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Arthritogenic actions of recombinant IL-1 and tumour necrosis factor α in the rabbit: evidence for synergistic interactions between cytokines *in vivo*

B. HENDERSON & E. R. PETTIPHER *Department of Pharmacology, Wellcome Research Laboratories, Langley Court, Beckenham, UK*

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SUMMARY

Intra-articular injection of highly purified or recombinant interleukin 1 (IL-1) into the rabbit knee induces a transient synovitis with leucocytic infiltration into the synovial lining and joint cavity and loss of proteoglycan from articular cartilage. Tumour necrosis factor α (TNF-α), which has many of the actions of IL-1, in the dose range 50–5 000 ng induced infiltration of leucocytes into the joint but failed to cause significant proteoglycan loss from cartilage. The nature of the leucocytic infiltrate induced by intra-articular TNF-α was predominantly monocytic compared with the mixed polymorphonuclear (PMN)/monocytic infiltrate induced by IL-1. Neither cytokine induced the accumulation of significant numbers of lymphocytes. In addition, on a molar basis, TNF-α was significantly less active than IL-1 in causing cell accumulation in the joint. Injection of submaximal doses of IL-1 and TNF into the rabbit resulted in a marked synergy with respect to the accumulation of PMN. The conclusion from these studies is that TNF-α could contribute to the PMN accumulation in the human joint in rheumatoid arthritis but is unlikely to be important in the destruction of articular cartilage.

Keywords interleukin 1 tumour necrosis factor α arthritis rabbits

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease predominantly afflicting the articulating (synovial) joints. The pathology of the joint lesion is complex with progressive fibrotic growth of the chronically inflamed synovial lining being found in association with the destruction of articular cartilage, subchondral bone and other intra-articular structures (Henderson & Edwards, 1987). The pioneering work of Fell & Jubb (1977) suggested that a soluble factor(s) produced by the synovial lining could stimulate chondrocytes to degrade their own extracellular matrix. This activity released by synovial lining tissue (termed catabolin) has been purified and shown to be interleukin 1 (IL-1) (Saklatvala *et al.*, 1984). There is now an extensive body of literature demonstrating that IL-1 can stimulate cultured articular tissues or cells derived from these tissues to release prostaglandins and metalloproteinases (reviewed by Henderson, Pettipher & Higgs, 1987). Such stimulation, if it occurred *in vivo*, could contribute to inflammation and tissue damage. Recently we have demonstrated that intra-articular injection of IL-1 into the rabbit knee induces a transient synovitis with the loss of proteoglycan from articular cartilage (Pettipher *et al.*, 1986).

It is unlikely that IL-1 alone can account for the pathology of RA. For example, tumour necrosis factor α (TNF-α) is also found in rheumatoid synovial fluids (Di Giovine, Nuki & Duff, 1988). In recent years it has been reported that TNF-α, one of the major cytokines produced by macrophages, has many of the pro-inflammatory properties of IL-1. It will stimulate human synovial cells to release PGE_2 and collagenase (Dayer, Beutler & Cerami, 1985) and cause resorption of bone and cartilage *in vitro* (Bertolini *et al.*, 1986; Saklatvala, 1986). There is also the strong suggestion from studies *in vitro* that IL-1 and TNF-α play a role in the leucocyte infiltration which occurs in rheumatoid joints. Addition of IL-1 to cultured vascular endothelial cell monolayers causes them to become adhesive for polymorphonuclear neutrophil leucocytes (PMNs), monocytes (Bevilacqua *et al.*, 1985) and lymphocytes (Cavender, *et al.*, 1986). This effect is caused by alterations in the surface properties of the endothelial cells rather than changes in the leucocytes (Bevilacqua *et al.*, 1985). TNF-α also promotes leucocyte adhesion (Gamble *et al.*, 1985; Bevilacqua *et al.*, 1987) but, unlike IL-1, it activates both the endothelium and the leucocytes to induce this process (Gamble *et al.*, 1985). IL-1 has been reported to be directly chemotactic *in vitro* for PMNs and monocytes (Luger *et al.*,

Correspondence: B. Henderson, Immunopharmacology Department, Celltech Limited, 216 Bath Road, Slough SL1 4EN, UK.

1983) and for lymphocytes (Miossec, Yu & Ziff, 1984). More recent studies using recombinant IL-1-α and IL-1-β have suggested that these molecules are only weak activators of PMN motility *in vitro* (Figari, Mori & Palladino, 1987; Georgilis *et al.*, 1987). TNF-α has also been reported to be chemotactic *in vitro* (Figari *et al.*, 1987; Ji Ming *et al.*, 1987). Irrespective of the *in vitro* evidence it is clear that IL-1 causes leucocyte accumulation *in vivo* (Pettipher, Higgs & Henderson, 1986; Movat *et al.*, 1987).

This range of *in vitro* activities suggests that TNF-α is likely to be important as a mediator of joint pathology in RA. There is also growing evidence that there are synergistic interactions between IL-1 and TNF-α, both *in vitro* (Elias *et al.*, 1987; Stashenko *et al.*, 1987) and *in vivo* (Movat *et al.*, 1987).

In this study the pro-inflammatory and cartilage-degrading activity of human recombinant (r) TNF-α in the rabbit has been assessed and compared with rIL-1. In addition, the possibility of synergistic interactions between these two cytokines has been examined.

MATERIALS AND METHODS

Materials

Human recombinant TNF-α (rTNF-α) was kindly provided by Genentech Inc. (San Francisco, CA). The specific activity was $3{\cdot}7 \times 10^7$ units/mg and endotoxin contamination was approximately 1 ng/mg protein. Recombinant IL-1 α and β were obtained commercially from Genzyme (Suffolk, UK) and IL-1-α was kindly donated by Dainippon Pharmaceuticals Ltd. (Osaka).

Assay of cytokine activity

To determine if there was loss of activity during storage, the activity of the TNF-α was assayed by a cytotoxicity assay using mouse L929 cells as described by Ruff & Gifford (1980). This assay confirmed the manufacturer's specific activity and showed negligible loss of activity during the course of the experiments described. To determine if rTNF-α could stimulate cells derived from the rabbit, fibroblasts were prepared from the synovial lining of normal rabbits. Briefly, the synovial lining was minced and digested with a mixture of bacterial collagenase, protease and hyaluronidase (Sigma). Cells were washed, filtered to remove debris and grown to confluency in 75 cm^2 flasks. After three passages cells were grown to confluency in 24-well plates. To assess the activity of cytokines, graded concentrations of IL-1-α (Dainippon) or TNF-α were added and the cells were incubated for 20 h. At the end of this time the culture fluids were collected and the amount of prostaglandin E_2 (PGE_2) assayed by radioimmunoassay as described by Salmon (1978).

Intra-articular injection of cytokines

Adult male New Zealand White rabbits (weight 2·5–3·5 kg) were used. Dilutions of IL-1 or TNF-α (or combinations of both cytokines) in phosphate-buffered saline (PBS) containing 0·02% heat-inactivated fetal calf serum (FCS: Hyclone, Utah, USA—low in endotoxin FCS) were injected into the knee through the suprapatellar ligament into the joint space. To minimize adsorption of proteins to plasticware the solutions of cytokines were prepared immediately before injection. To control for the effects of injection, contralateral knee joints were injected with an equal volume of vehicle. Animals were killed (by overdose of Euthetal: May and Baker) 4 h or 24 h after a single injection and the joint diameters were measured, just before killing, by calipers.

The synovial fluid from vehicle- and cytokine-injected knees was collected by injecting 1 ml of sterile saline. After gently manipulating the joint to mix the contents of the synovial cavity the joint was opened and the fluid (termed the joint wash) carefully collected. The jont wash was centrifuged for 30 seconds in an Eppendorf microcentrifuge to sediment infiltrating leucocytes. These were resuspended and used for the measurement of total and differential cell counts by conventional methods. The cell-free supernatants were used for the measurement of prostaglandin E_2 (PGE_2) and proteoglycan in the synovial fluid. Samples of the synovial lining from control and cytokine-injected joints were dissected and fixed in buffered formalin, embedded in paraffin wax, sectioned at 5 μm and stained with haematoxylin and eosin. The articular cartilage was dissected from the ends of the femurs of control and cytokine-injected joints for the assay of the proteoglycan content.

Assay of PGE_2

The PGE_2 concentration in the unextracted joint washes was measured as described by Salmon (1978). Briefly, rabbit antiserum to PGE_2 was incubated with test samples and tritiated standards. Free radioactive material was removed with dextran-coated charcoal and the residual bound activity was measured in a scintillation spectrometer. The limit of sensitivity of this assay is 0·05 ng/ml.

Assay of the proteoglycan content of articular cartilage

The articular cartilage slices dissected from the ends of the femurs of experimental or control joints were blotted dry, weighed and digested with papain at 65°C for 1 h to liberate the glycosaminoglycans (GAGs). The concentration of sulphated GAGs was measured by the 1,9-dimethylmethylene blue binding assay (Farndale, Sayers & Barrett, 1982). The loss of proteoglycan was then calculated on a percentage basis relative to the vehicle-injected control joints.

Assay of the proteoglycan content of synovial fluid

Aliquots of the synovial joint wash from vehicle- or cytokine-injected joints were digested with papain to liberate the GAGs and reacted with 1,9-dimethylmethylene blue as described by Farndale, Buttle & Barrett (1986).

RESULTS

Stimulation of rabbit synovial fibroblast PGE_2 synthesis by cytokines

Confluent cultures of rabbit synovial fibroblasts (fourth passage) were stimulated with graded concentrations of IL-1-α or TNF-α for 20 h and then the PGE_2 concentration in the culture supernatants was measured. Rabbit synovial fibroblasts normally show a maximal response with between 0·1 and 0·5 ng/ml IL-1 (either α or β). The peak response with TNF-α in this experiment occurred at 1 ng/ml. Therefore, with this cell population rTNF-α is some 2–10 times less potent than recombinant IL-1-α (Fig. 1).

Joint swelling and PGE_2 synthesis

As reported in an earlier study, single injections of IL-1 into the rabbit knee do not cause swelling of the joint or the appearance

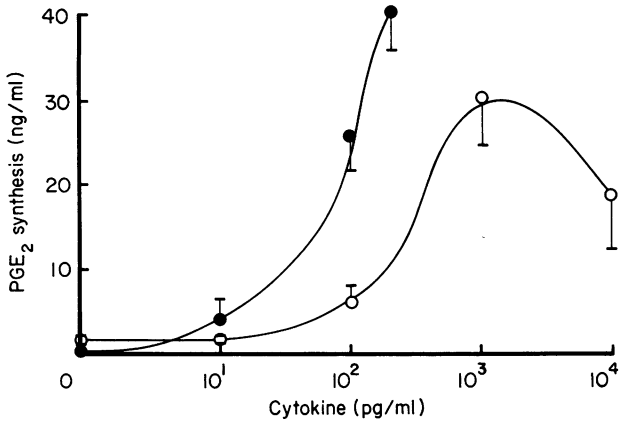

Fig. 1. Synthesis of PGE_2 by rabbit synovial fibroblasts exposed for 24 h to graded concentrations of IL-1-α (●) or TNF-α (○). Results are expressed as mean ± s.d. of triplicate cultures.

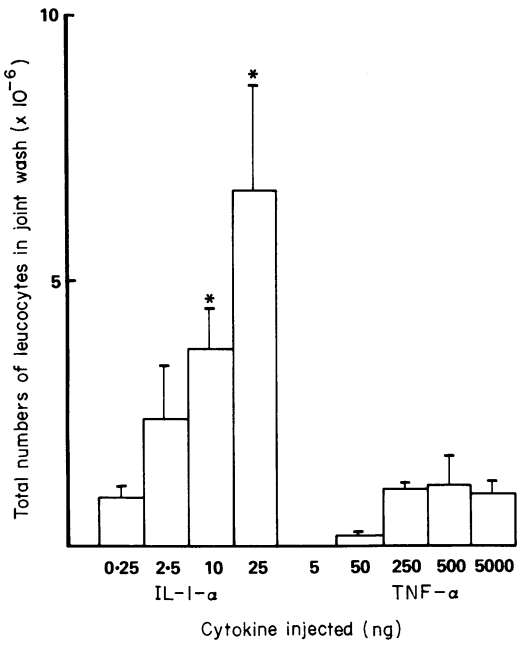

Fig. 2. The total numbers of leucocytes accumulating in the joints of rabbits injected with various doses of IL-1-α or TNF-α. Results are expressed as mean ± s.e.m. for groups of 3–6 animals at each dose of cytokine. The numbers of cells in contralateral vehicle-injected joints were $< 10^4$ cells. $^*P < 0{\cdot}01$ compared to maximal cell accumulation induced by TNF-α.

of PGE_2 in the joint wash (Pettipher, Higgs & Henderson, 1986). Injections of TNF-α at doses of up to 5 μg were also not associated with joint swelling or eicosanoid synthesis *in vivo*. This failure to stimulate swelling/PGE_2 synthesis was found both at 4 h and 24 h after injection of TNF-α.

Cellular accumulation in cytokine-injected joints

Injection of as little as 250 pg of IL-1 (α or β) into the knee joints of rabbits resulted in significant accumulation of leucocytes in the joint cavity and synovial lining both at 4 h and 24 h after injection. There was a dose-dependent increase in leucocyte

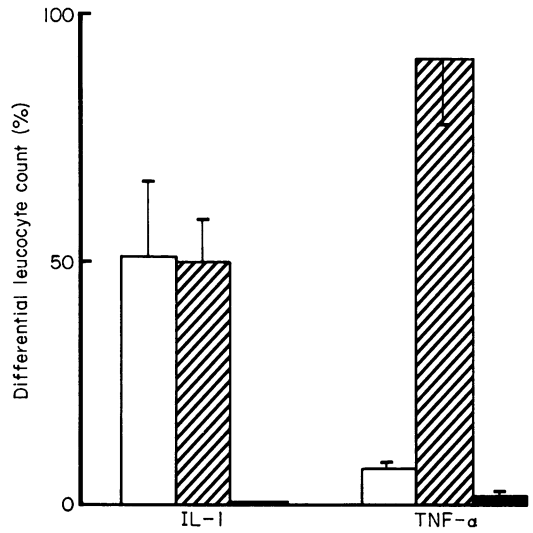

Fig. 3. The differential counts of the infiltrating leucocytes in the joints of rabbits 24 h after injection of 10 ng IL-1-α or 250 ng TNF-α. Results are expressed as the mean ± s.e.m. of nine animals. PMNs □, monocytes ▨, lymphocytes ■.

accumulation at 24 h over the range 0·25–25 ng IL-1 (Fig. 2). There were no qualitative or quantitative differences between IL-1-α and IL-1-β in this respect. In contrast to IL-1, significant accumulation of leucocytes was found only with doses of TNF greater than 50 ng. The maximum accumulation of cells occurred with 500 ng TNF-α with no further increase in cell numbers with 5 μg cytokine. The maximal number of cells induced to infiltrate the joint with TNF-α was significantly less than that induced by IL-1 (Fig. 2).

Injection of IL-1-α or IL-1-β into the rabbit knee reproducibly resulted in the accumulation of approximately equal numbers of PMN and monocytes in the joint cavity 24 h later. In three separate experiments, groups of three animals were injected with 10 ng IL-1-α or 250 ng TNF-α. In the TNF-α-injected joints only a small percentage of the infiltrating leucocytes were PMN, the majority of cells being monocytes (Fig. 3).

Effect of intra-articular cytokines on cartilage proteoglycan

The loss of proteoglycan from articular cartilage can be determined directly by measurement of the proteoglycan content of the cartilage, or indirectly by assay of the proteoglycan content of the synovial joint wash. Intra-articular injection of 10 units of highly purified IL-1 causes significant loss of proteoglycan from cartilage (Pettipher *et al.*, 1986). Injection of rIL-1s causes a dose-dependent loss of proteoglycan from cartilage and a dose-dependent increase in proteoglycan in the synovial fluid (Henderson & Pettipher, 1988). In 15 animals injected with TNF-α in the dose range 5–5 000 ng there was no significant loss of proteoglycan from articular cartilage. Analysis of the proteoglycan released into the synovial cavity confirmed the finding that TNF-α causes little cartilage proteoglycan breakdown.

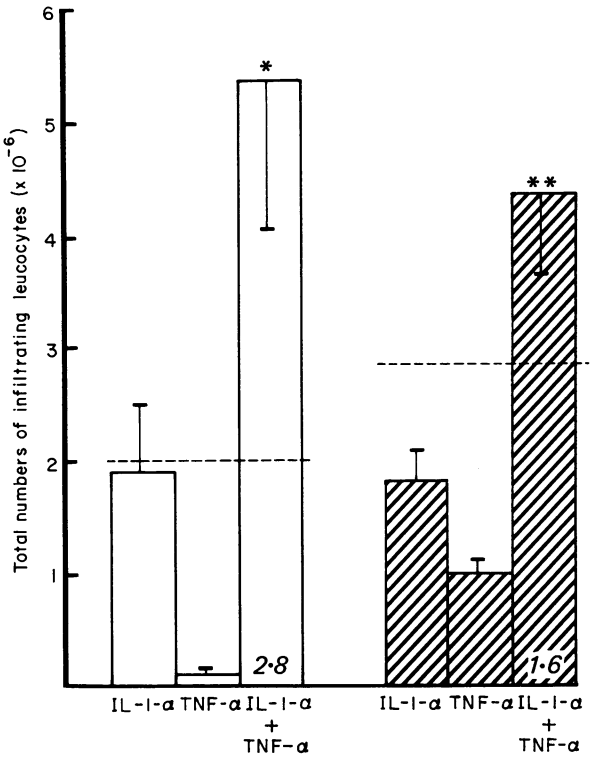

Fig. 4. Synergistic interaction between IL-1 and TNF. The total numbers of PMNs (□) or monocytes (▨) in the joint cavity 24 h after injecting 10 ng IL-1-α, 250 ng TNF-α or a combination of cytokines is shown. The results are expressed as mean ± s.e.m. of nine animals. The figures in the lower part of the right hand columns give the degree of synergy compared with the sum of cellular accumulation produced by individual cytokines. $*P<0{\cdot}02$ compared to the number of PMN induced by IL-1-α. $**P<0{\cdot}001$ compared to the number of monocytes induced by IL-1-α.

Effects of combinations of IL-1 and TNF-α

In a separate series of experiments the effect of intra-articular injections of combinations of IL-1 and TNF-α were compared with the effect of IL-1 or TNF-α alone.

There was no evidence of joint swelling or elevation of PGE_2 in joint fluid after injection of either cytokine alone or after injection of combinations of IL-1 and TNF-α. TNF-α caused little cartilage breakdown when injected alone and did not enhance that induced by IL-1. However, there was a dramatic synergy in regard to the leucocyte infiltration 24 h, but not 4 h after, the two cytokines were co-administered (Fig. 4). The infiltration of both PMN and monocytes was enhanced by co-administration of IL-1 and TNF-α but the PMN demonstrated a more dramatic synergy.

Examination of sections of synovial lining tissue from cytokine-injected joints demonstrated that there were more PMN and monocytes in the joints of animals injected with both cytokines than in those injected with either cytokine alone.

DISCUSSION

This study confirms that IL-1 is a potent inducer of PMN and monocyte accumulation *in vivo*. TNF-α was found to be significantly less active in causing leucocyte accumulation. Much more TNF-α was required to cause a significant infiltrate and the maximal response was much less than that of IL-1 (Fig. 2). In addition, the composition of the infiltrate produced by TNF-α was markedly different from that of IL-1. Twenty-four hours after injection of IL-1 the cellular infiltrate in the joint cavity consisted of approximately equal numbers of PMN and monocytes. In contrast, the infiltrate induced by TNF-α was predominantly monocytic. Neither IL-1 nor TNF-α caused the accumulation of significant numbers of lymphocytes in the synovial lining or joint cavity. This confirms recent studies by Issekutz, Stoltz & Meide (1988) who have shown that γ-interferon, but not IL-1-α or β, causes lymphocyte accumulation after intradermal injection in the rat.

In addition to promoting accumulation of leucocytes, the intra-articular injection of IL-1 induces a dose-dependent loss of proteoglycan from articular cartilage (Pettipher *et al.*, 1986; Henderson & Pettipher, 1988). This loss can be determined by direct assay of the proteoglycan content of cartilage or by measurement of proteoglycan in the synovial fluid, which is low in normal joints. The intra-articular injection of TNF-α did not cause significant loss of proteoglycan from articular cartilage. This may appear, at first sight, to be related to the numbers of infiltrating leucocytes induced to enter the joint by IL-1 or TNF-α. However, we established, by using leucopenic rabbits, that the infiltrating leucocytes are not responsible for the cartilage proteoglycan loss in IL-1-injected joints (Pettipher *et al.*, 1988). Recently, Schnyder, Payne & Dinarello (1987) reported that TNF-α can inhibit plasminogen activator release from cultured rabbit chondrocytes (a property shared with IL-1) but is unable to stimulate the release of metalloproteinase activity. This failure to induce the synthesis and release of enzymes that cause the breakdown of the extracellular matrix of cartilage may explain the inability of TNF-α to cause significant proteoglycan loss from rabbit cartilage *in vivo*.

When IL-1 and TNF-α were co-administered, there was a dramatic synergy with respect to the leucocyte accumulation in the synovial lining and cavity. Surprisingly, this synergy was only found 24 h after injection of cytokines, and not at 4 h. The injection of submaximal doses of IL-1-α and TNF-α together into the rabbit knee resulted in greater than twice the additive number of leucocytes in the joints of animals injected with single cytokines. When the numbers of PMN and monocytes were enumerated separately it was found that the injection of both cytokines resulted in a preferential increase in the former cell population. There were almost three times the number of PMN in joints injected with IL-1-α and TNF-α compared with the additive total for cytokines injected separately. Although the synergistic effect on monocyte accumulation was less pronounced, the injection of both cytokines did induce a significantly greater infiltration of these cells. Such synergy may occur in the rheumatoid joint and contribute to the marked infiltration of PMN in this chronic inflammatory state. Synergy between IL-1 and TNF-α in the local Shwartzman-like reaction in rabbit skin has recently been reported by Movat *et al.* (1987).

In summary, the injection of recombinant human TNF-α into the knee joints of rabbits results in a predominantly monocytic infiltrate with no joint swelling and little damage to cartilage. This suggests that TNF-α does not make a major contribution to the tissue damage seen in chronic arthritis. Injection of submaximal doses of IL-1-α and TNF-α causes a synergistic accumulation of PMN and monocytes and suggests

that the massive polymorphonuclear accumulation in the joints of rheumatoid patients may be caused by the interaction of these, and possibly other, cytokines.

REFERENCES

BERTOLINI, D.R., NEDWIN, G.E., BRINGMAN, T.S., SMITH, D.D. & MUNDY G.R. (1986) Stimulation of bone resorption and inhibition of bone formation *in vitro* by human tumour necrosis factors. *Nature* **319,** 516.

BEVILACQUA, M.P., POBER, J.S., WHEELER, M.E., COTRAN, R.S. & GIMBRONE, M.A. (1985) Interleukin 1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes and related leukocytic cell lines. *J. clin. Invest.* **76,** 2003.

BEVILACQUA, M.P., POBER, J.S., MENDRICK, D.L., COTRAN, R.S. & GIMBRONE, M.A. (1987) Identification of an inducible endothelial-leukocyte adhesion molecule. *Proc. natl. Acad. Sci. USA* **84,** 9238.

CAVENDER, D.E., HASKARD, D.O., JOSEPH, B. & ZIFF, M. (1986) Interleukin 1 increases the binding of human B and T lymphocytes to endothelial cell monolayers. *J. Immunol.* **136,** 203.

DAYER, J-M, BEUTLER, B. & CERAMI, A. (1985) Cachectin/tumour necrosis factor stimulates collagenase and prostaglandin E_2 production by human synovial cells and dermal fibroblasts. *J. exp. Med.* **162,** 2163.

DI GIOVINE, F.S., NUKI, G. & DUFF, G.W. (1988) Tumour necrosis factor in synovial exudates. *Ann. Rheum. Dis.* **47,** 768.

ELIAS, J.A., GUSTILO, K., BAEDER, W. & FREUNDLICH, B. (1987) Synergistic stimulation of fibroblast prostaglandin production by recombinant interleukin 1 and tumour necrosis factor. *J. Immunol.* **138,** 3812.

FARNDALE, R.W., SAYERS, C.A. & BARRETT, A.J. (1982) A direct spectrophotometric microassay for sulphated glycosaminoglycans in cartilage cultures. *Connective Tissue Res.* **9,** 247.

FARNDALE, R.W., BUTTLE, D.J. & BARRETT, A.J. (1986) Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. *Biochim. Biophys. Acta.* **883,** 173.

FELL, H.B. & JUBB, R.W. (1977) The effect of synovial tissue on the breakdown of articular cartilage in organ culture. *Arthritis Rheum.* **20,** 1359.

FIGARI, I.S., MORI, N.A. & PALLADINO, M.A. (1987) Regulation of neutrophil migration and superoxide production by recombinant tumour necrosis factors -α and -β: comparison to recombinant interferon-α and interleukin 1α. *Blood* **70,** 979.

GAMBLE, J.R., HARLAN, J.M., KLEBANOFF, S.J. & VADAS, M.A. (1985) Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumour necrosis factor. *Proc. natl. Acad. Sci. USA*, **82,** 8667.

GEORGILIS, K., SCHAEFFER, C., DINARELLO, C.A. & KLEMPNER, M.S. (1987) Human recombinant interleukin 1β has no effect on intracellular calcium or on functional responses of human neutrophils. *J. Immunol.* **138,** 3403.

HENDERSON, B. & EDWARDS, J.C.W. (1987) *The Synovial Lining in Health and Disease.* Chapman Hall, London.

HENDERSON, B., PETTIPHER, E.R. & HIGGS, C.A. (1987) Mediators of rheumatoid arthritis. *Brit. med. Bull.* **43,** 415.

HENDERSON, B. & PETTIPHER, E.R. (1988) Comparison of the *in vivo* inflammatory activities after intra-articular injection of natural and recombinant IL-1α and IL-1β in the rabbit. *Biochem. Pharmacol.* **37,** 4171.

ISSEKUTZ, T.B., STOLTZ, J.M. & MEIDE, P. (1988) Lymphocyte recruitment in delayed type hypersensitivity. The role of gamma IFN. *J. Immunol.* **140,** 2989.

JI MING, W., BERSANI, L. & MANTOVANI, A. (1987) Tumour necrosis factor is chemotactic for monocytes and polymorphonuclear leukocytes. *J. Immunol.* **138,** 1469.

LUGER, T.A., CHARON, J.A., COLOT, M., MICKSCHE, M. & OPPENHEIM, J.J. (1983) Chemotactic properties of partially purified human epidermal cell-derived thymocyte-activating factor (ETAF) for polymorphonuclear and mononuclear cells. *J. Immunol.* **131,** 816.

MIOSSEC, P., YU, C-L. & ZIFF, M. (1984) Lymphocyte chemotactic activity of human interleukin 1. *J. Immunol.* **133,** 2007.

MOVAT, H.Z., BURROWES, C.E., CYBULSKY, M.I. & DINARELLO, C.A. (1987) Acute inflammation and a Shwartzman-like reaction induced by interleukin-1 and tumour necrosis factor: Synergistic action of the cytokines in the induction of inflammation and microvascular injury. *Am. J. Pathol.* **129,** 463.

PETTIPHER, E.R., HIGGS, G.A. & HENDERSON, B. (1986). Interleukin 1 induces leukocyte infiltration and cartilage proteoglycan degradation in the synovial joint. *Proc. natl. Acad. Sci. USA* **83,** 8749.

PETTIPHER, E.R., HENDERSON, B., MONCADA, S. & HIGGS, G.A. (1988) Leukocyte infiltration and cartilage proteoglycan loss in immune arthritis in the rabbit. *Br. J. Pharmacol.* **95,** 169.

RUFF, M.R. & GIFFORD, G.E. (1980) Tumour necrosis factor. In: *Lymphokine Reports* (vol. II) (ed. by E. Pick) p. 235. Academic Press, NY.

SAKLATVALA, J., PILSWORTH, L.M.C., SARSFIELD, S.J., GAVRILOVIC, J. & HEATH, J.K. (1984) Pig catabolin is a form of interleukin 1. *Biochem. J.* **224,** 461.

SAKLATVALA, J. (1986) Tumour necrosis factor α stimulates resorption and inhibits synthesis of proteoglycans in cartilage. *Nature* **322,** 547.

SALMON, J.A. (1978) A radioimmunoassay for 6-keto-prostaglandin F1α. *Prostaglandins* **15,** 383.

SCHNYDER, J., PAYNE, T. & DINARELLO, C.A. (1987) Human monocyte or recombinant interleukin 1's are specific for the secretion of a metalloproteinase from chondrocytes. *J. Immunol.* **138,** 496.

STASHENKO, P., DEWHIRST, F.E., PEROS, W.J., KENT, R.L. & AGO, J.M. (1987) Synergistic interactions between interleukin 1, tumour necrosis factor, and lymphotoxin in bone resorption. *J. Immunol.* **138,** 1464.