Clin. exp. Immunol. (1984) **58,** 402–409.

Cytokines and the chronic inflammation of rheumatic disease. II. The presence of interleukin-2 in synovial fluids

A. M. E. NOURI, G. S. PANAYI & SALLY M. GOODMAN *Departments of Medicine and Rheumatology, Guy's Hospital Medical School, London, UK*

(Accepted for publication 25 June 1984)

SUMMARY

Sera and synovial fluids from patients with a variety of rheumatic diseases were assayed for interleukin-2 (IL-2) activity in a system utilizing the proliferative response of cultured human phytohaemagglutinin (PHA) T cell blasts. The synovial fluids from 14 rheumatoid arthritis, eight ankylosing spondylitis, three psoriatic arthritis and 11 osteoarthritis patients, all showed IL-2 activity. The amounts of IL-2 present in the fluids from different diseases were compared and ranged from 4·2 to 140·0 units/ml.

Keywords interleukin-2 rheumatic diseases synovial fluid cytokines chronic inflammation

INTRODUCTION

The rheumatic diseases are divided into two broad categories, (1) the immunoinflammatory group which includes rheumatoid arthritis (RA), ankylosing spondylitis (AS), psoriatic arthritis (PsAr) and systemic lupus erythematosus (SLE) and (2) the degenerative group like osteoarthritis (OA). However, in both groups there is clinical evidence of joint inflammation (hot, tender, painful and swollen joints) and inflammatory changes in the joint effusions characterized by the presence of neutrophils and mononuclear cells. In RA synovial membrane the predominant infiltrating lymphocyte is of the T cell type (Von Boxel & Paget, 1975; Abrahamson, Froland & Natvig, 1975), belongs to the OKT4 (helper/inducer) subpopulation (Duke *et al.*, 1983) and has the surface characteristics of an activated cell (Galili *et al.*, 1979; Burmester *et al.*, 1981; Poulter *et al.*, 1982). The situation with respect to OA is not so clear cut, the disease being considered to be a 'degenerative' or 'wear-and-tear' condition of the articular cartilage (Matthews, 1953). However, several pieces of evidence militate against this proposition. First, is the clear cut genetic influence in primary generalized OA. Second, there are the dramatic metabolic changes in articular cartilage seen in experimental as well as clinical OA (Harper & Nuki, 1980). Third, synovial membrane histology as well as synovial fluid cell analysis show changes compatible with inflammation and probably account for the therapeutic response to non-steroidal anti-inflammatory drugs (Dieppe, 1979). Finally, we have shown in a recent study (Nouri, Panayi & Goodman, 1983) that interleukin-1 (IL-1), a product of activated mononuclear phagocytes, is present to the same extent in the joint effusions from patients with category 1 and 2 diseases. We concluded from that study that, as far as IL-1 production and its role in inflammation was concerned, one could not distinguish between the two categories.

The process of lymphocyte proliferation requires generation of IL-2 and the acquisition of

Correspondence: Professor G. S. Panayi, Department of Rheumatology, Guy's Hospital Medical School, London SE1 9RT, UK.

responsiveness or receptors to this factor (Gronvik & Andersson, 1980). There are two necessary signals for such a process (a) the presence of IL-1 and (b) the presence of a second signal, usually antigenic stimuli. The existence of activated lymphocytes in the SF of RA patients (Galili *et al.*, 1979; Burmester *et al.*, 1981) prompted us to investigate the presence of IL-2 in the fluids of a variety of rheumatic diseases belonging to the two categories. The hypothesis was made that IL-2 would be found more frequently and in greater amounts in the effusions from patients with immuno/inflammatory diseases.

MATERIALS AND METHODS

Clinical material. Aspirated synovial fluids from knee joints and peripheral venous blood were transferred into sterile tubes containing preservative free heparin to give a final concentration of 10 iu/ml. After centrifugation at 1,000*g* for 10 min at 4°C the cell free supernatant was removed and stored at −20°C until used. The test samples were obtained from classical or definite RA (14), AS (eight), PsAr (three), OA (11), SLE (six) and normal (six).

Mononuclear cell preparation. Fifty millilitre blood samples were collected from patients or normal donors into sterile tubes containing preservative-free heparin to give a final concentration of 10 iu/ml. The samples were diluted three-fold with Dulbecco's modified Eagle's medium (DMEM, Gibco) containing antibiotics (100 units penicillin/ml and 100 ug streptomycin/ml, GIBCO). The mononuclear cells (MNC) were separated using a density gradient technique (Boyum 1968). After three washes in DMEM, the final cell pellet was resuspended in DMEM containing 10% heat inactivated (56°C, 30 min) foetal calf serum (FCS, Sera Labs) and adjusted to 1×10^6/ml.

Preparation and purification of IL-2. IL-2 rich supernatant was prepared by stimulating MNC (1×10^6/ml) with PHA (1 μg/ml) for 48 h at 37°C in a humidified atmosphere (5% CO_2/95% air). The cells were spun down and used for the IL-2 absorption study (see later), and the cell free supernatant which was designated 'IL-2 supernatant' was used for development of the IL-2-dependent cell line (CTC). Recombinant IL-2 obtained from Biogen was also used for part of this investigation (see text).

IL-2 was prepared using the supernatant of MNC stimulated with PHA. Saturated ammonium sulphate [$(NH_4)_2$ SO_4] solution was added drop by drop to the supernatant to give a final concentration of 40% and was left for 1 h at room temperature followed by centrifugation at 1,000*g* for 15 min. To this supernatant further $(NH_4)_2$ SO_4 solution was added to give a final concentration of 85%. After 1 h at room temperature and centrifugation at 1,000*g* for 20 min the pellet was dissolved in a minimal volume of phosphate-buffered saline and was used for gel chromatography on Sephadex G-100 (Pharmacia). The flow rate of the column was adjusted to 18 ml/h and 6 ml fractions were collected and millipore filtered (0·22 μm Millex) before use. The markers used for mol. wt calibration of the column were cytochrome C (Cyt C, 12,400), soya bean trypsin inhibitor (SBTI, 21,000) and bovine albumin (67,000). The column fractions were tested for IL-2 activity on CTC. The fractions showing stimulatory activity were pooled and dialysed against a large volume of distilled water at 4°C for 24 h. The dialysate was then freeze dried and kept at −20°C until used. When needed, a known weight of this preparation was dissolved in DMEM and millipore filtered before use as a standard purified IL-2.

IL-2-dependent T cells. These were prepared essentially as described by Bonnard, Yasaka & Maca (1980). The density gradient separated MNC were incubated with PHA (1 μg/ml, Sigma) for 48 h at 37°C in a humidified atmosphere (5% CO_2/95% air), centrifuged at 450*g* for 10 min a 4°C and resuspended in DMEM containing 20% FCS and 20% IL-2 supernatant to give a final cell concentration of 5×10^5/ml. After every 48 h of culture, the above procedure was repeated for a total of 14 days. The cells left at this time were designated as IL-2-dependent CTC and were assayed in the IL-2 assay; three CTC were developed and numbered 1 to 3. Another IL-2-dependent cell line of murine origin, i.e. HT-2 (Watson, 1979) which has absolute IL-2 requirement for its growth was also used in order to investigate the specificity of stimulatory factor(s) present in synovial fluids.

IL-2 assay. IL-2 was measured by its ability to stimulate proliferation of (i) CTC (Gillis *et al.*, 1978; Bonnard *et al.*, 1980). After 14 days of culture the CTC were centrifuged and the cells were

resuspended in DMEM to 1×10^6/ml. Aliquots of this cell suspension were dispensed into the flat bottomed wells of microtitre plates (Linbro, Titertek) in the presence or absence of test samples. After treatment with the appropriate test sample of IL-2 standards, the CTC were incubated for 24 h at 37°C in a humidified atmosphere (5% CO_2/95% air). The cells were then pulsed with tritiated thymidine (^{3}H-TdR, 0·2 μCi/well, specific activity 5 Ci/mmol, Amersham Intenational) and the incubation was continued for a further 24 h under the same conditions. (ii) HT-2 (Watson, 1979). These cells were aliquoted to give 5,000 cells/well in the presence or absence of test material or standard IL-2 for 24 h at 37°C. The ^{3}H-TdR was added at 0·2 μCi/well at the beginning of the culture. After termination of the incubation period the contents of each well were collected on Whatman (GF/C) filter paper using a multiple semi-automated cell harvester. The incorporation of ^{3}H-TdR to the cells was measured using a liquid scintillation counter (LKB, Wallac). All sera and synovial fluids in this study were assayed at four dilutions 1·25, 2·5, 7·5 and 15% (vol./vol.). The dilution giving the highest incorporation of ^{3}H-TdR by the TC or HT-2 was the one chosen as demonstrating the optimum level of IL-2 activity, and the number of units of IL-2 was calculated from the appropriate standard curves. The standard curves were constructed for each experiment using serial dilutions of standard IL-2 preparation.

IL-2 absorption. This was carried out essentially as reported by Bonnard, Yasaka & Jacobson (1979). The PHA stimulated MNC were prepared as described before. Aliquots of this cell suspension (20×10^6/ml) were added to known amounts of standard IL-2 (0·5–5·0 units) or to synovial fluids to give an equivalence of 1 or 5 units of IL-2 as measured by the IL-2 assay. The samples were incubated for 2 h at 37°C and then centrifuged at 450*g* for 10 min. The cell free supernatants were then assayed for the presence of IL-2 on CTC culture alongside the original sample for the purpose of comparison.

RESULTS

Response of CTC to IL-2. Although CTCl and 2 proliferated in response to standard IL-2, the magnitude of the response differs greatly between them (Fig. 1), so that the maximum response of CTCl was about 6,000 d/min, whereas that for CTC2 was 26,000 d/min. However, it was found that both cell lines peaked at 10 units of IL-2 and further increase in IL-2 concentration resulted in a decrease in the proliferative activity which is in accordance with the report by Gramatzki *et al.* (1982) that high levels of IL-2 will suppress CTC proliferation. A similar phenomenon was observed when three RA synovial fluids, thought to contain IL-2, were added to CTC. As can be seen from Table 1, the fluids gave maximum ^{3}H-TdR incorporation at 7·5% while at 15% CTC proliferation was inhibited.

IL-2 activity in patient materials

In sera. All sera apart from two (one AS and one SLE) failed to stimulate proliferation of CTC

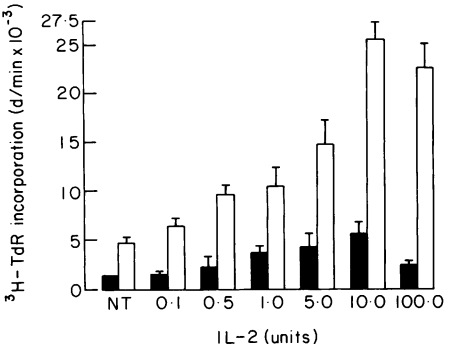

Fig. 1. The proliferative response of two separate T cell lines, CTCl (■); and CTC2 (□) to increasing amounts (0·1 to 100 units/ml) of a standard IL-2 preparation. NT stands for cells grown in tissue culture medium alone.

Table 1. The effect of different concentrations of synovial fluids on the proliferation of cultured T cells

Synovial fluid	Concentration of fluid			
	1·25%	2·5%	7·5%	15%
1	4,579±776	8,542±1,999	11,909±1,504	8,542±199
2	5,300±888	8,997±1,509	10,031±2,471	8,506±1,268
3	5,215±1,407	9,044±907	13,217±1,826	12,618±1,368

Results are expressed in d/min of incorporated ^{3}H-TdR. Background level of ^{3}H-TdR incorporation for CTC cultured in tissue culture medium alone was 4,844±213. Each value in this and subsequent tables and figures is the mean ±1 s.d. of triplicate cultures.

cultures. This prompted us to investigate whether the lack of activity in the sera was due to absence of IL-2 or the presence of inhibitory factor(s) capable of masking IL-2 activity. Therefore, 1 unit of IL-2 was added to CTC in the presence or absence of different volumes of sera. IL-2 alone resulted in ^{3}H-TdR incorporation by the CTC and the addition of sera did not give any significant degree of inhibition (data not shown). This experiment ruled out the possibility that inhibitory factor(s) were present in these sera.

In synovial fluids. The results are presented in Table 2. Fluids from all four groups of patients tested had detectable levels of IL-2 although the concentration varied from fluid to fluid. The level of IL-2 activity was calculated by testing a whole range of concentrations (1·25–15%) of synovial fluids on CTC cultures. The concentration at which maximum proliferative response was obtained was taken as the optimal level of IL-2 units, using a standard curve for IL-2. It is worth noting that synovial fluids obtained from OA patients also contained detectable levels of IL-2 and in some cases higher levels than those of RA fluids.

Characterization of IL-2 activity

In order to establish whether the stimulatory activity found in synovial fluids was IL-2 the following experiments were done.

Dialysis. Two synovial fluids (2 ml) from RA patients were dialysed against large volumes of

Table 2. IL-2 concentration (u/ml) present in the synovial fluids from different rheumatic diseases

Synovial fluid	No. positive/ No. tested	IL-2 (u/ml)
Rheumatoid arthritis	14/14	31·6±30·3* (4·2–106)†
Ankylosing spondylitis	8/8	37·3±43·6 (6·0–140)
Psoriatic arthritis	3/3	24·1±17·2 (5·7–40·0)
Osteoarthritis	11/11	28·3±33·5 (7·2–120)

* Mean ± 1 s.d., † range of values.

DMEM (2000 ml) for 24 h at 4°C. After completion of dialysis the samples were tested for their stimulatory activity on CTC and were compared with the untreated fluids. After dialysis fluids retain their proliferative activity (results are not shown), thus demonstrating that the stimulatory activity in the synovial fluids is non-dialysable.

Ammonium sulphate precipitation. Two RA fluids showed proliferative activity after precipitation with 40% followed by 85% $(NH_4)_2 SO_4$ (data not shown).

Column fractionation. Sephadex G-100 fractionation of synovial fluids was an additional step in the characterization of the mol. wt of the stimulatory activity present in them. The results of one such fluid is shown in Fig. 2. In column fractions 49 to 60, inclusive, corresponding to a mol. wt range of 10,000 to 20,000 daltons, maximum proliferative activity was observed. A similar profile of activity on the CTC was also observed for a synovial fluid from an OA patient (results not shown). It is of interest to note that some activity was also present in fractions 29 to 40, inclusive, corresponding to a mol. wt of greater than about 50,000 daltons. The nature of this large molecular weight stimulatory activity is presently under investigation.

IL-2 absorption

Under appropriate conditions T lymphocytes develop a receptor capable of recognizing the IL-2 molecule. This property of activated lymphocytes was exploited in order to investigate whether the stimulatory factor present in the synovial fluids can be absorbed out using these cells. Fig. 3 shows the proliferation response of CTC culture to standard IL-2 (a) and three synovial fluids, two RA (b,c) and one OA (d), before and after absorption with PHA blast cells. As expected, addition of increasing amounts of standard IL-2 and synovial fluids resulted in an increasing proliferative response. However, after absorption of the standard IL-2 and the fluids with the PHA blasts there was a significant decrease in the response (solid columns). For example, at 5 units of IL-2 (a) the level of ^{3}H-TdR incorporation by the CTC before and after absorption were about 5,000 and 1,800 d/min, respectively and the corresponding values at 1 unit of IL-2 were 3,000 and 1,200 d/min. This finding demonstrates that the blasts are capable of absorbing out the IL-2 activity with an efficiency of about 60%. This pattern of activity was also evident when blasts were added to synovial fluids. As can be seen all three fluids stimulated CTC proliferation at both 1 and 5 units of IL-2, 5 units being

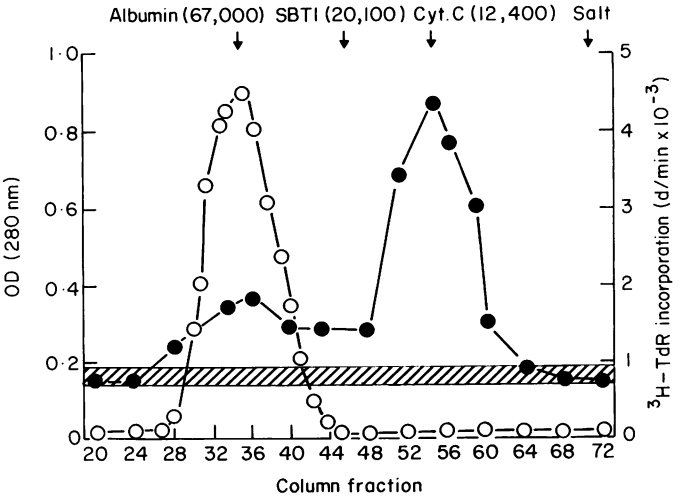

Fig. 2. Sephadex G-100 fractionation of 2 ml of RA synovial fluid. Eluted protein is shown as optical density (OD 280 nm o——o) and the proliferative response of CTC to the column fractions is shown in closed circles (●——●). Activity was found predominantly in the mol. wt range of 10,000–20,000 daltons. The hatched area shows the level of ^{3}H-TdR incorporation by unstimulated CC.

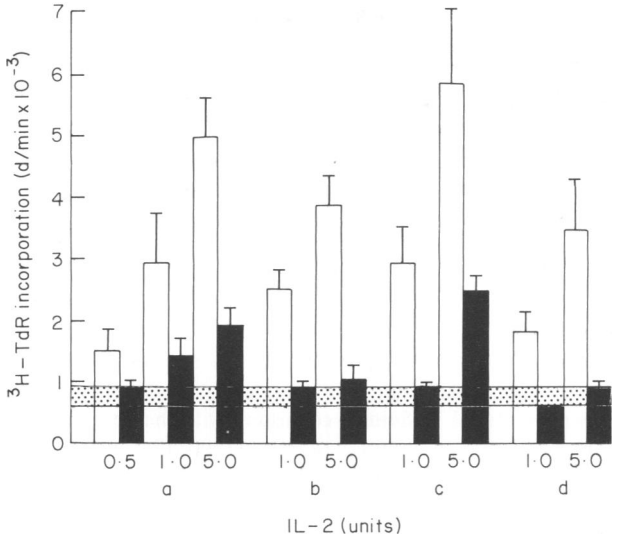

Fig. 3. The proliferative response of CTC culture to standard IL-2 (a) and three synovial fluids, two RA (b,c) and one OA (d) before (□) and after (■) absorption with PHA blast cells.

more stimulatory. After the absorption of the fluids with the blasts there is a universal decrease in the ability of these fluids to stimulate the CTC, although the decrease varied between the samples.

Thus, samples (b) and (d) at 1 unit of IL-2 almost completely lost their stimulatory activities, but at 5 units there was still enough IL-2 activity to stimulate CTC by about two-fold over the unstimulated cells. These findings indicate that stimulatory activity present in the fluids can be absorbed out by IL-2 bearing lymphocytes (Bonnard *et al.*, 1979).

HT-2 proliferation

In order to investigate the nature of the stimulatory factor(s) present in synovial fluids in more detail parallel study of these fluids and recombinant IL-2 (Biogen) on proliferation response of CTC and HT-2 were carried and the following results were obtained. First, both cells lines responded in dose related manner to IL-2 with the peak of proliferative activity at about 200 units of IL-2 (results are not shown). Second, both cell lines were found to respond in a bell shaped dose–response manner to three RA fluids investigated. As can be seen in Table 3, the number of units of IL-2 obtained by both cell lines in response to fluids '1' and '2' are very close although in the case of fluid '3' it looks as if the CTC line is more sensitive to the factor(s) present in the fluid.

Table 3. IL-2 concentration (units/ml) in three RA synovial fluids assayed on both CTC and HT-2 cell lines

	IL-2 (u/ml)	
RA fluids	CTC	HT-2
1	13·9 ± 1·2	11·5 ± 1·5
2	18·0 ± 3·7	13·0 ± 3·0
3	70·0 ± 7·0	42·0 ± 8·2

Each value is mean ± 1 s.d.

DISCUSSION

This study has shown that SF from patients with immunoinflammatory joint diseases RA, AS and PsAr and from patients with degenerative OA have IL-2 activity which varies in individual SF from 4·2 to 140·0 units/ml. There was no significant difference in the concentration of IL-2 between the different disease groups. This activity was shown to be IL-2 as it was not dialysable, was precipitated by 85% $(NH_4)_2SO_4$ and had a mol. wt of 10,000–20,000 daltons on Sephadex G-100 chromatography. Furthermore, it could be absorbed out without PHA blast lymphocytes which is an accepted criterion for IL-2 activity (Gillis *et al.*, 1978). Further confirmation about the nature of CTC stimulatory factor(s) present in the fluids being IL-2 came from the finding that these fluids are capable of stimulating the well established murine cell line i.e. HT-2 to proliferate.

When fractionated on Sephadex G-100, the bulk of proliferative activity on CTC was found to occur in a mol. wt range of 10,000–20,000. However, a second but smaller peak of activity corresponding to a mol. wt of greater than 50,000 was also observed (Fig. 2). The presence of this larger mol. wt 'blastogenic material' in fluids is consistent with the observation made by Stastny *et al.* (1975) who demonstrated that RA fluids tested at 1:200 dilution were able to stimulate the incorporation of ^{3}H-TdR into mouse spleen cells. After fractionation of fluids on Sephadex G-200, the mol. wt of the stimulatory activity was found in peak III which corresponded to a molecular size of greater than 50,000 daltons and was designated as 'blastogenic activity'. Although the nature of this factor(s) is not clear, it is conceivable that it might be a polymeric form of IL-2 or of IL-2 complexed with other molecules or that this is due to a lymphokine mitogenic factor (Gillis, Smith & Watson, 1980). These possibilities are currently under investigation.

The inability of sera to stimulate CTC proliferation may have been due either to the absence of IL-2 or to the presence of inhibitory factor(s). The latter is unlikely since the addition of an increasing volume of serum to CTC in the presence of a known amount of IL-2 did not lead to a decrease in CTC proliferation. The absence of IL-2 in sera is in accord with the findings of Donohue & Rosenberg (1983) who demonstrated that IL-2, after i.v. injection of large quantities into the mouse, could be removed from the circulation within 5 min. One possible explanation regarding the presence of IL-2 in two of the sera tested in this study (one SLE and one AS) might be the presence of large amounts of IL-2 in the circulation of the patients at the time of sample collection. Unlike our study, Wilkins *et al.* (1983) were able to find IL-2 activity in only 10 of 14 RA SF and in none of two OA fluids. Three possible explanations exist for this discrepancy. First, they considered a sample to be positive if it stimulated the proliferative response of CT6 to twice that of unstimulated cells while in our study we estimate the amount of IL-2 activity present in the sample. Second, they used the mouse CT6 cell line while we used our own PHA generated human CTC for IL-2 assay. Third, the highest concentration of SF used by them was only 10% unlike the 15% used by us. In a more recent report by Wood *et al.* (1983) it was claimed that the stimulatory factor present in synovial fluids is IL-1. Although such a conclusion is in accordance with our previous findings (Nouri *et al.*, 1983) their inference regarding the absence of IL-2 in fluids is questionable since they draw their conclusion from testing one single RA fluid at a single dilution.

IL-2 is a product of activated lymphocytes (Gillis *et al.*, 1978). In immunoinflammatory rheumatic diseases, and particularly in RA, there are activated lymphocytes in SF and synovial membrane (Burmester *et al.*, 1981; Poulter *et al.*, 1982). Thus it is not surprising to find IL-2 in the SF from such diseases. However, the situation with OA is not so clear. There are few if any immunological mechanisms operating in this disease, although SF and synovial membrane can have a minor degree of infiltrating inflammatory cells. It has been proposed that phagocytic cells can be activated by phagocytosing of damaged cartilage, bone fragments and cartilage matrix products (Dieppe, 1980) which could result in the production of IL-1 and other inflammatory mediators. In this study, however, the finding that the presence of IL-2 activity in immunoinflammatory fluid is the same as in OA fluids requires explanation. This raises the following possibilities; (a) that MNC infiltrating OA fluids and membranes are much more efficient IL-2 producers than corresponding RA MNC, (b) that there are more activated lymphocytes in the SF and membrane of RA and other immunoinflammatory diseases capable of absorbing out any IL-2 produced by activated T cells compared to OA. These possibilities are currently under investigation.

REFERENCES

ABRAHAMSEN, T.G., FRØLAND, S.S. & NATVIG, J.B. (1975) Elution and characterisation of lymphocytes from rheumatoid inflammatory tissue. *Scand. J. Immunol.* **4,** 823.

BONNARD, G.D., YASAKA, K. & JACOBSON, D. (1979) Lignand-activated T-cell growth factor induced proliferation: absorption of T-cell growth factor by activated T cells. *J. Immunol.* **123,** 2704.

BONNARD, G.D., YASAKA, K. & MACA, R.D. (1980) Continued growth of functional human T lymphocytes: Production of human T cell growth factor. *Cell. Immunol.* **51,** 390.

BÖYUM, A. (1968) Separation of leukocytes from blood and bone marrow. *Scand. J. clin. lab. Invest.* **21,** 97.

BURMESTER, G.R., YU, D.T.Y., IRANI, A.M., KUNKEL, H. & WINCHESTER, R. (1981) Ia^+ T-cells in synovial fluid and tissues of patients with rheumatoid arthritis. *Arthrit. Rheum.* **24,** 1370.

DIEPPE, P. (1982) In *Scientific Basis of Rheumatology* (ed. by G. S. Panayi) Chap. 13, p. 255. Churchill-Livingston, Edinburgh.

DONOHUE, J.H. & ROSENBERG, S.A. (1983) The fate of interleukin-2 after *in vivo* administration. *J. Immunl.* **130,** 2203.

DUKE, O., PANAYI, G.S., JANOSSY, G., POULTER, L.W. & TIDMAN, N. (1983) Analysis of T-cell subsets in the peripheral blood and synovial fluid of patients with rheumatoid arthritis by means of monoclonal antibodies. *Ann. Rheum. Dis.* **38,** 63.

GALILI, U., ROSENTHAL, L., GALILI, N. & KLEIN, E. (1979) Activated T cells in the synovial fluid of arthritic patients: characterisation and comparison with *in vitro* activated human and murine T cells in cooperation with monocytes in cytotoxicity. *J. Immunol.* **122,** 878.

GILLIS, S., FERN, M.M., OU, W. & SMITH, K.A.A (1978) T cell growth. Parameters of productio and a quantitative microassay for activity. *J. Immunol.* **120,** 2027.

GILLIS, S., SMITH, K.A. & WATSON, J. (1980) Biochemical characterisation of lymphocyte regulatory molecule. II. Purification of a class of rat and human lymphokines. *J. Immunol.* **124,** 1954.

GRAMATZKI, M., STRONG, D.M., GROVE, S.B. & BONNARD, G.D. (1982) Cryopreserved human cultured T-cells as responder cells for the quantitative measurement of interleukin-2: improvement of the assay. *J. Immunol. Meth.* **53,** 209.

GRONVIK, K.O. & ANDERSSON, J. (1980). The role of T-cell growth stimulating factors in T-cell triggering. *Immunol. Rev.* **51,** 35.

HARPER, P. & NUKI, G. (1980) Genetic factors in osteoarthritis. In *Aetiopathogenesis of osteoarthritis* (ed. by G. Nuki) pp. 184–202. Pitman Medical, London.

MATTHEWS, B.F. (1953) Composition of articular cartilage in osteoarthritis; changes in collagen/chondroitin sulphate ratio. *Br. Med. J.* **ii,** 660.

NOURI, A.M.E., PANAYI, G.S. & GOODMAN, S.M. (1984) Cytokines and the chronic inflammation of rheumatic disease I. The presence of interleukin-1 in synovial fluids.

POULTER, L.W., DUKE, O., HOBBS, S., JANOSSY, G. & PANAYI, G.S. (1982) Histochemical discrimination of HLA-DR positive cell populations in the arthritic synovial lining. *Clin. exp. Immunol.* **48,** 381.

ROSENSTEIN, M., EBERLEIN, T., KEMENY, M.M., SUGARBAKER, P.H. & ROSENBERG, S.A. (1981) *In vitro* growth of murine T-cells VI. Accelerated skin graft rejection caused by adoptive transferred cells expanded in T cell growth factor. *J. Immunol.* **127,** 566.

STASNY, P., ROSENTHAL, M., ANDREIS, M. & ZIFF, M. (1975) Lymphokines in the rheumatoid joint. *Arthrit. Rheum.* **18,** 237.

VON BOXEL, J.A. & PAGET, S.A. (1975) Predominantly T cell infiltrate in rheumatoid synovial membranes. *N. Engl. J. Med.* **293,** 517.

WATSON, J. (1979) Continuous proliferation of murine antigen specific helper T-lymphocytes in culture. *J. Immunol.* **9,** 592.

WILKINS, J.A., WARRINGTON, R.J., SIGURDSON, S.L. & RUTHERFORD W.J. (1983) The demonstration of an interleukin-2 like activity in the synovial fluids of rheumatoid arthritis patients. *J. Rheumatol.* **10,** 1.

WOOD, D.D., IHRIE, E.J., DINARELLO, C.A. & COHEN, P.L. (1983) Isolation of an interleukin-1-like factor from human joint effusions. *Arthrit. Rheum.* **26,** 975.