

***In vivo* effects of propranolol on some cellular and humoral immune functions in a group of patients with lepromatous leprosy**

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Summary Certain functions of blood neutrophils and lymphocytes were investigated at varying time intervals after the addition of propranolol to standard therapy in a group of patients with lepromatous leprosy. A control group of patients received standard therapy only. The leucocyte functions tested were neutrophil chemotaxis, phagocytosis and NBT reduction and lymphocyte mitogen induced transformation, leucocyte inhibitory factor production and number of spontaneous E, E and EAC rosettes. Serum immunoglobulins, complement components and total haemolytic complement were also measured in both groups. Over a 3-month period neutrophil chemotaxis, numbers of active-E and E rosettes, lymphocyte transformation and lymphokine production improved on standard therapy alone. However, although the propranolol group had the highest mean responses, there were no significant differences between the two groups of patients after one month and three months. Likewise there was no difference between the two groups with respect to other cellular or humoral investigations. Neutrophil chemotaxis appeared to be the best functional correlate of clinical improvement.

Introduction

Cell-mediated immune responses such as lymphocyte transformation to mitogens and antigens (Bullock and Fasal, 1971;¹ Rea and Levan, 1977;² Nath *et al.*, 1977),³ lymphokine production (Godal, 1972;⁴ Talwar, 1972;⁵ Myrvang, 1973)⁶ and numbers of circulating T (Dwyer *et al.*, 1974;⁷ Sher *et al.*, 1976)⁸ and B (Mendes *et al.*, 1974;⁹ Sher *et al.*, 1976)⁸ lymphocytes are depressed in

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patients with lepromatous leprosy. Likewise phagocytic cell functions such as neutrophil motility (Bullock *et al.*, 1974;¹⁰ Ward *et al.*, 1976;¹¹ Sher *et al.*, 1978)¹² and macrophage ingestion of *Mycobacterium leprae* (Barbieri and Correa, 1967;¹³ Convit *et al.*, 1974)¹⁴ have been reported to be abnormal in these patients.

Recently propranolol has been shown to possess immunostimulatory activity. This drug has been reported to increase normal and abnormal neutrophil motility *in vitro* (Anderson and Van Rensburg, 1978;¹⁵ Anderson and Van Rensburg, 1979).¹⁶ Buckley and McGregor (1978)¹⁷ have reported that propranolol increased the number of survivors, decreased the duration of disease and increased the number of peritoneal exudate cells in rats which had been experimentally infected with type III pneumococci by increasing granulocyte adhesion. Furthermore propranolol can cause increased antibody synthesis in mice (Nakazawa *et al.*, 1976)¹⁸ and rats (Benner *et al.*, 1968).¹⁹

We have previously reported (Anderson and Van Rensburg, 1979)¹⁶ that the profile of effects of propranolol on leucocyte function *in vitro* is similar to that of levamisole. However the concentration of propranolol required to achieve stimulation of leucocyte function *in vitro* is approximately ten-fold less (Anderson and Van Rensburg, 1979).¹⁶ For this reason a pilot study was undertaken to assess the immunologic status of a group of patients with lepromatous leprosy, also receiving standard therapy, before and after the administration of propranolol. No attempt was made to carry out a formalized clinical trial but changes in the clinical and histopathological picture were compared to the laboratory findings.

Patients

Twelve new untreated admissions (six borderline (BL), two sub-polar (LI) and four lepromatous (LL) were paired according to type of leprosy, age and sex (six males and six females). The classification was based on clinical and histopathological criteria of Ridley and Jopling (1966). All patients showed negative skin tests to lepromin. Patients in the control group received standard therapy of rifampicin (600 mg daily) for 6 weeks and dapsone (DDS, 100 mg daily) thereafter. The effects of treatment on immune functions were assessed by comparison of pre-treatment results with those obtained during treatment. Thus each patient served as his own control.

Propranolol

Each patient in the experimental group in addition to standard therapy received the beta-adrenoreceptor blocking agent propranolol in a fixed dose

of 40 mg t.i.d. over a twelve-week period. Clinical evaluation and immunological testing were done at the outset and during (one and three months) propranolol therapy. Histopathology on biopsy specimens was performed at the outset and after 3 months. They were assessed for cellular composition, bacterial index (BI) and the granularity index (GI) of the bacteria.

Cellular studies

NEUTROPHIL MOTILITY

Neutrophils were obtained from heparinized venous blood (5 units heparin/ml), washed twice with Hank's balanced salt solution (HBSS, Grand Island Biological Company, NY, USA) supplemented with 1 g/l of Hepes (Sigma, St Louis, Missouri, USA) to give a final pH of 7.2 and resuspended to a final concentration of 5×10^6 /ml. Two leucoattractants were used in the study:

- (a) Fresh pooled normal sera activated with 100 μ g/ml of bacterial endotoxin (*E. coli*: 0127:B8 Difco, Detroit, Mich, USA).
- (b) Endotoxin activated autologous serum. Both types of EAS were incubated for 30 min at 37°C followed by an eight-fold dilution with HBSS. Neutrophil motility was assessed as previously described (Anderson and Van Rensburg, 1979).¹⁶ Chambers were incubated and the results expressed as the average number of cells reaching the lower surface of the 5 μ pore size Millipore after 3 hours incubation and expressed as an average for triplicate filters.

PHAGOCYTOSIS

This was assessed as previously described, by the ingestion of *Candida albicans* (Anderson and Van Rensburg, 1979).¹⁶ Results are expressed as percentage *C. albicans* ingested in the presence of 10% fresh autologous serum.

NITRO BLUE TETRAZOLIUM REDUCTION

Tests of resting and stimulated (semi-quantitative) NBT reduction were performed according to the method of Sher *et al.* (1974).²¹ The percentage of NBT (reduced) containing PMN was evaluated. For the resting test the normal result is < 10% positive PMN and in the stimulated test > 90%.

LYMPHOCYTE TRANSFORMATION

Blood for studies of lymphocyte function was defibrinated and fractionated by density gradient centrifugation (Ficoll-sodium metrizoate) at 400 g for 25 min.

Mononuclear cells were washed twice in RPMI (Grand Island Biological Co, NY, USA). 2×10^5 cells (50 μ l) were used in the assay which was done as previously described using PHA and CON A as the mitogens at concentrations of 25 and 50 μ g/ml (Anderson *et al.*, 1979).²²

LEUCOCYTE MIGRATION INHIBITION FACTOR (LIF) PRODUCTION

To 17 \times 100 mm polypropylene culture tubes was added 0.5 ml mononuclear cell suspension (4×10^6 /ml), 0.1 ml TC199 or 0.1 ml PHA (25 μ g and 50 μ g/ml) and the volume adjusted to 1 ml by the addition of serum supplemented TC199. The tubes were incubated at 37°C/96 hrs in a humidified atmosphere of 3% CO₂ in air. The cell free supernatant was removed and assayed for LIF according to the method of Weisbart and Mickey (1977).²³ Using human blood neutrophils as the indicator cells. Migration zones in the presence and absence of LIF containing supernatants were measured microscopically using a \times 2.5 objective and a calibrated eyepiece graticule. The results are expressed as percentage inhibition of migration as calculated by comparison with the control system.

E AND EAC ROSETTES

Rosetting techniques were performed according to the method of Brain and Marston (1973).²⁴ Briefly, lymphocytes obtained by density gradient centrifugation were further purified by the elimination of contaminating phagocytic cells by iron adherence. The ratio of lymphocytes to E and EAC was 1:10. For EAC rosettes pooled human AB serum was used as a sub-lytic source of complement. Active E-rosettes were measured according to the method of Wybran and Fudenberg (1973).²⁵ The lymphocyte : sheep red blood cell ratio was 1:10. The mixture was centrifuged at 250g/5 min before immediate counting of rosettes. A lymphocyte which had bound 3 or more red cells constituted a rosette.

Serological investigations

IMMUNOGLOBULINS AND COMPLEMENT COMPONENTS

The serum levels of IgG, IgA and IgM were determined by radial immunodiffusion using commercial plates and standards (Behring Institute). Serum C-3 and C-4 complement levels were assayed by rocket immunoelectrophoresis (Axelsen, Kroll, Weeke, 1973;²⁶ Laurell, 1972)²⁷ Results of immunoglobulin and complement estimations are expressed as mg/100ml and mg/1000 ml respectively. Serum IgE levels were measured by radioimmunoassay (Phadebas

IgE test, Pharmacia diagnostics) and results expressed as IU/ml. The upper limit of normal is taken as 150 IU/ml. Serum CRP levels were measured by a semi-quantitative latex agglutination procedure, the normal value being $< 13.2 \mu\text{g/ml}$. ASO antibodies were measured by inhibition of lysis of sheep red blood cells by streptolysin-O, and results expressed as the reciprocal of the titre (normal value 200).

Results

CALCULATION AND EXPRESSION OF RESULTS

Results are expressed as the mean value with standard error of the control and experimental groups for each investigation at each time interval tested. The results of each immunological test for control and experimental groups were compared by the Student's t test.

Neutrophil function

Results are shown in Table 1. Neutrophil chemotaxis was markedly impaired prior to the commencement of therapy and steadily increased throughout the three-month period, being most noticeable after the inclusion of dapsone. Although the increase in chemotactic responsiveness at one month and 3 months was greatest in the group receiving propranolol, the increase was not significant. The restoration of chemotactic responsiveness appeared to correlate with clinical improvement. Two patients in the control group and one in the experimental group showed no increase in chemotaxis throughout the trial period. These three also had less obvious clinical response to therapy. There was a slight but insignificant impairment of phagocytosis of *C. albicans* which normalized to a similar extent in both groups following therapy. Resting and stimulated NBT reduction was normal in both groups and remained unaffected with therapy.

Lymphocyte function

Results are shown in Table 2. Three patients in the control group and 3 in the experimental group showed reduced responsiveness to PHA (results $< 30,000$ cpm) and CON A (results $< 8,000$ cpm). The mean responsiveness to the mitogens increased to a similar extent in both groups following therapy, indicating that the improvement was due to standard therapy alone. A similar improvement in mitogen induced production of LIF, independent of propranolol supplementation, was also observed. Numbers of active-E, E and EAC rosettes were normal prior to therapy (with the exception of 2 with reduced

Table 1. Neutrophil chemotaxis to EAS, phagocytosis and nitro-blue tetrazolium reduction in a control group of patients with lepromatous leprosy and an experimental group who received propranolol in addition to standard therapy

Investigations	Control Group*			Experimental group†		
	Pre-treatment	1-month post-treatment	3 months post-treatment	Pre-treatment	1 month Post-treatment	3 months post-treatment
<i>Chemotaxis to:</i>						
a. Normal EAS (cells/HPF) (Normal value > 180 cells/HPF)‡	56 ± 19.7§	95 ± 45.0	131 ± 36.0	67 ± 25.5.	106 ± 16	232 ± 27.7
b. Autologous EAS (Cells/HPF) (Normal value > 180 cells/HPF)	26 ± 8.1	52 ± 31.0	129 ± 43.3	46 ± 15.2	73.1 ± 19.3	191 ± 26.8
<i>Phagocytosis of C. albicans</i> (% ingestion/25 min) (Normal value > 90%)						
	80 ± 6.4	82 ± 3.9	94 ± 1.6	86 ± 1.6	86 ± 2.7	95 ± 0.7
<i>NBT Reduction</i>						
a. Resting (Normal value < 10%)	10 ± 4.6	4.6 ± 1.9	8.7 ± 2.6	11 ± 3.0	8.7 ± 3.0	10.3 ± 3.8
b. Stimulated (Normal value > 90%)	97 ± 0.9	92 ± 1.1	95 ± 1.1	92 ± 0.5	95 ± 1.3	95 ± 1.1

*Receiving standard therapy only.

†Receiving standard therapy and propranolol 40 mg t.d.s.

‡The lower limit of the normal range of values shown here is taken as being one standard deviation below the mean of 100 control subjects.

§Results expressed as mean and standard error for six individuals.

Table 2. Lymphocyte mitogen induced transformation and relative percentages of active E, E and EAC rosetting cells in a control group of patients with lepromatous leprosy and an experimental group who received propranolol in addition to standard therapy

Investigation	Control Group*			Experimental group†		
	Pre-treatment	1 month post-treatment	3 months post-treatment	Pre-treatment	1 month post-treatment	3 months post-treatment
<i>Lymphocyte transformation to:</i>						
a. 25 µg/ml PHA (cpm)	26.264 ± 8.645‡	47.698 ± 19.483	45.482 ± 17.338	25.584 ± 5.746	59.904 ± 16.710	58.821 ± 20.980
b. 50 µg/ml PHA (cpm)	30.405 ± 8.323	45.216 ± 16.095	43.798 ± 15.125	23.386 ± 5.028	48.005 ± 11.592	50.994 ± 21.466
c. 25 µg/ml CON A (cpm)	7.639 ± 2.781	12.340 ± 8.042	18.462 ± 8.893	1.941 ± 612	12.255 ± 3.735	9.35 ± 2.010
d. 50 µg/ml CON A (cpm)	8.205 ± 4.494	14.934 ± 7.814	15.702 ± 7.979	4.199 ± 1.503	15.862 ± 5.230	12.156 ± 2.683
<i>Rosetting cells</i>						
a. Active E-rosettes (%)	47 ± 8.6	57 ± 3.0	54 ± 8.3	38 ± 10.0	46 ± 4.0	67 ± 8.1
b. E-rosettes (%)	62 ± 5.0	57 ± 4.5	66 ± 2.8	57 ± 7.1	60 ± 7.1	70 ± 4.1
c. EAC-rosettes (%)	34 ± 8.0	25 ± 8.0	28 ± 4.3	27 ± 6.5	27 ± 4.4	19 ± 3.5

*Receiving standard therapy only.

†Receiving standard therapy and propranolol 40 mg t.d.s.

‡Results expressed as mean and standard error for six individuals.

Table 3. Serum immunoglobulin and complement levels in a control group of patients with lepromatous leprosy and an experimental group who received propranolol in addition to standard therapy for a three-month period

Investigation	Control group*			Experimental group†		
	Pre-treatment	1 month post-treatment	3 months post-treatment	Pre-treatment	1 month post-treatment	3 months post-treatment
Serum IgG (g/l)	33.3 ± 3.3‡	33.3 ± 3.8	29.8 ± 4.0	33.8 ± 4.3	37.8 ± 4.3	31.3 ± 3.7
Serum IgA (g/l)	5.1 ± 1.1	5.6 ± 1.2	5.4 ± 1.1	5.4 ± 1.0	6.2 ± 1.0	5.9 ± 1.1
Serum IgM (g/l)	5.0 ± 1.1	4.3 ± 0.8	3.8 ± 0.6	5.0 ± 1.0	6.4 ± 1.4	4.1 ± 0.8
Serum IgE (IU/ml)	595 ± 248	485 ± 222	422 ± 780	1,700 ± 780	778 ± 276	1,350 ± 546
THC (CH50)	158 ± 8.1	162 ± 9.9	147 ± 6.6	166 ± 15.0	170 ± 20.0	145 ± 9.2
C'3 (mg/dl)	71 ± 7.1	66 ± 4.5	69 ± 7.0	65 ± 3.8	75 ± 8.4	66 ± 8.7
C'4 (mg/dl)	31 ± 4.9	36 ± 4.0	25 ± 3.0	25.3 ± 6.1	25.5 ± 4.4	28.6 ± 7.2

*Receiving standard therapy only

†Receiving standard therapy and propranolol 40 mg t.d.s.

‡Results expressed as mean and standard error for six individuals

numbers of EAC rosettes and with reduced numbers of E-rosettes in the control group) and remained unaltered throughout in both groups.

Serological studies

Serum immunoglobulins, including IgE, were elevated in both groups and remained increased to a similar extent throughout the 3-month period. Likewise levels of C3 and C4 and total haemolytic complement were normal at the outset and remained unaffected.

Histological results

There were no detectable differences between the control and experimental groups with regard to cellular composition of the biopsies, bacterial index and granularity index after 3 months of therapy.

Skin testing

All patients in each group remained anergic to lepromin after 3 months of therapy.

Patients

Using clinical criteria there appeared to be no differences between the two groups, although clinical improvement appeared to correlate with the extent of recovery of neutrophil chemotactic responsiveness. There were no adverse reactions to propranolol in the experimental group. The incidence of ENL was the same in both groups (2/6 in the control and 2/6 in the experimental groups).

Discussion

This study has confirmed previous reports that some patients with lepromatous leprosy have reduced lymphocyte transformation (Bullock and Fasal, 1971;¹ Rea and Levan, 1977;² Nath *et al.*, 1977)³ and LIF production (Godal, 1972;⁴ Talwar, 1972;⁵ Myrvang, 1973)⁶ to mitogens and depressed neutrophil migratory responses to leucoattractants (Bullock *et al.*, 1974;¹⁰ Ward *et al.*, 1976;¹¹ Sher *et al.*, 1978).¹² However these functions of circulating leucocytes improved, and in almost all cases normalized, following 3 months of standard therapy with rifampicin and dapsone. The inclusion of propranolol, which has been reported to possess immunostimulatory properties, with standard therapy caused further improvement of neutrophil chemotactic responsiveness and

lymphocyte functions. However the magnitudes of the increases were not significant and did not correlate with any clinical, histopathological or bacteriological differences between the two groups. Likewise skin test unresponsiveness to lepromin was still evident in all members of both groups after the 3-month period.

These results indicate that immunostimulation with presently available agents may be of limited value in patients with lepromatous leprosy. In agreement with this contention is a recent report by Faber *et al.* (1979)²⁸ who were unable to detect any significant improvement in *in vitro* and *in vivo* immunological functions in a group of patients receiving transfer factor.

Of interest is the recovery of normal blood leucocyte function with standard therapy alone. Increased leucocyte responsiveness *in vitro* was most evident when dapsone was introduced to the therapeutic regime. Apart from its bacteriostatic effect (McCullough and Maren, 1973),²⁹ which may be directly related to improved leucocyte function by lessening the antigenic load and removing the source of serum inhibitors of leucotaxis (Sher *et al.*, 1978)¹² dapsone may have other modes of action (McDougall, 1979).³⁰ One possibility currently being investigated in this laboratory, is that dapsone *per se* possesses immunostimulatory activity, which may invalidate studies assessing the value of immunostimulants in patients with lepromatous leprosy.

Recovery of neutrophil chemotactic responsiveness appeared to correlate with clinical improvement since the three patients who did not recover normal neutrophil locomotion had the least clinical improvement. This apparent correlation between recovery of chemotaxis and clinical response to therapy is presently being further investigated.

Although no striking immunological or clinical benefits were observed by the addition of propranolol to standard therapy in patients with lepromatous leprosy it is possible that the duration of the trial period was insufficient. However after 3 months of standard therapy \pm propranolol blood neutrophil and lymphocyte functions had normalized in most patients, which indicates the likelihood that at this stage non-specific immunostimulation may be of little value.

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