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Cell-mediated immunological reactivity in neonates and infants with congenital syphilis

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SUMMARY

Thirty-eight mothers and babies were assessed by the lymphocyte transformation test for their cell-mediated immune responses to *Treponema pallidum* and to purified protein derivative of tuberculin (PPD). All babies, including controls, appeared to be reactive when results of [^{3}H]-thymidine incorporation were expressed as net counts per minute. This was because of high background activity, which was most marked in cells from neonates. When results were expressed as stimulation ratios, it was revealed that cells from neonates were unreactive to both antigens, even when those from their mothers were reactive. Cells from some older babies with active congenital syphilis were reactive to both antigens.

INTRODUCTION

Syphilis is one of the diseases in which the causative organism is known to be capable of crossing the placenta and establishing congenital infection. The relative contributions of humoral and cellular immune mechanisms in determining the outcome of the disease are far from clear. Humoral mechanisms must play some part, since in rabbits the passive transfer of immune serum caused delayed onset and attenuation in severity of syphilitic lesions (Bishop & Miller, 1976). However, serum was unable to confer complete protection, even in high doses. It is therefore probable that cellular mechanisms are also necessary for protection.

In congenital infection with *Treponema pallidum*, maternal antibody (IgG) is passively transferred to the infant. If the foetus survives to term, then clinical manifestations of congenital syphilis usually appear after the first few weeks of life (Willcox, 1964). It is thought that this time coincides with the reduction of residual maternal antibody below a critical level.

In general, cell-mediated immune reactivity does not appear to be passively transferred to the foetus (Leikin & Oppenheim, 1971). It was suggested that active sensitization induced by transplacental passage of antigens is necessary for its development (Brody, Oski & Wallach, 1968). Congenital syphilis provides a model for testing the capacity of the foetus to mount an immunological response to an invading pathogen. In this study, the cell-mediated immune response of babies with potential, or active, congenital syphilis was investigated by means of the lymphocyte transformation test (LTT). Reactivity to *T. pallidum* and to an unrelated bacterial antigen, purified protein derivative of tuberculin (PPD), was assessed and the results were compared with those of their mothers.

MATERIALS AND METHODS

Patients. Patients came from the Ministry of Public Health Venereal Disease Demonstration Centre (VD Centre) and the labour ward of St Paul's Hospital, Addis Ababa. Blood samples were taken from thirty-eight mothers and their babies. Serological tests on all mothers with syphilis were positive. Group (a) comprised seven mothers and babies with active congenital infection. The babies had papular rashes which contained spirochaetes when examined by dark-field microscopy

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at the VD Centre. Six were aged 2–4 months; the seventh was 2 years old. Six mothers had no clinical signs of infection but the seventh had secondary syphilis. Group (b) comprised seventeen mothers and babies in St Paul's Hospital labour ward The babies were classed as 'potential congenital syphilis' since serological tests on their mothers were positive. Assessment of disease activity in the mothers was difficult, however, as accounts of previous exposure to antibiotics were unreliable. Only one of the babies had a positive IgM fluorescent treponemal antibody test at a serum dilution of 1/200. Group (c) comprised fourteen normal mothers and babies in the labour ward. Blood samples were obtained from subjects in group (a) when they were first seen at the VD Centre and from subjects in group (b) within 24 hr of birth, after the results of serological tests on the mothers were known. Maternal venous and foetal cord blood samples were taken at parturition from subjects in group (c). Aliquots were kept for serological tests. In all cases, samples were obtained with the informed consent of the mother.

Serological tests. All samples were tested by the slide VDRL test with carbon antigen and by the fluorescent treponemal antibody test at a serum dilution of 1/200 (FTA_{200}). Both tests were performed in accordance with the USPHS Manual of Serological Tests (1969).

Antigens. A washed saline suspension of Nichols strain *T. pallidum* (kindly provided by Dr Nafra Johnston of the VD Reference Laboratory, London) was stored at −70°C. It was used at a final concentration of 10^5–10^6 organisms per ml.

PPD free of preservative (Statens Serum Institute, Copenhagen) was used at a final concentration of 10 μg/ml.

Lymphocyte transformation tests. Lymphocyte transformation tests (LTT) were performed by a micromethod (Friedmann & Turk, 1975). Lymphocytes were separated, washed and cultured in Cooke microtitre plates at a concentration of 10^6/ml in RPMI 1640, supplemented with 10% pooled normal human serum. Cultures, in triplicate, were incubated in the presence of appropriate concentrations of antigen for 5 days at 37°C in a gassed, humidified atmosphere. They were given a 4 hr pulse of 0·2 μCi [^{3}H]thymidine (sp. act. 2 Ci/mmol) before aspiration onto glass-fibre paper with an automatic harvester. [^{3}H]Thymidine uptake was determined by liquid scintillation counting in an Intertechnique SL 31 automatic spectrometer.

Results of antigen-induced lymphocyte transformation, as shown by incorporation of [^{3}H]thymidine, are expressed as mean net counts per minute (ct/min) per culture. This value is obtained by subtracting the mean ct/min of antigen-free control cultures from the mean ct/min of cultures containing antigen. Stimulation ratios were calculated as mean ct/min of cultures containing antigen divided by mean ct/min of cultures without antigen.

Results were analysed by the non-parametric test of Kolmogorov–Smirnov (Siegel, 1956).

RESULTS

Serological tests

Serum from all mothers in group (a) gave positive VDRL tests at dilutions of 1/16 or 1/32, while their babies were positive in the range 1/16–1/256. The FTA_{200} test was positive for all members of the group.

Similarly, all mothers in group (b) gave positive results in the VDRL test (range 1/2–1/32), and the FTA_{200} test was positive for all. All but one of their babies gave positive VDRL tests (range 1/1–1/16). Serum from one baby gave a positive result in the FTA_{200} test with fluorescein-conjugated anti-IgM antiserum.

Serum from all control mothers, group (c), was negative in both VDRL and FTA_{200} tests.

Spontaneous activity of lymphocytes

Lymphocytes from the babies of each group, cultured in the absence of antigen, gave different background values of [^{3}H]thymidine incorporation (Table 1). Spontaneous activity was highest in the cells from cord blood of normal control babies (median 2660 ct/min, range 1475 to 9994). The next most active cells were those from babies up to 24 hr old (median 1997 ct/min, range 550 to 5063), although the activity was not significantly different from that of cord blood. The lowest background activity was found in cells from infants of 2 months or more (median 115 ct/min, range 741 to 3352). Group (a) had significantly lower counts than group (c), the control group ($P<0·001$). Spontaneous activity of cells from adults was lower still, and was similar for all three groups of mothers: the median of group (a) was 850 ct/min, of group (b) it was 453 ct/min and of group (c) it was 409 ct/min.

Lymphocyte responses to T. pallidum

The net ct/min of lymphocytes cultures in the presence of *T. pallidum* are shown in Fig. 1. The median value for lymphocytes from mothers of group (a) was 996 ct/min (range 257 to 2232) and for babies 1993 ct/min (range 590–2810). They were not significantly different from each other or from the

TABLE 1. Background activity of lymphocytes from the three different groups of babies

Group	Babies	Number	Age	Source of blood	[^{3}H]Thymidine uptake (ct/min)	
					Median	Range
(a)	Active congenital syphilis	7	2 months–2 years	Peripheral venous	1115	741–3352
(b)	Potential congenital syphilis	17	12–24 hr	Peripheral venous	1997	550–5063
(c)	Control	14	Newborn	Umbilical cord	2660	1475–9994

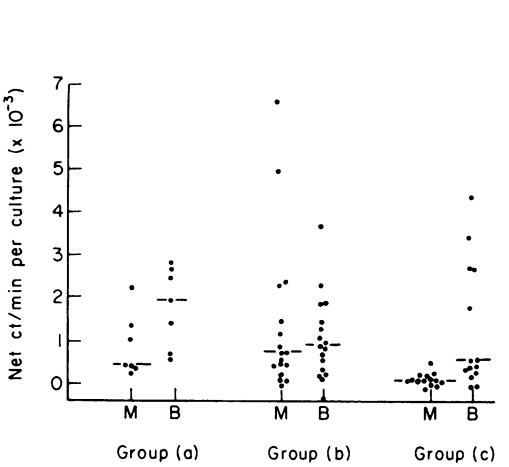

FIG. 1.

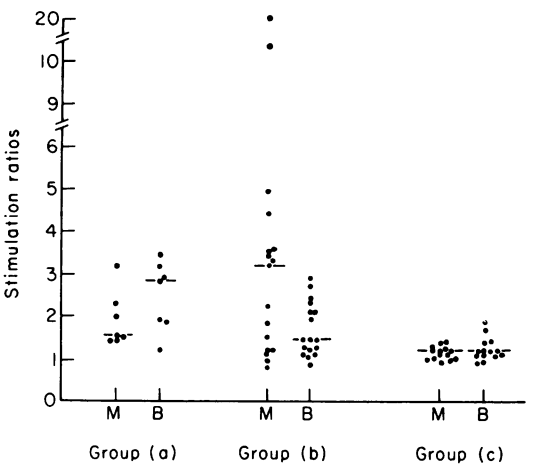

FIG. 2.

FIG. 1. Net [^{3}H]thymidine incorporation by lymphocytes cultured with *T. pallidum*. Group (a), babies had active congenital syphilis; group (b), mothers had positive serology, babies had 'potential' congenital syphilis; group (c), controls. M, Mothers; B, babies. Lines show medians.

FIG. 2. Responses of lymphocytes cultured with *T. pallidum* expressed as stimulation ratics. See Fig. 1 for legend.

response of cells from mothers and babies of group (b), which were 734 ct/min (range −37 to 6540) and 887 ct/min (range −487 to 3679), respectively. Cells from normal babies, group (c), did not differ significantly from those of either mothers or babies of groups with actual or potential syphilis: median 517 ct/min (range −110 to 4321). Lymphocytes from the mothers of group (c), however, were very much less reactive than those from their own babies or from any other patients: median 42 ct/min (range −177 to 495) ($P<0{\cdot}001$ for all comparisons).

The paradox that lymphocytes from some normal babies reacted to *T. pallidum* is resolved when results are plotted as stimulation ratios. Ratios for normal mothers and babies were all less than 2 (Fig. 2). Lymphocytes from neonates with potential congenital syphilis, group (b), gave a median ratio of 1·45 (range 0·83 to 2·9), which was significantly less than that for their mothers, median 3·2 (range 0·84 to 20) ($P<0{\cdot}05$). The latter values were significantly higher than those of either of the other groups of mothers ($P<0{\cdot}001$). Lymphocytes from babies with active congenital syphilis, group (a), gave a median ratio of 2·8 (range 1·2 to 3·4), which was significantly higher than that given by cells from control babies ($P<0{\cdot}001$).

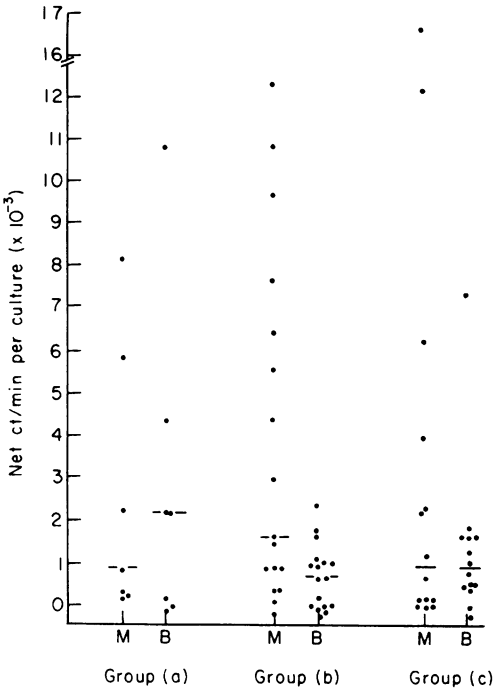

FIG. 3.

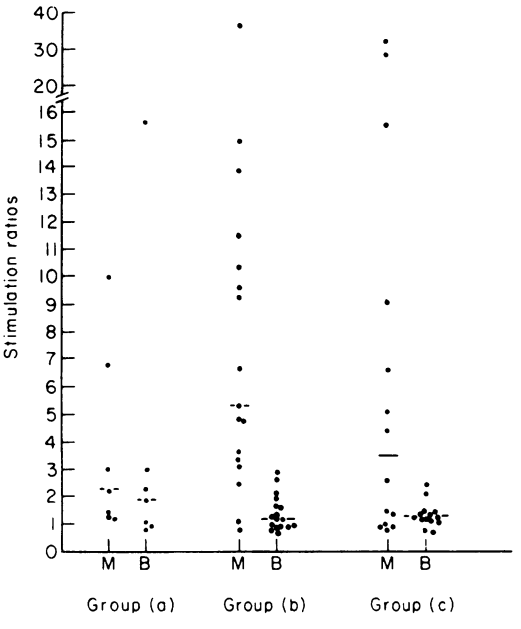

FIG. 4.

FIG. 3. Net [^{3}H]thymidine incorporation by lymphocytes cultured with PDD. See Fig. 1 for legend.

FIG. 4. Response of lymphocytes cultured with PPD expressed as stimulation ratios. See Fig. 1 for legend.

Responses to PPD

As it was possible that cells from a proportion of patients with syphilis might not respond to *T. pallidum* (Friedmann & Turk, 1977), they were also tested for their ability to react to the unrelated microbial antigen, PPD, since it was known to elicit positive responses from most Ethiopians tested (Bjune, personal communication). None of the mothers had signs or symptoms of tuberculosis; it was not possible to perform Mantoux tests.

When cultured with PPD, lymphocytes from mothers and babies of all groups gave values for [^{3}H]-thymidine incorporation which were not significantly different from each other (Fig. 3). The ranges of net ct/min for cells from mothers in groups (b) and (c) were, however, wider than those for their babies: for mothers and babies of group (b) the ranges were -1645 to 12,384 ct/min and -521 to 2318 ct/min; and for mothers and babies of group (c) the ranges were -170 to 16,608 ct/min and -2537 to 7281 ct/min, respectively. When results are plotted as stimulation ratios, there were no significant differences between the values for all three groups of mothers (Fig. 4). Ratios for cells from neonates, however, were significantly lower than those for their mothers ($P<0{\cdot}001$ for group (b), $P<0{\cdot}05$ for group (c)). The ratios for babies in group (a) were not significantly different from those of their mothers.

DISCUSSION

The development of cell-mediated immune reactivity is of major importance for the survival of the newborn (Editorial, 1969). Although most cellular mechanisms are immunologically competent in the neonate (Campbell *et al.*, 1974), specific cellular immune reactivity is not passively acquired from the mother.

Cord blood lymphocytes respond to PHA, and are, in fact, more sensitive to lower concentrations of mitogen than adult cells (Carr, Stites & Fundenberg, 1972). When pregnant women suffered infection

with *Escherichia coli*, cord blood lymphocytes from their babies proliferated when cultured with an extract of the organism, whereas cells from uninfected controls did not (Brody *et al.*, 1968). However, when expectant mothers were immunized with diphtheria or tetanus toxoids, although their own lymphocytes responded to those antigens *in vitro*, cells from their babies did not (Leikin & Oppenheim, 1971). It is possible that cell-mediated reactivity develops in the foetus if enough antigen crosses the placenta. This seems more likely to occur when active infection is present in the mother, than when antigens are administered for the purpose of immunization.

The interpretation of results obtained with neonatal lymphocytes in the LTT is complicated by their high levels of spontaneous proliferation (Pulvertaft & Pulvertaft, 1966; Alford, Cartwright & Sell, 1976; Barnetson, Bjune & Duncan, 1976). The present study confirmed that this activity was highest in lymphocytes from cord blood, but decreased as the age of the donors increased (Alford *et al.*, 1976). It was still appreciably raised in children of 2–4 months compared with adult values.

Results of net [^{3}H]thymidine incorporation by lymphocytes cultured with *T. pallidum* indicated that only cells from control mothers were unresponsive, while those of their babies seemed, like the rest, to be reactive. When the results were plotted as stimulation ratios, however, cells from both groups of neonates were revealed to be unresponsive, especially when compared to women with latent syphilis. In this study, stimulation ratios are probably the only useful way of comparing responses in the neonatal populations, as apparent positive [^{3}H]thymidine uptakes are shown to be related to high background activity. The same effect was true for neonatal lymphocyte responses to PPD. There were no significant differences in the net ct/min for any group. Stimulation ratios, however, show that the cells from both groups of neonates were unresponsive compared to their mothers; furthermore, the most reactive mothers did not give birth to the most reactive babies. Thus even though mothers with latent syphilis, group (b), had cellular reactivity to *T. pallidum*, and mothers of both groups (b) and (c) had lymphocyte reactivity to PPD, cells from their babies were unresponsive to both antigens. With regard to PPD, these findings confirm those of Barnetson *et al.* (1976). Presumably there was no intrauterine exposure to that antigen, although those authors thought that reactivity to another antigen, *Mycobacterium leprae*, might be transferred by some lymphokine-like substance. It seems highly unlikely that transfer of maternal reactivity to different mycobacterial antigens should be so selective.

If transplacental passage of antigen is necessary for the induction of cellular reactivity in the foetus, it might be expected that in cases of potential congenital syphilis some babies would have shown such reactivity to *T. pallidum*, since they would have been exposed to spirochaetal antigens *in utero*. That they did not, however, accords with our finding that most adult Ethiopians in the early infectious stages of syphilis have only a poorly developed cellular reactivity to *T. pallidum* (Friedmann & Turk, 1977). As congenital syphilis corresponds to the infectious stage in adults (King & Nichol, 1975), infants with congenital infection and whose lymphocytes are unresponsive to *T. pallidum* may be regarded as behaving like their adult counterparts. Some of the older infants, group (a), did show more marked lymphocyte reactivity to PPD than did neonates, but this could well reflect sensitization by post-natal exposure to antigen.

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