Clin. exp. Immunol. (1984) **57**, 324–330.

Anergy in sarcoidosis: the role of interleukin-1 and prostaglandins in the depressed *in vitro* lymphocyte response

B. N. HUDSPITH,* J. BROSTOFF,* M. W. McNICOL† & N. McJ. JOHNSON‡
*Departments of *Immunolology and †Medicine, Middlesex Hospital Medical School and ‡Willesden Chest, Clinic, London, UK*

(Accepted for publication 8 March 1984)

SUMMARY

We have shown that peripheral blood monocytes from patients with sarcoidosis release reduced amounts of interleukin-1 (IL-1) when compared with normals. In part, this defect explains the relative *in vitro* unresponsiveness of T lymphocytes from patients with sarcoidosis as measured by mitogen- or antigen-induced lymphocyte transformation. The addition of supernatants containing pre-formed IL-1 partially restored this defect. This enhancement was found to be additive to the previously described effect of indomethacin, an inhibitor of prostaglandin synthesis. Thus, it would appear that the activated peripheral blood monocytes found in sarcoidosis not only cause reduced lymphocyte proliferation by acting as suppressor cells but are also unable to act as accessory cells in producing IL-1.

Keywords sarcoidosis monocytes interleukin-1 prostaglandins

INTRODUCTION

Sarcoidosis is a disease of unknown cause characterized by multisystem granuloma formation, the major immunological features of which are depressed *in vivo* and *in vitro* delayed type hypersensitivity (Siltzbach, 1971) with increased *in vivo* B cell proliferation and immunoglobulin production (Lawrence *et al.*, 1980).

Cells of the monocyte–macrophage lineage have been shown to be activated at both the site of granuloma formation, where they constitute the major cell type (Pinkston, Bitterman & Crystal, 1983) and in the peripheral blood (Johnson *et al.*, 1981). Although the specific trigger of this activation remains unknown, it is clear that this cell is of importance in the modulation of the immunological defects found in sarcoidosis (Johnson *et al.*, 1981; Hunninghake *et al.*, 1981).

Previous studies by ourselves (Johnson *et al.*, 1981) and others (Goodwin *et al.*, 1979) have demonstrated that prostaglandins (PG) released by activated monocytes account, in part for the observed lymphocyte depression. We now show that these cells also produce less interleukin-1 (IL-1), a monokine required for the activation of T lymphocytes and it is this monocyte cell defect which appears responsible for the anergy observed in sarcoidosis.

Correspondence: Dr J. Brostoff, Department of Immunology, Middlesex Hospital Medical School, 40–50 Tottenham Street, London W1P 9PG, UK.

MATERIALS AND METHODS

Patients. We studied a total of 22 patients with sarcoidosis (nine male and 13 female), mean age 42 years (range 20–70). Patients were taken at random from either Willesden Chest Clinic or from in- and out-patients at the Middlesex Hospital. The diagnosis of sarcoidosis was based on a positive Kveim test of characteristic tissue histology in all cases. Eighteen (10 male and eight female) healthy laboratory or medical personnel, mean age 31 years (range 22–49) receiving no medication acted as controls.

Lymphocyte separation. Heparinized venous blood was diluted 1:1 with balanced salt solution (BSS) and layered onto Ficoll-Hypaque gradients and centrifuged for 35 min at 400*g* at room temperature. The total mononuclear cells were recovered from the interface, washed twice in BSS and resuspended in RPMI.

Generation of IL-1. The total mononuclear cells were counted and adjusted to 4×10^6 cells/ml in RPMI + 5% fetal calf serum (FCS). Two millilitres of this cell suspension was dispensed into wells of Costar plates and incubated in 5% CO_2 at 37°C. After 1 h the non-adherent cells were removed and the wells were washed twice with RPMI + 2% FCS. These adherent cells were stimulated with lipopolysaccaride (LPS) to generate IL-1. In experiments using adherent cells from normal donors 20 μg/ml gave the maximum mitogenic response. In cultures where the effect of drugs was studied, a submaximal dose of 5 μg/ml was used (Fig. 1). In experiments where the effect of PG were measured, indomethacin was added to give a final concentration of 1 μg/ml and PGE_2 was used at a final concentration of 10^{-6} and 10^{-8} M. LPS free cultures acted as controls. Each well was then made up to a final volume of 2 ml with RPMI + 2% FCS.

The plates were then incubated in 5% CO_2 in air at 37°C for 24 h. After this time the supernatants were collected, centrifuged and dialysed for 48 h against 100 ml sterile RPMI which was changed five times over this period. The samples were then stored at −20°C until assayed.

Assay for IL-1. The assay used in these experiments was the enhancement of mitogen stimulation of mouse thymocytes (Gery & Waksman, 1972). A 200 μl culture of 4×10^6 adherent cell depleted mouse thymocytes in 5% FCS were plated into flat bottomed microtitre plates. IL-1 preparations or control supernatants were added at a 1:4 dilution and then half of the wells were stimulated with PHA at a final concentration of 0·01 mitogenic unit/ml. The plates were then incubated in 5% CO_2 in air at 37°C for 3 days and for the final 18 h of culture the plates were pulsed with ^{3}H-thymidine, at 0·4 μCi/well. The cells were harvested onto glass fibre discs using a titertek harvester and counted using a toluene based scintillation fluid on a Packard β-counter.

The IL-1 containing supernatants were tested for interleukin-2 (IL-2) activity using the IL-2-dependent PHA blast culture method (Paganelli *et al.*, 1983). Our supernatants failed to support proliferation in these cultures as measured by ^{3}H-thymidine incorporation after 48 h incubation, thus excluding the presence of IL-2.

Mitogen assay of human peripheral blood lymphocytes. Total mononuclear cells were plated into flat bottomed microtitre plates at a concentration of 2×10^5 cells/well in 200 μl, these were then incubated and assayed using the same conditions as the mouse thymocytes. A suboptimal dose of concanavalin A (Con A, 2 μg/ml) and drugs were added at the initiation of the culture.

In experiments testing the effect of pre-formed IL-1 the supernatants were added at final dilution of 1:4.

Mitogens and drugs. Con A (ICN Pharmaceuticals Inc, USA), phytohaemagglutinin (Wellcome Reagents Ltd, UK) and *E. coli* lipopolysaccharide (Difco Ltd., UK) were dissolved in RPMI. Indomethacin and PGE_2 (Sigma Chemicals Ltd, UK) were dissolved in ethanol and made up to the required concentration with RPMI. RPMI plus ethanol was used as a control.

RESULTS

IL-1 production

It was found that IL-1 levels were reduced in the supernatants derived from sarcoid monocytes over

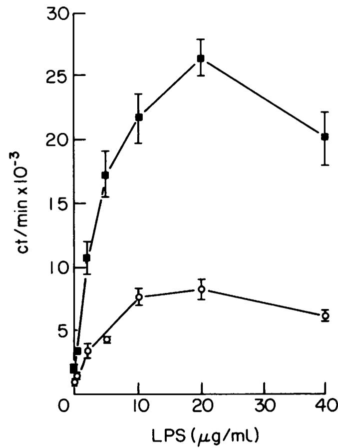

Fig. 1. An example of lipopolysaccharide (LPS) stimulation of lymphocytes from a patient with sarcoidosis (○) and a normal control (■). The thymidine incorporation of lymphocytes from the patient is less than in normal control, and the background incorporation of unstimulated cultures is similarly reduced. In subsequent experiments, a submaximal stimulus of 5 μg/ml of LPS was used.

the whole LPS dose–response used although the background production in these patients was similar to normals (Fig. 1). This finding was independent of therapy and did not appear to correlate with disease activity.

As we have previously shown that the proliferation of lymphocytes can be partially restored by indomethacin in sarcoidosis, we tested the effect of this inhibitor of PG synthesis on IL-1 production. Indomethacin added at the time of LPS stimulation had no effect on IL-1 production in either sarcoidosis or normal subjects (Fig. 2). To test the possibility that the cyclo-oxygenase pathway was being activated prior to LPS stimulation we added indomethacin at both the

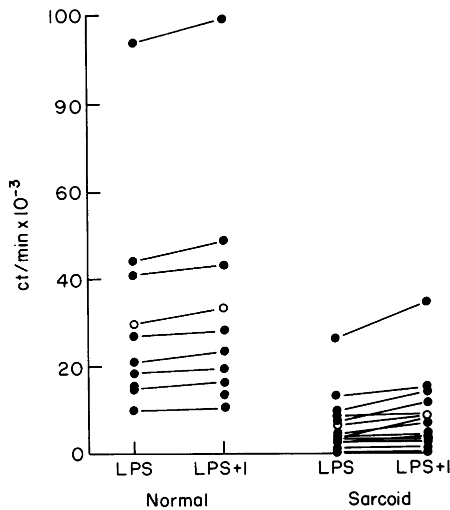

Fig. 2. IL-1 production in sarcoidosis and normal subjects and the effect of indomethacin. Plastic adherent cells from the sarcoid patients when stimulated with LPS released significantly reduced levels of interleukin ($P < 0{\cdot}001$). This impaired production of IL-1 could not be reversed by treating the cells with indomethacin (LPS + I). The level of IL-1 released by the sarcoid patients appeared to be independent of activity and therapy. LPS = 5 μg/ml; indomethacin = 1 μg/ml; ○——○ = mean.

Table 1. The effect of indomethacin pre-treatment of adherent cells on IL-1 production

	Normal ($n=4$)	Sarcoid ($n=5$)
	mean ± s.e.	
LPS only	12,494 ± 3,041	5,766 ± 768
LPS + indomethacin treatment of adherent cells	12,352 ± 2,390 (99%)*	5,887 ± 768 (102%)*
Indomethacin treatment of TMC (1 h) + LPS stimulation of adherent cells	12,798 ± 3,150 (102%)*	6,438 ± 829 (112%)*

TMC = Total mononuclear cells; * Figure in brackets represent % of controls.

adherence stage and the LPS stimulation stage (Table 1). In both cases no increase in IL-1 levels could be detected.

Effect of PGE_2

We found that PGE_2 reduced the IL-1 production by 30% with cells from normal subjects and this prostanoid also reduced IL-1 activity when assayed by the mouse thymocyte assay (Table 2). However, in both cases the only significant suppression was seen at 10^{-6}M, considerably higher than reported levels produced in this type of culture, ie 10^{-8}M (Goodwin *et al.*, 1979) at which dose no effect was seen.

Table 2. (a) The effect of PGE_2 on IL-1 production from controls and (b) the effect of PGE_2 on IL-1 function

	Mean ± s.e.	*P*
(a) Production of IL-1 in five control subjects		
LPS only	12,494 ± 3,041	
LPS + PGE_2 (10^{-6} M)	8,872 ± 2,729 (71%)*	0·05
LPS + PGE_2 (10^{-8} M)	12,839 ± 2,283	NS
(b) Function of IL-1 on mouse thymocytes using IL-1 preparations from five control subjects		
IL-1 preparation only	12,494 ± 3,041	
IL-1 preparation + PGE_2 (10^{-6} M)	7,062 ± 1,365 (57%)	0·01
IL-1 preparation + PGE_2 (10^{-8} M)	11,835 ± 4,318	NS

* Figure in brackets represent % control.

Restorative effect of pre-formed IL-1 on the mitogenic response

It was found that IL-1 containing supernatants enhanced the Con A response of sarcoid lymphocytes by approximately 30%, a similar percentage enhancement to that obtained by indomethacin treatment alone of these cultures. When both IL-1 and indomethacin were added together, the results were additive (Fig. 3).

The effect of IL-1 plus indomethacin treatment of the sarcoid cultures restored thymidine incorporation to that seen in control cultures (Fig. 4). This effect was not due to a direct mitogenic effect of the IL-1 preparation as the preparation actually caused a slight suppression of the Con A response in the control subjects.

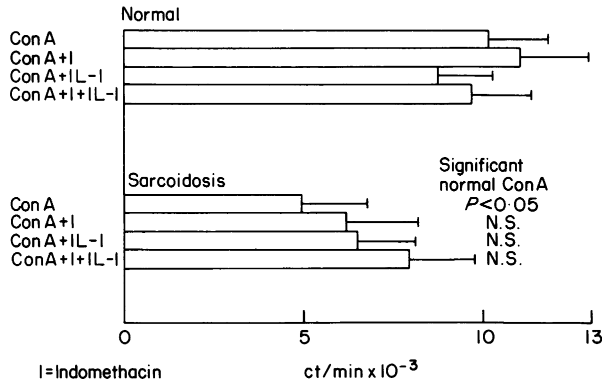

Fig. 3. The effect of IL-1 and indomethacin on thymidine incorporation in sarcoidosis. The Con A response was significantly reduced in sarcoidosis. The addition of either indomethacin (1 μg/ml) or IL-1 (50 μl IL-1 supernatant to each well) increased the mitogenic response to an equal degree. The simultaneous addition of both indomethacin and IL-1 restored the Con A responses to that seen in cultures from normal subjects.

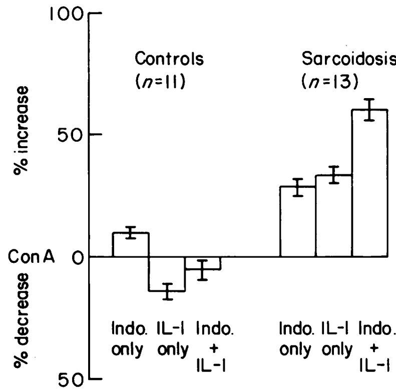

Fig. 4. The effect of IL-1 and indomethacin on thymidine incorporation in sarcoidosis—expressed as a percent of culture with mitogen alone. Il-1 containing supernatant enhanced the Con A response of lymphocytes from sarcoid patients by 34%. A similar enhancement was achieved by indomethacin (29%). The addition of both IL-1 and indomethacin to these cultures had an additive effect (61%). Addition of IL-1 to control cell cultures lead to a slight suppression of the Con A response, indicating that the IL-1 was not enhancing the patient group via a direct mitogenic effect. The effect of indomethacin and IL-1 were again additive but the overall effect was one of slight suppression.

DISCUSSION

We have shown that the activated monocytes found in the peripheral blood in sarcoidosis have a reduced capacity to produce IL-1. This monokine is required as a signal by helper T lymphocytes which then synthesize and release IL-2, which induces proliferation of activated T lymphocytes (Smith *et al.*, 1980).

It has been shown that the PG released by these monocytes are responsible, in part, for the observed depression of lymphocyte responses (Johnson *et al.*, 1981). This observation was believed to explain the major immunological defects as it has been shown that PGE_2 can increase immunoglobulin production (Lydyard *et al.*, 1982) and inhibit T lymphocyte proliferation (Goodwin *et al.*, 1979). However, as indomethacin only partially restored the mitogen response we examined other possible mechanisms which could explain the suppression.

We therefore tested whether another defect could be present, i.e. reduced IL-1 production by sarcoid monocytes. This was indeed the case and the mitogenic response could be partially restored

by adding pre-formed IL-1 to the cultures and completely so by adding both IL-1 and indomethacin together.

The enhancement due to IL-1 was independent of PG, as the actual increase due to IL-1 was the same in the presence or absence of indomethacin, and additive with indomethacin in restoring the mitogen response to near normal. The result was not expected as we have shown that PGE_2 suppressed both the production of IL-1 and its function (Table 2), presumably by suppressing IL-2 synthesis (Rappaport & Dodge, 1982) and so were expecting a synergistic effect, that is exogenous IL-1 on its own would be blocked from having its full effect by the presence of endogenously produced PG, the addition of indomethacin to this system would remove the inhibitory effect and so allow the IL-1 to initiate full T lymphocyte proliferation. However, our results indicated that PG suppression and lack of IL-1 production are independent of each other.

Because we found that indomethacin did not reverse the defective IL-1 production we believe that the monocytes found in peripheral blood in sarcoidosis have been activated *in vivo* and that this activation has caused maturation of the monocyte to a stage beyond that where IL-1 is synthesized and onto a stage where PG release predominates. This defect is not due to a decreased blood monocyte count as in sarcoidosis there is an increase in the absolute number of circulatory mononuclear cells. The addition of inhibitors of the cyclo-oxygenase enzyme system can inhibit prostaglandin synthesis but cannot cause reversal of maturation back to an IL-1 producing cell. Similar results were obtained by Wyler, Oppenheim & Koonlz (1979) who, using a mouse malaria system, showed that the adherent cells released IL-1 during the initial phase of infection, this subsequently declining to be replaced by a suppressor factor.

Our findings differ from the results of Hunninghake *et al.* (1981) and Pinkston *et al.* (1983) who found increased IL-1 and IL-2 production in cells from sarcoid patients. However, they obtained their cells from the lung by bronchoalveolar lavage and we have used cells separated from peripheral blood. This suggests that cells from different sites are compartmentalised and it is possible that the cells capable of providing help ($OKT4^+$) have migrated from the blood to the site of disease activity, thus giving rise to the altered ratios of helper suppressor cells in blood compared with lavage cells. It is also of interest to note that these defects of increased PG and decreased IL-1 production as seen in the sarcoid patients, are independent of disease activity and therapy, whereas the finding of increased helper cell activity in the lung correlates with disease activity (Pinkston *et al.*, 1983).

In the light of our results showing reduced Il-1 production by blood mononuclear cells and the increased interleukin production by alveolar cells (Hunninghake *et al.*, 1981; Pinkston *et al.*, 1983) it is possible that cells at the site of granuloma formation control the traffic of immunocompetent cells from blood to the site of disease activity and it is this relative depletion that leads to the observed peripheral anergy. Another possibility is that the hyperactive cells at the site of the granuloma are synthesizing interleukins spontaneously and continuously, and it is this over production that leads to feed back inhibition of peripheral blood cells with the capacity to synthesize the interleukins and this down regulation may lead to the depressed responses seen in sarcoidosis. Thus, it is important that the different cell compartments should be looked at separately, and we are presently studying the effects of bronchoalveolar cells on peripheral blood cell function. Treatment of sarcoidosis by immunotherapy based on treatment with interleukins would prove difficult and actual treatment with IL-1 would be inadvisable due to its pyrogenic effects (Duff & Durum, 1983). Therapy would need to be aimed at the inappropriate activation of monocytes.

REFERENCES

Duff, G.W. & Durum, S.K. (1983) The pyrogenic and mitogenic actions of interleukin-1 are related. *Nature*, **304,** 449.

Gery, I. & Waksman, B.H. (1972) Potentiation of the T-lymphocyte response to mitogens. II. The cellular source of potentiating mediators. *J. exp. Med.* **136,** 143.

Goodwin, J.S., DeHoratius, R., Israel, H., Peak, G.T. & Messner, R.P. (1979) Suppressor cell function in sarcoidosis. *Ann. Intern. Med.* **90,** 167.

Hunninghake, G.W., Broska, P., Haber, R., Keogh, B.A., Line, B. & Crystal, R.G. (1981) Correlation of lung T-cell and macrophage function with disease activity in pulmonary sarcoid. *Clin. Res.* **29,** 550A.

Johnson, N.McI., Brostoff, J., Hudspith, B.N.,

Boot, R.J. & McNicol, M.W. (1981) Tγ cells in sarcoidosis: E rosetting monocytes suppress lymphocyte transformation. *Clin. exp. Immunol.* **43,** 491.

Lawrence, E.C., Martin, R.R., Blaese, R.M., Teague, B.B., Awe, R.J., Wilson, R.K., Deaton, W.J., Bloom, K., Greenberg, S.D. & Stevens, P.M. (1980) Increased bronchoalveolar IgG secreting cells in interstitial lung disease. *N. Engl. J. Med.* **302,** 1186.

Lydyard, P.M., Brostoff, J., Hudspith, B.N. & Parry, H. (1982) Prostaglandin E_2 mediated enhancement of human plasma cell differentiation. *Immunol. Lett.* **4,** 113.

Rappaport, R.S. & Dodge, G.R. (1982) Prostaglandin E inhibits the production of human interleukin-2. *J. exp. Med.* **155,** 943.

Pinkston, P., Bitterman, P.B. & Crystal, R.G. (1983) Spontaneous release of interleukin-2 by lung T-lymphocytes in active pulmonary sarcoidosis. *N. Engl. J. Med.* **308,** 793.

Paganelli, R., Aivli, F., Beverley, P.C.L. & Levinsky, R.J. (1983) Impaired production of interleukins in patients with cell-mediated immunodeficiencies. *Clin. exp. Immunol.* **51,** 338.

Siltzbach, L. (1973) Sarcoidosis. In *Immunological Diseases* (ed. by M. Samter) p. 581. Little, Brown & Co, Boston.

Smith, K.A., Lachman, L.B., Oppenheim, J.J. & Farata, M.F. (1980) The functional relationship of the interleukins. *J. exp. Med.* **151,** 1551.

Wyler, D.J., Oppenheim, J.J. & Koonlz, L.C. (1979) Influence of malaria infection on the elaboration of soluble mediators by adherent mononuclear cells. *Infect. Immunol.* **24,** 151.