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Prevalence and specificity of the enhancing effect of three types of interleukin 2 on T cell responsiveness in 97 lepromatous leprosy patients of mixed ethnic origin

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SUMMARY

Peripheral blood mononuclear cells from 97 predominantly lepromatous leprosy patients and 11 control subjects were tested in a lymphoproliferative assay for response to *Mycobacterium leprae* (whole and sonicated), and sonicated *M. vaccae*, *M. tuberculosis*, and *M. scrofulaceum*, in the presence and absence of three types of interleukin 2 (IL-2) (crude, purified, and recombinant). IL-2 enhanced the response to sonicated *M. tuberculosis* and *M. leprae* organisms more often in patients than in control subjects, but not significantly so and only in a minority of patients. This effect was significantly more common (though still only found in a minority of 46%) using *M. leprae* organisms as antigen, than when using sonicates of *M. leprae* (19%) or *M. vaccae* (19%). However it was nearly as frequent using sonicated *M. tuberculosis*, or *M. scrofulaceum*. Thus in only nine patients was the effect specific to *M. leprae*.

Enhancement by IL-2 could not be related to the type of IL-2 used, the dose of antigen, or the amount of endogenous IL-2 released by the cells tested. Similarly it was not related to the extent to which IL-2 caused increased background proliferation in control wells, which occurred to an equal extent using cells from control subjects, nor was it related to the extent of antigen-driven proliferation.

The data have also been analysed in relation to duration of disease (50 years to a few weeks) and ethnic origin. No correlations have been revealed.

Thus enhancement by IL-2 of the lymphoproliferative response to mycobacterial antigens does occur using cells from lepromatous leprosy patients, but it is found in a minority of patients, it is not specific to *M. leprae*, and can occur with cells from normal donors.

Keywords interleukin 2 lepromatous leprosy mycobacterial antigens

INTRODUCTION

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* (*M. leprae*). At one end of the spectrum of disease, tuberculoid leprosy, patients have good cell mediated immunity and rapidly clear *M. leprae* from the tissues. At the more severe end of the spectrum, lepromatous leprosy, there is antigen-specific cell-mediated immunological unresponsiveness.

Recent publications (Haregewoin *et al.*, 1983; 1984; Nath *et al.*, 1984) have indicated that this

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anergy can, in some patients, be at least partly reversed by the addition of exogenous T cell growth factor or purified human interleukin 2 (IL-2) *in vitro*, though other workers have had difficulty reproducing their results (Ottenhoff, Elferink & De Vries, 1984; Mohagheghpour *et al.*, 1985: McAdam, Bloom, personal communications), and indeed other reports suggest that IL-2 can reverse deficient immune responses in rheumatoid arthritis (Emery, Panayi & Nouri, 1984) and schistosomiasis (Gastl *et al.*, 1984). In the original studies, Haregewoin *et al.* (1983) studied 17 Ethiopian lepromatous patients, all but three of whom had been treated for up to 18 years, and four healthy contacts: 13 patients, but none of the controls, showed enhancement of response to *M. leprae* in the presence of T cell conditioned medium. Nath *et al.* (1984) studied 30 Indian lepromatous patients, of whom 24 were untreated and six had been treated for up to 5 years, and no control subjects: 21 patients showed enhanced response to *M. leprae* (though whether to soluble or particulate antigen is not stated) in the presence of IL-2. In the second study reported by Haregewoin *et al.* (1984) the response to *M. leprae* organisms of lepromatous leprosy patients from England, Holland and Ethiopia, but no controls, were studied; details of disease duration, therapy and numbers in each ethnic group were not given. Thirty-three of 53 patients showed enhanced response in the presence of T cell conditioned medium, eight of eight using purified IL-2, and 10 of 11 using recombinant IL-2. Our first aim therefore was to study a large number of patients and to include those of various racial groups and showing a wide range of duration of disease.

Secondly we wanted to determine the specificity of the effect demonstrated by the other groups. Haregewoin *et al.* had used purified protein derivative (PPD), a soluble antigen, as a control antigen for *M. leprae* organisms in their first study (1983), but no other mycobacterial antigens in the second study (1984), whereas Nath *et al.* (1984) did not compare undefined *M. leprae* antigen with any other. We chose to compare *M. leprae* organisms and sonicate with *M. vaccae* sonicate (skin test responses to this organism can correlate with skin test responses to *M. leprae* in leprosy; Paul, Stanford & Carswell, 1975), *M. scrofulaceum* sonicate (a representative slow grower), *M. tuberculosis* sonicate (an antigen to which leprosy patients respond), and PPD (for comparison with the results of Haregewoin *et al.* 1983; 1984).

Thirdly, we investigated the suggestion by Kaplan *et al.* (1985) that lepromatous leprosy patients form two groups, namely, non-responders and low-responders to *M. leprae* antigens and it is only in the low-responder group that addition of IL-2 drives further expansion of *M. leprae*-specific T cells.

MATERIALS AND METHODS

Test subjects. The leprosy patients studied were from two sources: (a) In-patients and out-patients under the care of Dr M. F. R. Waters at The Hospital for Tropical Diseases, St Pancras, London, UK and (b) in-patients at the Sanatorio de Fontilles, Alicante, Spain. They were classified clinically and histologically by the system of Ridley and Jopling (1966), except in the case of long-standing patients who were designated lepromatous (L) on clinical grounds. Treatment was with standard regimens of dapsone, rifampicin and clofazimine or ethionamide. A number of patients were also undergoing treatment for erythema nodosum leprosum (ENL).

Control subjects. These were healthy laboratory staff at The Middlesex Hospital Medical School, London, UK.

Antigens. Sonicated *M. leprae* and whole dead *M. leprae* organism were a kind gift from Dr R. J. W. Rees (National Institute for Medical Research, London, UK). Sonicated *M. leprae*, *M. tuberculosis*, *M. vaccae*, *M. scrofulaceum* and purified protein derivative (PPD) were used at a final concentration of 10 μg protein/ml, and *M. leprae* organisms were used at a final concentration of 10^6 organisms/well.

Interleukin 2 preparations. Three sources of IL-2 were used. Recombinant IL-2 was gift from the Cetus Corporation to Dr Tore Godal, to whom we are grateful for a small sample. It was used at 5 and 20 u/ml, as recommended by Dr Godal. Purified IL-2 (Genzyme) was obtained from Koch Light laboratories, (Haverhill, Suffolk, UK) and used at 40 and 160 u/ml (units as defined by the manufacturer). At these concentrations it was comparable to the Cetus preparation used at 5 and 20

u/ml in the IL-2 assay. Crude IL-2 was the supernatant from the MLA 144 IL-2 secreting Gibbon cell line (Rabin *et al.*, 1981). The cells were seeded at 10^5/ml in 10% pooled normal serum in RPMI 1640 (GIBCO, Grand Island, NY, USA). Supernatant was collected at 72 h, and passed through a 0·22 μm filter and stored at 4°C. It was used at a final concentration of 1% unless otherwise stated. At this concentration it was equivalent to 20 u/ml of the Cetus preparation in the IL-2 assay, and in its stimulatory effect on human peripheral blood lymphocytes (see Results).

Assay of lymphocyte proliferation. Lymphocyte transformation tests (LTTs) were used: peripheral blood mononuclear cells (PBMC), obtained from defibrinated blood, separated over a Ficoll-Hypaque (Flow Laboratories, Irvine, Ayrshire, UK) gradient and washed three times, were cultured in triplicate at 100,000 PBMC/well in 96 well microtitre plates (Nunclon 1-67008) in the presence and absence of antigens and IL-2. All cultures were in RPMI 1640 buffered with 25 mM HEPES (GIBCO, Grand Island, NY, USA), supplemented with standard concentrations of L-glutamine, penicillin and streptomycin, and 10% pooled normal human serum.

Cultures were incubated at 37°C in 5% CO_2 in air for seven days and labelled with 0·1 μCi ^{3}H-thymidine (specific activity 25 Ci/Mol, Amersham, Buckinghamshire, UK, TRK 120) for 18 h prior to harvesting (Ilacon harvester) on to glass fibre paper (Whatman 1822 915) and counting for 1 min

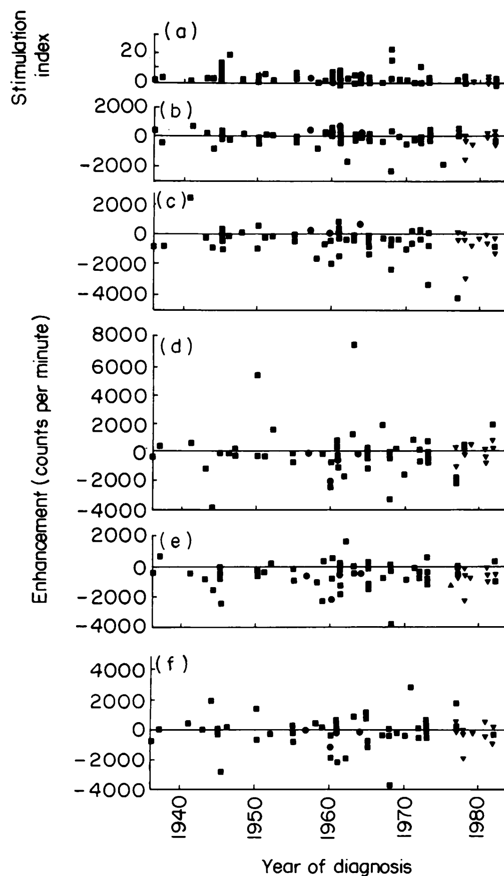

Fig. 1. Effect of MLA 144 IL-2 on (a) background lymphoproliferation (see text), and comparison of enhancement (see text) of response to (b) *M. leprae* organisms, (c) *M. leprae* sonicate, (d) *M. tuberculosis* sonicate, (e) *M. vaccae* sonicate and (f) *M. scrofulaceum* sonicate in bacteria-negative (■) and bacteria-positive (▼) Spanish patients. (●) Denotes bacteria-negative patients showing *M. leprae*-specific enhancement of response.

in a liquid scintillation counter (LKB 1211) using Betafluor scintillation fluid (National Diagnostics, Somerville, NJ, USA).

Calculation of the increment in tritiated thymidine incorporation attributable to enhancement by IL-2 of the response to antigen. The results are expressed in Figs 1 to 4 as counts per minute per well, after subtraction from the value obtained in the presence of both antigen and IL-2, of the following three control values: (a) the background in well containing cells only, (b) the counts caused by IL-2 alone and (c) the counts caused by antigen alone.

The value is therefore zero if the effects of IL-2 and antigen were additive, with no evidence of synergy, and negative if their combined effect was less than additive.

The stimulation of background by IL-2. The increase in background proliferation caused by IL-2 alone was calculated as a stimulation ratio by dividing the counts in wells with IL-2 by the counts in control well containing cells only.

Assay of IL-2. IL-2 was assayed as described by Gillis *et al.* (1982.

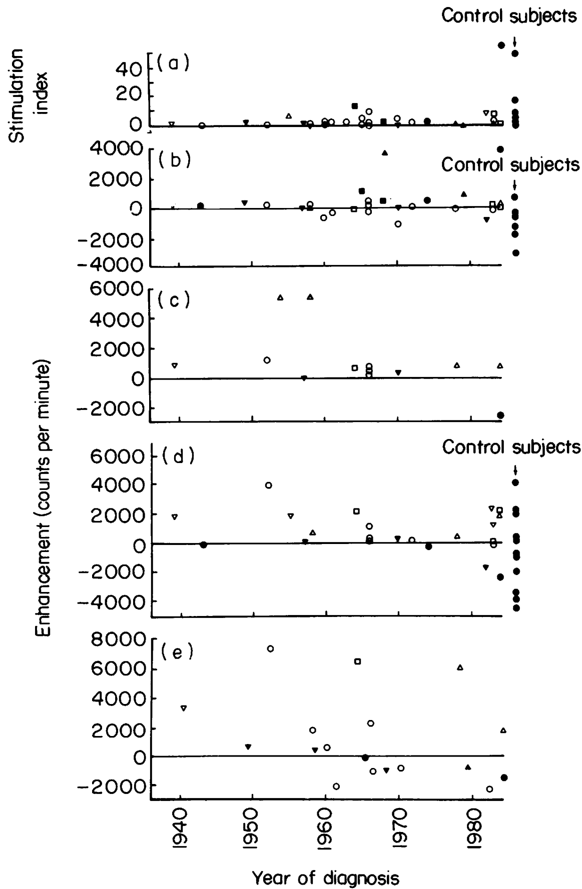

Fig. 2. Effect of MLA 144 IL-2 on (a) background lymphoproliferation, and comparison of enhancement of response to (b) *M. leprae* organisms, (c) *M. vaccae* sonicate, (d) *M. tuberculosis* sonicate and (e) PPD in subjects of different ethnic origin. (●) Indian patients (includes patients of Indian, Bengali, Anglo-Indian and Pakistani origin) showing *M. leprae*-specific enhancement of response; (○) other Indian patients; (■) African patients showing *M. leprae*-specific enhancement of response; (□) other African patients; (▽) West Indian patients; (▼) Caucasian patients (includes patients of Moroccan and Cypriot origin); (▲) Far Eastern patients (includes patients of Vietnamese, Philippino and Anglo-Burmese origin) showing *M. leprae*-specific enhancement of response; (△) other Far Eastern Patients.

RESULTS

The effects of three IL-2 preparations on background proliferation

The parts of Figs 1–4 labelled (a) show the effects of IL-2 alone, due to triggering of expansion of T cells activated by antigen presenting cells primed by mycobacterial or other antigens. The proliferation of cells from Spanish patients (Fig. 1a) was increased 4·7-fold (range 1·3- to 23·2-fold) by 1% MLA 144 IL-2. For the other patients the figure was 3·8-fold (range 1·0- to 13·9-fold, with one exceptional value of 232·1-fold). These groups did not differ significantly from each other or from the controls (mean 4·3-fold, range 1·1- to 16·1-fold, with one exceptional value of 48·7-fold).

The mean values for stimulation of patients' cells by Cetus recombinant IL-2 were 2·7 fold at 5 u/ml, and 3·9-fold at 20 u/ml. The Genzyme purified IL-2 caused 2·5-fold stimulation at 40 u/ml, and 3·4-fold at 160 u/ml. Thus the three preparations were comparable in this respect at the concentrations used.

The size of the stimulatory effect of IL-2 alone was not related to ethnic group, or to the presence or duration of disease.

The effect of IL-2 on the response to mycobacterial antigens

The effect of 1% MLA-144 IL-2 on antigen-driven proliferation of cells from the Spanish patients is shown in Fig. 1, and its effect on cells from the mixed group of non-Spanish patients, and a group of controls set up in parallel is shown in Fig. 2. The Cetus (Fig. 3) and Genzyme (Fig. 4) preparations were tested on some individuals from the mixed patient group. Sample data for cells from two Anglo-Indian patients tested in parallel are shown in Table 1.

The results are similar for all preparations and concentrations of IL-2:

(a) IL-2 can cause enhancement of the responses of cells from both normals and patients, but often the response to antigen in the presence of IL-2 is less than the sum of the responses to IL-2 and antigen used separately, resulting in negative values.

(b) Enhancement can occur with mycobacterial antigens other than *M. leprae*.

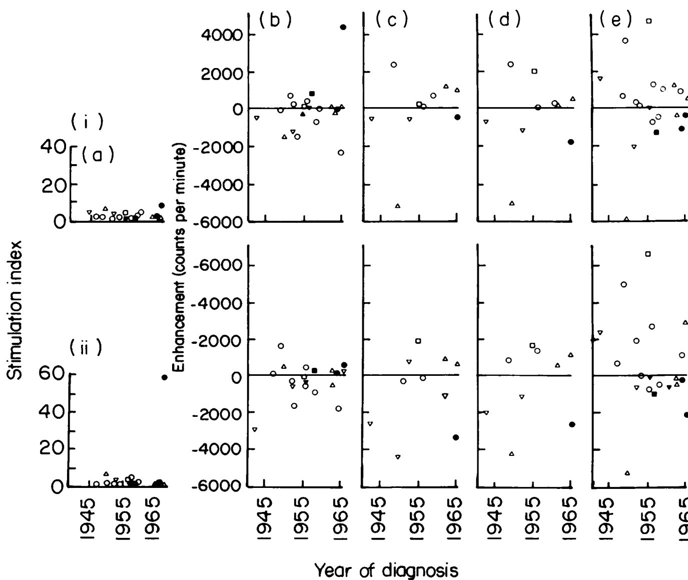

Fig. 3. Effect of (i) 5 u/ml and (ii) 20 u/ml Cetus IL-2 on (a) background lymphoproliferation, and enhancement of response to (b) *M. leprae* organisms, (c) *M. vaccae* sonicate, (d) *M. tuberculosis* sonicate and (e) PPD in subjects of different ethnic origins. For symbols see legend to Fig. 2.

Table 1. Sample data showing the effect of three types of IL-2 on antigen-driven proliferation of cells from two patients

	MLA 144 IL-2 (1%)				Genzyme IL-2 (40 u/ml)				Cetus IL-2 (5 u/ml)			
Patient	Cells	IL-2	Antigen	Antigen and IL-2	Cells	IL-2	Antigen	Antigen and IL-2	Cells	IL-2	Antigen	Antigen and IL-2
E.L.	177±42	951±274	MLO 450±101	2489±495	151±6	226±65	MLO 233±111	435±260	177±42	290±38	MLO 450±101	468±280
			PPD 1870±230	2499±361			MV 1288±693	1456±296			PPD 1870±230	1796±397
							MTB 1191±172	1592±302				
M.B.	193±30	495±131	MLO 240±44	850±514	75±24	75±33	MLO 55±10	67±21	193±30	285±81	MLO 240±44	235±86
			PPD 1826±733	2744±919			MV 230±100	1159±875			PPD 1826±733	2651±299
							MTB 1096±3	829±146				

MLO *M. leprae* organisms.
PPD Purified protein derivative.
MV *M. vaccae* sonicate.
MTB *M. tuberculosis* sonicate.

Table 2. Effect of MLA 144 IL-2 on mycobacterial antigen responses (columns a–c), and differences in response between the various antigens tested, expressed as *P* values, as determined by Fisher's exact test (columns d–g), in Spanish patients

Antigen	Number of patients showing a synergistic response with IL-2 (a)	Number of patients showing an additive response with IL-2 (b)	Number of patients showing a less than additive response with IL-2 (c)	*M. leprae* organisms (d)	*M. leprae* sonicate (e)	*M. tuberculosis* sonicate (f)	*M. vaccae* sonicate (g)
M. leprae organisms	28 (46%)	1 (2%)	32 (52%)	–	–	–	–
M. leprae sonicate	12 (19%)	2 (3%)	49 (78%)	0·0012	–	–	–
M. tuberculosis sonicate	24 (39%)	0 (0%)	38 (61%)	ns	0·0125	–	–
M. vaccae sonicate	12 (19%)	2 (3%)	48 (78%)	0·0015	ns	0·0143	–
M. scrofulaceum sonicate	23 (37%)	6 (10%)	33 (53%)	ns	0·0199	ns	0·0085

ns Not significant.

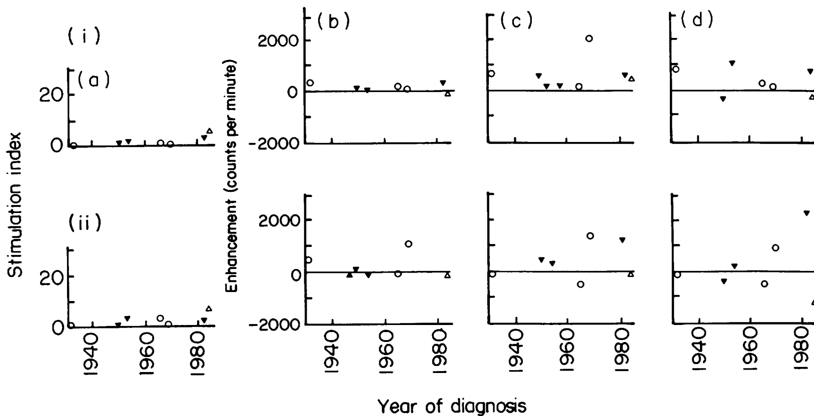

Fig. 4. Effect of (i) 40 u/ml and (ii) 160 u/ml Genzyme IL-2 on (a) background lymphoproliferation, and enhancement of response to (b) *M. leprae* organisms, (c) *M. vaccae* sonicate and (d) *M. tuberculosis* sonicate in subjects of different ethnic origins. For symbols see legend to Fig. 2.

(c) Enhancement using either *M. leprae* organisms or sonicated *M. tuberculosis* is more common with cells from patients than with cells from normal donors set up in parallel, but not significantly so, and is still only found in a minority of patients.
(d) Significant enhancement specific for *M. leprae* was rare (9 out of 97 patients).
(e) There is no relationship between duration of disease (or smear positivity) and the presence of enhancement.

Data for the largest and most homogenous patient group are summarized in Table 2. Enhancement was commoner when using *M. leprae* organisms (46%), or sonicates of *M. scrofulaceum* or *M. tuberculosis*, than when using sonicated *M. leprae* or *M. vaccae*.

Enhancement in cells from Spanish patients showing some response to *M. leprae* sonicate occurred with the same frequency (4 of 17) as in non-responding cells (8 of 34). Expansion of cells to *M. leprae* organisms did not occur significantly more often in low responders (7 of 14) than in non-responders (21 of 47) ($P=0{\cdot}25$, Fisher's exact test).

The effect of varying IL-2 and antigen concentration

The concentrations of IL-2 used were those suggested by Dr T. Godal, which in our hands cause some background proliferation of cells in wells without antigen. With cells from five patients and six normal donors we also studied a series of greater dilutions of IL-2, which were below the threshold of mitogenicity for normal cells. These concentrations had no enhancing effect on the response to *M. leprae* or *M. tuberculosis* of cells from patients or controls (data not shown).

Since the optimum concentration of antigen for triggering release of IL-2 is usually higher than the optimum for induction of lymphoproliferation, we examined the possibility that exogenous IL-2 would enhance responses only at low antigen concentrations which were suboptimal for IL-2 release. Use of antigen concentrations down to 0·1 μg/ml with cells from four normal donors and six patients did not reveal IL-2-dependent enhancement which was greater than seen with the standard dose of antigen used in the studies described above (data not shown).

DISCUSSION

The observation by Haregewoin *et al.* (1983) that addition of an exogenous source of IL-2 would enhance the response to *M. leprae* of lymphocytes from lepromatous leprosy patients, appeared to localize the defect in these individuals, and prove the existence in their peripheral blood of *M. leprae*-responsive T cells.

The results of this larger study partially confirm their observations. However enhancement of the lymphoproliferative response to mycobacterial antigens following addition of IL-2 was seen with cells from a minority of patients, and it occurred also (though less frequently) with cells from normal donors. In neither patients nor normals was it restricted to the antigens of *M. leprae*.

Our results do not appear to depend on the type of IL-2 used because we have used crude, purified, and recombinant material with similar results.

Similarly we have been unable to detect any relationship to dose of antigen, or concentration of IL-2 used.

Explanations put forward by other authors (Ottenhoff *et al.*, 1984; Mustafa, Haregewoin & Godal, 1984) to explain an inability to show that IL-2 mediated enhancement is a general phenomenon in lepromatous patients are race and duration of disease. The data on the large number of patients presented here allowed a careful search for such correlations, but none were seen. In addition concurrent ENL and therapy with thalidomide or prednisolone did not influence the effect of IL-2 on antigen response.

Neither do our data support the suggestion of Kaplan *et al.* (1985) that IL-2 can drive further expansion of *M. leprae*-specific T cells in low responders.

We conclude that enhancement by IL-2 of lymphoproliferative responses to *M. leprae* in lepromatous leprosy patients is not quite as common as the earlier reports suggested (Haregewoin *et al.*, 1983; 1984; Nath *et al.*, 1984), and the effect is restricted neither to leprosy patients, nor to the antigens of *M. leprae*. It is unlikely to be the fundamental defect in lepromatous leprosy.

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