (N.S.)	0.99 \pm 0.13
0.01 ml	3125 \pm 946 (<i>P</i> < 0.01)	3.56 \pm 1.17 (<i>P</i> < 0.001)	1009 \pm 260 (N.S.)	1.20 \pm 0.42
0.005 ml	1962 \pm 879 (<i>P</i> < 0.01)	1.87 \pm 0.65 (<i>P</i> < 0.01)	830 \pm 197 (N.S.)	0.95 \pm 0.24
Medium	902 \pm 145			

¹ Fourteen different hyaluronidase-treated synovial effusions from rheumatoid arthritis patients and 10 synovial effusions from non-rheumatoid patients were assayed on eight different normal lymphocyte donors. The non-rheumatoid fluids were obtained from two patients with ankylosing spondylitis, six with osteoarthritis, one with Reiter's syndrome and one with post-traumatic effusion. All experiments were performed in duplicate.

² Counts per minute per 0.1 ml of culture supernatant. The significance is determined as compared to medium control.

³ Stimulation Index = $\frac{\text{ct./min experimental culture}}{\text{ct./min medium control}}$

The significance determined between rheumatoid and non-rheumatoid effusions containing cultures.

of non-rheumatoid effusion shows no significant changes. However, rheumatoid synovial effusions exert profound effects on the production of immunoglobulins. Low concentrations, such as 0.005 ml added to 2 ml lymphocyte cultures are already actively enhancing. Maximal stimulation is present in cultures to which 0.01 ml of joint effusions was added. Increasing amounts of rheumatoid synovial fluids added to the cultures reduced the stimulatory effect with, eventually, no immunoglobulin synthesis upon addition of 0.25 ml of synovial fluid.

DISCUSSION

The rheumatoid synovium is a playground for multiple immunological and metabolic events resulting in a chronic perpetuating inflammation. Both humoral and cellular immune mechanisms have been shown to be involved in this process (Pekin *et al.*, 1964; Smiley *et al.*, 1968; Winchester *et al.*, 1969; Rodman *et al.*, 1967; Bacon *et al.*, 1973; Waxman *et al.*, 1973; Rothenberger and Thiele, 1970; Clot *et al.*, 1973). It is therefore to be expected that synovial effusions will contain material released during such events. In earlier publications, we have demonstrated that mediators of delayed hypersensitivity can be found in rheumatoid synovial effusions (Stastny *et al.*, 1975) and intra-articular injections of such mediator preparations evoke the development of a chronic synovitis resembling to some extent rheumatoid arthritis (Andreis *et al.*, 1974). In a recent publication we adduced evidence for the presence of factors enhancing the synthesis of immunoglobulins in rheumatoid joint effusions (Rosenthal *et al.*, 1976).

The immune response to certain antigens is controlled not only by a T cell population with enhancing capacity on immunoglobulin synthesis, but also by a subpopulation of T cells with suppressive effect on antibody response. Recent work done by Katz, Paul and Benacerraf (1973) interpreted the observation of inhibition of antibody response as due to release of soluble inhibitory mediators most likely by T lymphocytes. In this communication, evidence was brought that the two antagonistic activities can be demonstrated in synovial fluids produced *in vivo*. To what extent suppressive or enhancing activities are manifested depends upon the dilution of the synovial effusion while exposed to lymphocyte cultures. The presence of 12.5% rheumatoid synovial fluids in the lymphocyte cultures inhibits the immunoglobulin synthesis specifically without exerting any detectable toxic effect on the viability or impairment of the protein synthesis in the lymphocytes. Subsequent dilution of the synovial fluid gives rise to extensive production of immunoglobulin reaching an optimum concentration of 0.5% of synovial effusion present in the lymphocyte cultures. The effect observed here does not seem to be mediated by a specific factor because it can be demonstrated in normal, unsensitized lymphocytes. The question of whether the suppression or stimulation of immunoglobulin synthesis is mediated by the same factor in different concentrations or by two different agents is the subject of further studies.

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