Clin. exp. Immunol. (1976) 26, 397–402.

Plasma factors in delayed-type hypersensitivity

AUGMENTATION OF LYMPHOCYTE RESPONSES IN BORDERLINE LEPROSY REACTIONS

G. BJUNE* & R. StC. BARNETSON† *Armauer Hansen Research Institute, Addis Ababa and †Medical Research Council Leprosy Project, Addis Ababa, Ethiopia

(*Received* 16 *July* 1976)

SUMMARY

The phytohaemagglutinin-induced responses of lymphocytes were found to be inhibited by plasma from patients with leprosy when compared with their responses in pooled serum from healthy donors. When patients developed reversal reactions, the initial inhibitory effect of their plasma was replaced by an augmentary effect on the responses to phytohaemagglutinin. The period of augmentation coincided with that of the reversal reaction in patients with borderline tuberculoid leprosy, but was delayed in patients with borderline lepromatous leprosy. The plasma from each leprosy patient was also observed to have the same effect on lymphocytes from unrelated individuals, showing that the inhibition and augmentation were due to factors in the plasma and not to a change in lymphocyte receptors.

It is possible that the normal stable state of leprosy results from the presence of factors in plasma which act as a control mechanism, and that delayed hypersensitivity reactions may be caused by a breakdown of this control.

INTRODUCTION

Cell-mediated immune (CMI) responses are essential for resistance to various intracellular organisms and to neoplastic change in the tissues. Under certain circumstances, however, the CMI responses are not beneficial to the host, and they are then termed 'delayed hypersensitivity reactions' (Coombs and Gell type IV hypersensitivity reactions).

Possibly the most clear-cut clinical example of delayed-type hypersensitivity is the reversal reaction in borderline leprosy which often leads to permanent deformity due to granuloma formation in nerves. Ridley (1969) found the histology of these reactions typical of delayed-type hypersensitivity, and Godal *et al.* (1973), Barnetson *et al.* (1975), and Bjune *et al.* (1976), found a marked rise in *in vitro* lymphocyte transformation responses to antigens from *Mycobacterium leprae* during reaction.

The reason for the emergence of hypersensitivity from a state of comparative non-reactivity is still unclear. One possibility, however, is that reaction occurs when there is a breakdown of control mechanisms which normally regulate CMI responses and prevent them from becoming overactive and harmful.

Humoral factors influencing CMI responses can be studied *in vitro* using the lymphocyte transformation (LT) test. Phytohaemagglutinin (PHA) stimulates T lymphocytes predominantly and comparison of responses in autologous plasma and in standard serum may demonstrate any modifying effect of the patient's own plasma. In this investigation we have utilized such a system to demonstrate the effect of plasma factors on T-cell responses in patients with borderline leprosy who developed reversal reaction.

Correspondence: Dr G. Bjune, Institute for Experimental Medical Research, Ullevaal Hospital, Oslo 1, Norway.

PATIENTS AND METHODS

Twenty-four patients with borderline leprosy who developed reversal reaction during their 1st year of treatment were studied. All patients were classified clinically and histologically according to the Ridley–Jopling scale (Ridley & Jopling, 1966). Sixteen were borderline tuberculoid (BT), four, borderline (BB) and four, borderline lepromatous (BL). Twelve of the patients were male, and twelve female; their ages ranged from 13–56 years. All patients received treatment with dapsone, which was continued in unaltered dosage throughout the study. When they developed reaction they were treated with prednisolone at an initial dosage of 30–40 mg daily, and this was gradually reduced over a period of 1–9 months depending on the duration and severity of the reaction. Clinical assessments and LT tests were carried out at intervals before, during and after the reaction. The degree of inflammation in skin lesions and nerves was carefully recorded and graded on an arbitrary scale as described earlier (Bjune *et al.*, 1976). When erythema and oedema involved normal skin around the lesions, and/or nerves were markedly tender and enlarged with electrophysiological and functional evidence of substantial acute involvement, the inflammation was considered a fully developed reaction. The diagnosis of reaction was confirmed by biopsy of a skin lesion and the radial cutaneous nerve.

Nineteen patients with borderline leprosy (BT:14, BL:5) matched for age, sex and duration of treatment who had quiescent hypopigmented skin lesions and no evidence of neuritis for at least 6 months, were used as controls. All tests and clinical assessments were carried out in the same way as for the reactional patients.

In forty-seven of the LT tests in reactional patients before, during and after the reaction, the effect of the patients' plasma was tested on lymphocytes from another individual (healthy or leprosy patient) in parallel with the autologous lymphocytes. The foreign lymphocytes and the patients' own cells were cultured identically on the same tray.

Lymphocyte transformation tests were carried out by a micro method as described by Closs (1975). Peripheral blood lymphocytes were separated by centrifugation on Ficoll–Isopaque (Nyegaard, Oslo, Norway) as described by Böyum (1968) and cultured in a concentration of $0{\cdot}5 \times 10^6$/ml. Stimulation with PHA (Reagent Grade, Wellcome, Beckenham) was carried out at a dilution of 10^{-2} from stock, found to be optimal in previous experiments. The standard serum was collected from twenty healthy donors from countries where leprosy was not endemic. It was filtered through a 0·45 μ Millipore filter and stored in small aliquots at −70°C. The patients' plasma was recovered from the blood sample after the separation of cells, and was kept for not longer than 4 hr before being mixed with the cells for culture. Lymphocyte cultures were carried out in triplicate in medium TC 199 (Gibco, Glasgow) with 10% of either standard serum or patient's plasma on the same micro tray (Linbro, IS-FB-96-TC, New Haven, Connetticut). Control cultures without PHA added were also included in the tray. Lymphocyte cultures were incubated at 37°C, 100% humidity and 5% CO_2 for 96 hr. The cells were labelled with 0·5 μCi of tritiated thymidine (specific activity, 2 Ci/mM; Radiochemical Centre, Amersham) 16 hr before they were harvested onto glass-fibre filters (Gelman, Ann Arbor, Michigan), washed with distilled water, dried and the thymidine uptake recorded as ct/min in a liquid scintillation counter (Inter-technique SL 31, Plaisire, France).

The recorded values for stimulation by PHA were calculated as the mean of stimulated triplicate cultures, with the mean of unstimulated triplicates subtracted. Statistical significance of differences between groups was estimated with the Wilcoxon matched-paired-signed-rank-test (W), or the Kolmogorov Smirnov two-sample-test (KS) when observations were not paired. Estimates of linear regression with statistical calculations for paired data were done on a Canon Canola F-20P computer.

RESULTS

The PHA-induced responses of lymphocytes from the nineteen control patients who had stable borderline leprosy gave a median value of 34,700 ct/min when cultured in 10% autologous plasma. This value was much lower than when the cells were cultured in 10% standard serum i.e. median: 48,200 ct/min ($P<0{\cdot}001$ (W)). The degree of inhibition by autologous plasma remained fairly constant in the nine clinically stable males, while the ten stable females showed considerable variation from test to test (Fig. 1).

Plasma from the twenty-four patients who developed reactions produced a similar degree of suppression of PHA responses both before and after reaction, while by contrast for the period related to the reaction they augmented the responses. The duration of the augmenting effect of plasma and its degree showed great individual variation, as did the intensity and duration of the clinical reaction. In ten of the patients we were able to perform several tests before, during and after the reaction (Fig. 2). When the effect of autologous plasma from BT patients on the lymphocyte responses to PHA (recorded as a percentage of the responses in standard serum) was compared before, during and after reaction, a highly significant difference appeared (Fig. 3). The median value before reaction was 52%, during reaction 128% and after reaction 57% ($P<0{\cdot}001$ (KS) for both rise and fall).

The augmenting effect of plasma from BL patients appeared later than that from BT patients. Post-reactional values recorded up to 2 months after the clinical reaction had subsided, were significantly

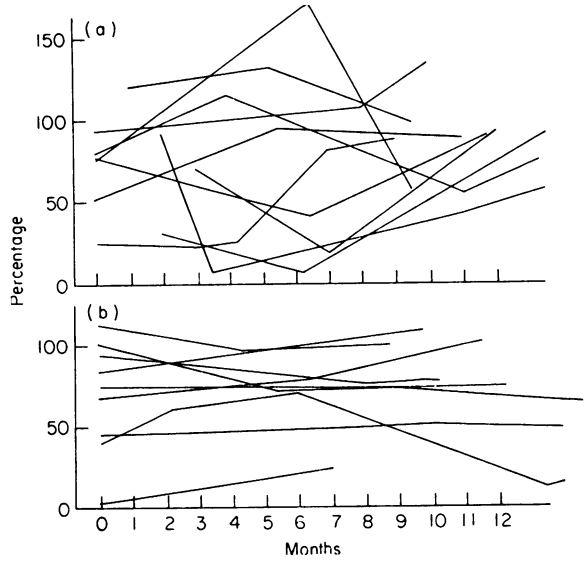

FIG. 1. Effect of autologous plasma on lymphocyte responses to PHA, recorded as a percentage of the parallel responses in standard serum. Variation with time in ten clinically stable females (a) and nine stable males (b).

higher (median: 90%) than the reactional values (median: 36%), ($P = 0{\cdot}005$ (KS)). The degree of inhibition observed in stable BL patients (median: 61%) was not significantly different from the inhibition by plasma from BL patients in reaction.

The changes in the effect of plasma on PHA responses during the course of reversal reactions were not a direct effect of steroid treatment. Reactional BT patients on steroid treatment had a median response of 126% and postreactional ones 52·5%, which were not significantly different from patients in the same groups without such treatment (125% and 57% respectively).

The effect of plasma in each case from reactional patients, taken before, during and after reaction, was tested simultaneously on autologous lymphocyte responses and lymphocytes from an unrelated

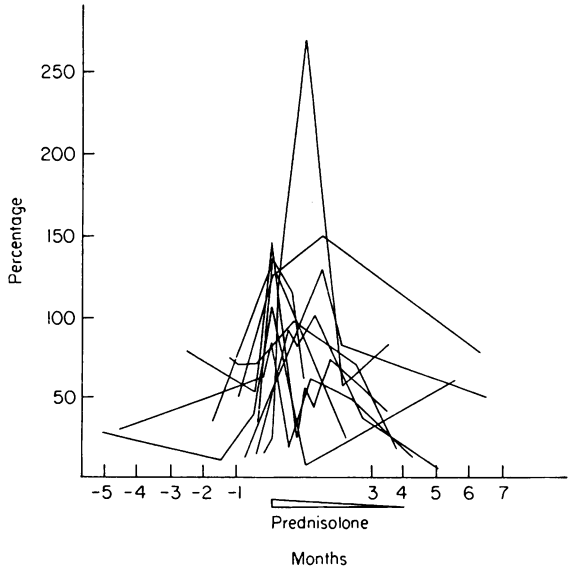

FIG. 2. Effect of autologous plasma on lymphocyte responses to PHA, recorded as a percentage of the parallel responses in standard serum. Variation with time in ten patients going through a reversal reaction. 0 = height of reaction clinically, when steroid treatment was initiated.

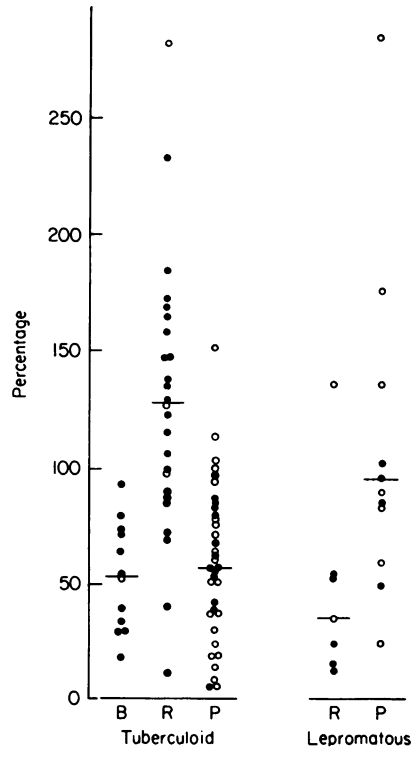

FIG. 3. Effect of autologous plasma on lymphocyte responses to PHA, recorded as a percentage of the parallel responses in standard serum. Comparison of responses before reaction (B), during reaction (R) and post reaction (P), in sixteen borderline tuberculoid and five borderline patients. (○) patients on steroid treatment; (●) patients on dapsone only; (———) median effect on the group.

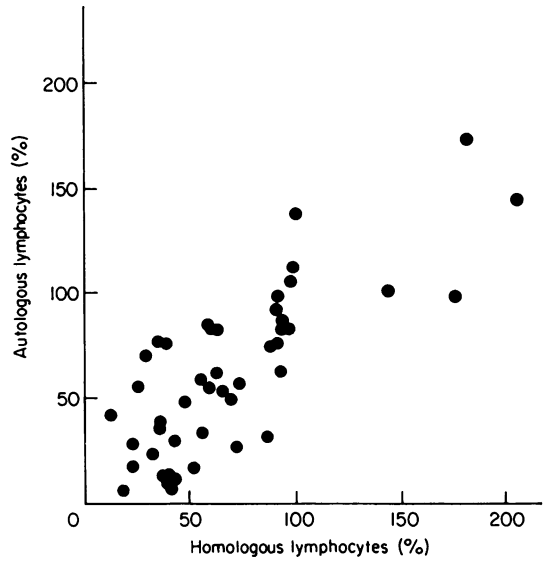

FIG. 4. Effect of reactional patients' plasma on lymphocyte responses to PHA, recorded as a percentage of the parallel responses in standard serum: correlation between the effect on autologous lymphocytes and lymphocytes from unrelated individuals (homologous). Correlation coefficient, $r = 0{\cdot}81$ ($P < 0{\cdot}001$).

individual. The relationship of the effect of reactional patients' plasma on autologous and homologous lymphocytes is shown in Fig. 4. From this it is seen that the plasma had the same effect on both types of test cells.

DISCUSSION

Factors in the blood inhibiting lymphocyte responses to PHA have been demonstrated in leprosy by Nelson *et al.* (1971), in recurrent infections in childhood by Fitzgerald & Hosking (1976), various neoplasms by Sucio-Foca *et al.* (1973), in pregnancy by Gatti (1971), and also in several other conditions. Common to many of these conditions is a prolonged stimulation of the immune system.

BT patients often show surprisingly little inflammatory response despite the presence of bacilli in the tissues for many years. Plasma from such patients caused inhibition of lymphocyte responses to PHA compared with the responses in standard serum (Fig. 1). In those patients with BT leprosy who developed a reversal reaction, this inhibitory effect disappeared during the reaction and was replaced by an augmenting effect of plasma on lymphocyte responses (Figs 2 and 3).

That both the augmentation and inhibition of lymphocyte responses were due to factors in the plasma and not to a change in lymphocyte receptors, is supported by the observation that the plasma had the same effect on both homologous and autologous lymphocytes (Fig. 4). Control experiments with different concentrations of PHA gave no indication of a changed sensitivity of lymphocytes during reaction nor was there any significant change in the actual height of the PHA responses in standard serum in relation to the reaction. So the augmentation of responses by autologous plasma during reaction was not due to a change in the lymphocytes' responsiveness to PHA.

Although clinically stable male patients had a constant degree of inhibition during the period of observation, the effect of plasma from stable female patients on PHA responses showed a great variation from test to test (Fig. 1). Further studies on normal individuals (Bjune, unpublished data) have shown that females have pronounced variations of plasma effect on the PHA response related to the menstrual cycle. The magnitude of these variations considerably surpass the basic level of suppression due to the leprosy infection.

It has been observed by Weddell & Pearson (1975) that bacilli can lie unrecognized in Schwann cells of dermal and peripheral nerves, while they are completely cleared from skin by macrophages. A reversal reaction could therefore be caused by a sudden detection of formerly hidden antigen as well as by a breakdown of control mechanisms possibly reflected by the plasma factor(s) described here.

The finding in BL patients that the period of augmenting effect of autologous plasma was delayed in relation to the onset of clinical reaction, could indicate that the plasma factor(s) are secondary to the reaction. However, it is probable that the plasma factor(s) described here will initially occupy lymphocyte receptors and exert their effect there, before they can appear free in the blood. Therefore the delayed appearance in the blood of the augmentary effect does not necessarily negate their primary importance in transforming the CMI response from a well controlled fight against bacillary multiplication to an overactive and self-destructive hypersensitivity reaction.

We would like to thank Lena Lundin and Wollelaw Ejigu for skilled technical assistance, Dr Dennis Ridley for the histological classification, Dr Ben Naafs for nerve conduction studies and Drs John Pearson, Peter Friedmann and Göran Kronvall for help in preparing the manuscript. Armauer Hansen Research Institute is run by the Swedish and Norwegian Save the Children organizations and is affiliated with the All Africa Leprosy and Rehabilitation Training Centre (ALERT), Addis Ababa, Ethiopia, and the University of Bergen, Norway.

REFERENCES

BARNETSON, R.StC., BJUNE, G., PEARSON, J.M.M. & KRONVALL, G. (1975) Antigenic heterogeneity of patients with reactions in borderline leprosy. *Brit. med. J.* iv, 435.

BJUNE, G., BARNETSON, R.StC., RIDLEY, D.S. & KRONVALL, G. (1976) Lymphocyte transformation test in leprosy; correlation of the response with inflammation of lesions. *Clin. exp. Immunol.* **25**, 85.

BÖYUM, A. (1968) Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. clin. Lab. Invest.* **21**, supplement 97, 77.

CLOSS, O. (1975) *In vitro* lymphocyte response to purified protein derivative, BCG and *Mycobacterium leprae* in a population not exposed to leprosy. *Infect. Immunol.* **11**, 1163.

FITZGERALD, M.G. & HOSKING, C.S. (1976) Plasma inhibitors of lymphocyte responses to phytohaemagglutinin in children with recurrent infections. *Immunology*, **30**, 33.

GATTI, R.A. (1971) Serum inhibitors of lymphocyte responses. *Lancet*, **i**, 1351.

GELL, P.G.H., COOMBS, R.A. & LACHMANN, P. (eds.) (1974) *Clinical Aspects of Immunology*, 3rd. edn, Section IV. Blackwell Scientific Publications, Oxford.

GODAL, T., MYRVANG, B., SAMUEL, D.R., ROSS, W.F. & LÖFGREN, M. (1973) Mechanism of 'reactions' in borderline tuberculoid (BT) leprosy. *Acta Path. Microbiol. Scand.* Supplement 236, 45.

NELSON, D.S., NELSON, M., THURSTON, J.M., WATERS, M.F.R. & PEARSON, J.M.H. (1971) Phytohaemagglutinin-induced lymphocyte transformation in leprosy. *Clin. exp. Immunol.* **9**, 33.

RIDLEY, D.S. & JOPLING, W.H. (1966) Classification of leprosy according to immunity. A five-group system. *Int. J. Leprosy* **34**, 255.

RIDLEY, D.S. (1969) Reactions in leprosy. *Lepr. Rev.* **40**, 77.

SUCIO-FOCA, N., BUDA, J., MCMANUS, J., THIEM, T. & REEMTSMA, K. (1973) Impaired responsiveness of lymphocytes and serum-inhibitory factors in patients with cancer. *Cancer Res.* **33**, 2373.

WEDDELL, *A*.G.M. & PEARSON, J.M.H. (1975) *Topics of Tropical Neurology*, (ed. by R.W. Hornabrook), Davis, Philadelphia.