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Defective cell mediated immunity in sarcoidosis: effect of interleukin-2

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ABSTRACT Interleukin-2 has been reported to enhance the immune response in diseases characterised by defective cell mediated immunity. The effect of exogenous recombinant interleukin-2 was studied on the proliferative and cytotoxic responses of peripheral blood mononuclear cells from 32 patients with sarcoidosis and 14 healthy control subjects. The proliferative response to purified protein derivative was smaller in patients than in control subjects ($p < 0.001$) whereas the response to 80 U interleukin-2 alone and to purified protein derivative and interleukin-2 did not differ significantly between the two groups. In addition, in eight patients but no control subjects tritiated thymidine incorporation induced by the combination of purified protein derivative and interleukin-2 was more than twice the sum of that induced by purified protein derivative and interleukin separately. Cytotoxic activity occurring spontaneously and induced by purified protein derivative and interleukin-2 in blood mononuclear cells was significantly less for patients with sarcoidosis than for control subjects ($p < 0.05$ spontaneous, < 0.001 purified protein derivative induced, < 0.02 interleukin induced). Synergism between antigen and interleukin did not occur with respect to the cytotoxic response in either patients or controls. Defective interleukin-2 production may contribute to, but does not entirely explain, the functional abnormalities of peripheral blood lymphocytes from patients with sarcoidosis.

Introduction

Sarcoidosis is characterised by granuloma formation and by the simultaneous occurrence of cellular hyperactivation and lack of responsiveness. These two aspects divide on anatomical lines. In the lung T lymphocytes are activated, as indicated by unstimulated proliferation[1] and release of interleukin-2[2]; and alveolar macrophages are also activated, showing increased antigen presentation[3] and release of γ interferon.[4] In contrast, outside the lung delayed hypersensitivity skin reactions are decreased, and peripheral blood mononuclear cells have been reported to respond poorly when stimulated with mitogens in vitro.[5]

Interleukin-2 is an important component in the proliferative and cytotoxic responses of lymphocytes when activated by antigens or mitogens.[6] Evidence of reduced production of interleukin-2 at sites of immunological unresponsiveness[7] and increased production at sites of active disease[2] led us to postulate that defective interleukin-2 production might explain the observed discrepancy in cellular activation between blood and lung. To test this hypothesis we studied the in vitro effect of exogenous human recombinant interleukin-2 on cytotoxic and proliferative responses of peripheral blood mononuclear cells from patients with sarcoidosis.

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Methods

STUDY POPULATIONS

Unselected patients with sarcoidosis were studied. All patients had biopsy proved disease or a positive Kveim test response, or both. The disease was considered to be active if a deterioration in the chest radiograph, a fall in transfer factor for carbon monoxide, or an increase in serum angiotensin converting enzyme activity had occurred in the last six months. Healthy control subjects were members of staff at the Clinical Research Centre, Harrow.

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Table 1 *Details of the patients*

Patient No	Age (y)	Sex	Race	Duration of disease	Clinical details	Disease activity	Treatment
1	64	F	EU	18 months	BHL	Inactive	None
2	39	F	EU	2 years	BHL	Inactive	None
3	57	F	EU	1 year	L	Active	None
4	62	F	EU	2 years	C	Active	None
5	38	F	EU	3 years	PI, A	Active	NSAID
6	25	F	EU	4 years	CNS	Active	Steroid
7	39	F	EU	1 year	BHL	Active	None
8	41	F	EU	6 years	PI, L	Inactive	None
9	52	F	EU	1 year	EN, A	Active	None
10	31	F	EU	1 month	EN, BHL	Active	NSAID
11	25	F	EU	9 months	PI	Active	None
12	35	F	EU	3 years	BHL	Active	NSAID
13	38	F	EU	1 year	BHL, C	Active	None
14	28	M	EU	3 months	PI, A	Active	Steroid
15	29	M	EU	6 months	BHL, PI	Active	None
16	30	M	EU	6 months	BHL	Active	None
17	28	M	EU	2 years	C, A	Active	Steroid
18	27	M	EU	6 months	BHL	Active	None
19	37	M	EU	3 years	BHL	Active	None
20	34	M	EU	3 months	BHL, EN	Active	Steroid
21	31	M	EU	1 month	BHL, EN	Active	None
22	31	M	EU	1 year	BHL	Active	None
23	37	M	EU	3 years	BHL	Inactive	NSAID
24	31	F	AC	3 months	PI	Active	NSAID
25	42	F	AC	1 year	PI, L	Active	None
26	46	F	AC	3 years	L	Active	None
27	53	F	A	2 years	L	Active	None
28	57	F	AC	7 years	BHL, PI	Inactive	Steroid
29	52	F	AC	10 years	PI	Active	None
30	25	F	AC	1 year	BHL, L	Active	None
31	43	F	A	1 year	BHL, A	Active	None
32	52	M	AC	2 years	BHL, L	Inactive	Steroid
33	52	M	AC	2 years	PI	Inactive	None
34	35	M	AC	3 years	PI	Active	None
35	30	M	A	2 months	BHL	Active	None
36	49	M	AC	1 year	BHL	Inactive	None
37	20	M	A	1 year	BHL	Active	None
38	49	M	A	18 months	BHL, PI	Active	Steroid
39	30	M	AC	6 years	PI	Inactive	None

EU—European; A—Asian; AC—Afrocaribbean; BHL—bilateral hilar lymphadenopathy; PI—pulmonary infiltrate; L—lymphadenopathy; C—skin manifestations; CNS—central nervous system affected; A—arthropathy; EN—erythema nodosum; NSAID—non-steroid anti-inflammatory drug.

PROLIFERATIVE RESPONSE OF PERIPHERAL BLOOD MONONUCLEAR CELLS

Venous blood anticoagulated with heparin was diluted 1:1 with phosphate buffered saline, layered on to Ficoll-Paque (Pharmacia, Upsala, Sweden), and centrifuged at room temperature for 35 minutes at 600 *g*. Peripheral blood mononuclear cells from the interface were collected, washed twice, and suspended at a concentration of 10^6/ml in tissue culture medium (RPMI 1640) with 10% heat inactivated fetal calf serum, penicillin 100 IU/ml, streptomycin 100 μg/ml, and glutamine 2 mM.

The proliferative response of the peripheral blood mononuclear cells was measured by uptake of radiolabelled thymidine. The cells were suspended at 10^6/ml and plated out in 96 well microtitre plates (Becton Dickinson) at 0·2 ml/well. Cells were incubated with medium alone for control values, or were stimulated with purified protein derivative of tuberculin (PPD) 40 μg/ml (Serumsinstitute, Copenhagen), human recombinant interleukin-2 (IL-2) 80 U/ml (Biogen Medical Research, Switzerland), or both together. Triplicate cultures were maintained at 37°C in 5% carbon dioxide for seven days.

For the last six hours of culture 1 μCi/well of ^{3}H-methylthymidine (specific activity 2 Ci/nmol) (TRA 319, Amersham) was added and cells were then harvested on to glass fibre paper (Skatron AS, Norway). Filters were counted in a gamma counter (Wallac LKB, Helsinki). Results are expressed as counts per minute (cpm).

Enhancement of the proliferative response in the presence of PPD and interleukin-2 together was arbitrarily defined as a response of two or more times the sum of the separate responses to PPD and to interleukin-2.

GENERATION OF CYTOTOXIC EFFECTOR CELLS

Peripheral blood mononuclear cells were separated on Ficoll-Paque and were cultured in RPMI in flat bottomed microculture plates with 10% fetal calf serum. For spontaneous cytotoxic activity the cells

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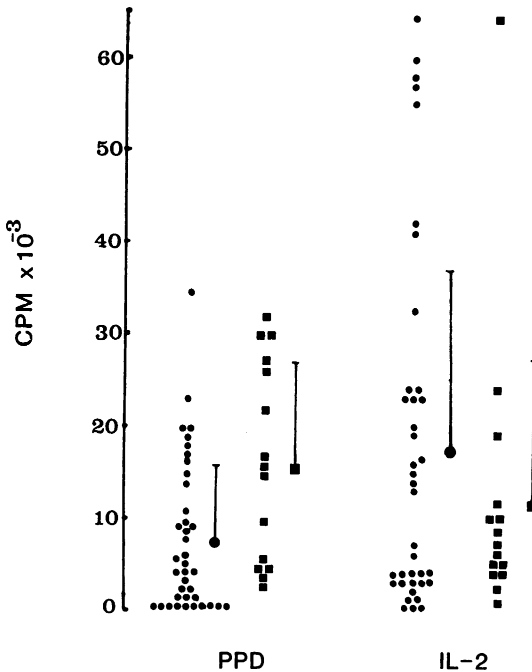

Fig 1 *Proliferative response to purified protein derivative (PPD) and to interleukin-2 (IL-2) for patients (●) and controls (■). Group means with SD are shown (see table 2 for significant values).*

were unstimulated and for antigen induced activity they were cultured in the presence of PPD 40 μg/ml. For interleukin-2 induced cytotoxicity the cells were cultured with IL-2 500 U/ml. Cytotoxicity was measured at 24 hours.

CYTOTOXICITY ASSAYS

Cytotoxicity was assessed against T24, a human bladder carcinoma cell line,[8] in a four hour chromium-51 release assay. Target cells were labelled with 100 μCi $Na_2{}^{51}CrO_4$ (Amersham) at a concentration of 10^7/ml for 90 minutes at 37°C, followed by four washings with balanced salt solution. Ten thousand target cells were mixed with effector cells at an effector:target ratio of 20:1. The plates were centrifuged at 100 *g* for five minutes and incubated for four hours. After incubation 100 μl of supernatant from each well was removed and counted in a gamma counter (Wallac 800). Maximum ^{51}Cr release was produced by incubation of the target cells with Triton X-100. Minimum release was produced by incubation of targets with medium alone. Results are expressed as the percentage of specific lysis, which was calculated by the formula:

$$\frac{\text{Experimental release} - \text{minimum release}}{\text{maximum release} - \text{minimum release}} \times 100.$$

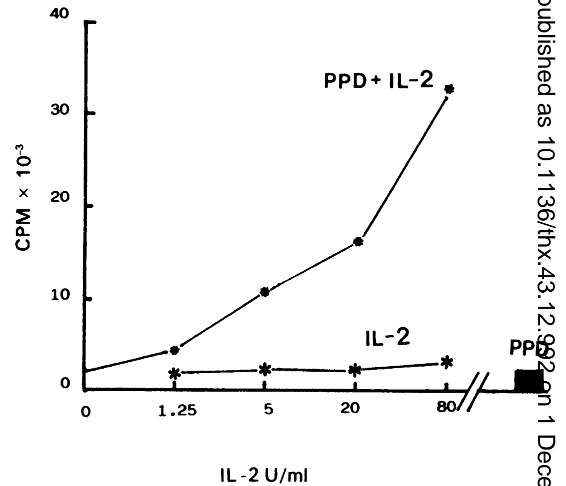

Fig 2 *Effect of IL-2 on the proliferative response to purified protein derivative (PPD): data from patient 10 (table 1), showing the proliferative response to PPD 40 μg/ml, PPD 40 μg/ml in the presence of recombinant IL-2 1·25–80 U/ml, and recombinant IL-2 1·25–80 U/ml alone.*

STATISTICAL ANALYSIS

Data were tested for normality by using normal scores,[9] and were found to be non-normally distributed both untransformed and after logarithmic

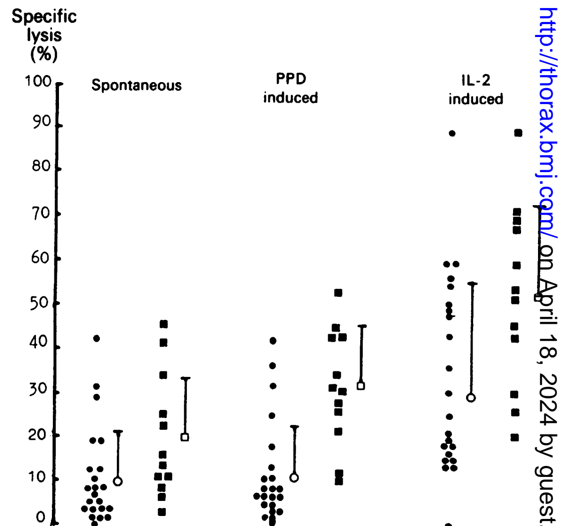

Fig 3 *Cytotoxic function assessed at 24 h in patients with sarcoidosis (●) and healthy controls (■). Cytotoxicity was unstimulated or induced by incubation with purified protein derivative (PPD) 40 μg/ml or by IL-2 (500 U/ml). All modes of cytotoxicity are significantly reduced in sarcoidosis (p unstimulated $< 0·05$, purified protein derivative induced $< 0·001$, interleukin-2 induced $< 0·01$).*

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Table 2 *Proliferative and cytotoxic responses (mean (SD)): patients and controls*

	Proliferative responses (cpm)		*Cytotoxic response (% specific lysis)*	
Stimulus	*Sarcoidosis (n = 39)*	*Control (n = 14)*	*Sarcoidosis (n = 22)*	*Control (n = 12)*
[Unstimulated]	1011 (1032)	1782 (1849)	11 (3)	20 (13)*
PPD	7272 (7729)	15174 (10874)***	12 (12)	32 (13)***
IL-2	17079 (19655)	11472 (16105)	35 (22)	53 (21)**
IL-2 + PPD	23998 (16409)	21462 (12998)	31 (22)	55 (16)**

Significant difference between groups: *$p < 0.05$; **$p < 0.02$; ***$p < 0.001$. PPD—purified protein derivative; IL-2—interleukin 2.

square root, and reciprocal transformation. The Mann-Whitney U test was therefore used.

Results

PROLIFERATIVE RESPONSE OF PATIENTS AND CONTROLS

We studied 39 patients and 14 healthy control subjects. Clinical details of the patients are provided in table 1. The proliferative response of peripheral blood mononuclear cells to purified protein derivative was significantly reduced in the patients with sarcoidosis (table 2). In contrast, proliferation of blood mononuclear cells induced by interleukin-2 did not differ significantly in the two groups (table 2). The responses of individual patients and controls are shown in figure 1, and group data with significance values in table 2.

Although blood mononuclear cells from some patients with sarcoidosis responded poorly to purified protein derivative and interleukin-2 separately there was a good response when the two were combined. Enhancement of response (see under "Methods") occurred in eight patients (Nos 1, 7, 8, 10, 14, 32, 33, 35; table 1) but in no control subjects. Enhancement of the response was dose dependent on interleukin-2 in five of the eight patients (see fig 2), whereas in the remaining three enhancement occurred only with the highest dose of interleukin (80 U/ml).

CYTOTOXIC RESPONSES

The cytotoxic activity of unstimulated peripheral blood mononuclear cells from the patients with sarcoidosis was less than that of the control subjects (table 2, fig 3). The addition of purified protein derivative significantly increased the cytotoxicity of cells from the control subjects but not that of cells from the patients with sarcoidosis. Exogenous interleukin-2 increased cytotoxicity of cells from both groups, although interleukin induced cytotoxic activity in patients with sarcoidosis remained less than that observed in the control group. The cytotoxic response to purified protein derivative was measured in the presence or absence of interleukin in patients and controls. In both groups the response was similar to that obtained with interleukin alone (table 2). In no subject in either group was an enhancement of cytotoxic response seen in the presence of purified protein derivative and interleukin together.

Discussion

Non-specific cytotoxicity of peripheral blood lymphocytes in sarcoidosis has been the subject of studies with contradictory findings. Agostini has shown that natural killer cells, as identified by a panel of monoclonal antibodies, occur with increased frequency in peripheral blood in sarcoidosis, and that this correlates with evidence of significantly increased lysis of K-562 target cells.[10] The effector population used by Agostini was depleted of adherent cells. Adherent macrophage-monocytes have previously been shown to have a suppressive effect on cellular activation in sarcoidosis.[11 12] Hall also found increased cytotoxicity in cells from patients with sarcoidosis when he used an effector population depleted of adherent cells.[13] A Japanese study showed increased natural killer activity in a functional assay.[14] In contrast, Tartoff, using an unfractionated population, found that natural killer cytotoxicity and natural killer like cytotoxicity induced by antigen were reduced in women with sarcoidosis.[15] Antonaci, using a phagocyte depleted effector population, found decreased cytotoxic activity.[16] Recently studies from this laboratory have shown impaired interleukin-2 induced cytotoxic activity in sarcoidosis.[17]

The present results show that in sarcoidosis unstimulated, antigen induced, and interleukin-2 induced cytotoxicity are significantly impaired. Although extrapolation from in vitro to in vivo studies must be regarded with caution, a defect in cytotoxic function may be relevant to the increased incidence of lymphomas[18] occurring in sarcoidosis.

In this study the proliferative response of the patients with sarcoidosis to purified protein derivative was clearly impaired, being less than half that of controls. In the presence of exogenous interleukin-2, however, an enhancement of proliferative response was seen in some patients. A striking example is illustrated in figure 2. In some cases this enhancement appeared to represent a synergistic effect between antigen and interleukin-2, which was dose dependent on interleukin. The magnitude of this effect varied

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greatly. Our impression was that enhancement was more likely to occur in subjects with a low response to both reagents when given separately, though this could not be shown statistically. Skin sensitivity to tuberculin was not assessed routinely at the time of study and we cannot therefore say how it may relate to the phenomenon of in vitro enhancement.

In diseases characterised by immunological unresponsiveness, interleukin-2 has been shown to enhance cellular responses in vitro.[19-24] It has been suggested that exogenous interleukin-2 compensates for poor endogenous interleukin-2 production, and therefore restores proliferative responses.[20 21] The same phenomenon, however, occurs in apparently normal subjects.[19 25] An alternative explanation might be that interleukin-2 acts by antagonising the production or action of other inhibitory molecules, such as prostaglandins of the E series. Further studies are required to test this hypothesis. Evidence from the present study shows that enhancement of antigen induced proliferative responses in the presence of interleukin occurs only in a few sarcoidosis patients. Consequently, although a deficiency of interleukin-2 production may be a contributing factor in some patients, it is unlikely to account for the "immunological unresponsiveness" observed in patients.

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