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Transfer of maternal specific cell-mediated immunity to the fetus

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SUMMARY

The extent of specific cell-mediated immunity was measured in 67 consecutive newborns and their mothers. The stimulation index of blast transformation of the infants' lymphocytes in the presence of purified protein derivative, *Candida* extract and streptokinase was $>2{\cdot}0$ in 54%, 18% and 23% respectively. This was seen only in infants whose mothers' index was also $>2{\cdot}0$ to the same antigen. Leucocyte inhibition factor generated from lymphocytes of four babies in the presence of purified protein derivative inhibited migration of indicator cells over 50%; their stimulation index with purified protein derivative was $>2{\cdot}0$. Newborns have cell mediated immunity to the same antigens as their mothers, and this wanes during the first few months of life.

Keywords cell mediated immunity transplacental fetal lymphocyte transformation leukocyte inhibition factor

INTRODUCTION

Cell mediated immunity (CMI) in the newborn of the same specificity as the mother's was first demonstrated by electrophoretic macrophage migration inhibition (Field & Caspary, 1971) and later by blast transformation of cord lymphocytes (Cutler & Pabst, 1972; Leiken, Whang-Pen & Oppenheim, 1974; Scott *et al.*, 1981; Gallagher *et al.*, 1981). Teleologically, this state would benefit the baby by protecting it against infections to which the mother has responded with active CMI.

The practical significance of these findings lies in their relevance to immunization of infants with vaccines which require full participation of the cell-mediated immune response to specific antigens for adequate immunity induction. For example, the effectiveness of BCG immunization of infants may be influenced by preexisting CMI to mycobacterial antigen through interference with processing of the BCG organisms. We have therefore investigated the specificity of this phenomenon by two independent CMI assays to establish the extent of correlation between maternal and infant sensitization.

MATERIALS AND METHODS

Lymphocyte stimulation with specific antigens. Cord blood was obtained from infants at birth and in some cases from venous blood at several weeks of age. Venous blood from their mothers was obtained within a week of delivery. Ficoll-separated peripheral blood lymphocytes (PBL) were cultured in microtitre wells in triplicates as described previously (Bowen & Pabst, 1976). Briefly,

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2×10^5 cells were cultured in 0·2 ml RPMI in 15% autologous plasma or in 15% human AB serum in the presence or absence (control background) of purified protein derivative (PPD, preservative free, Connaught), or *Candida* extract (Hollister Stier) or streptokinase (SK, Hoechst). After preliminary dose and time response assays, concentrations used for PPD were 0·016, 0·16, and 1·6 μg/well, *Candida* and SK 4 and 8 μl/well of 1/100 dilution of each antigen. Thus, if cell numbers permitted, 14 variables for each sample were explored: three concentrations of PPD, two each of *Candida* and SK, cultured in AB serum or in homologous plasma.

Cultures were pulsed with 1·0 μCi tritiated thymidine for 7 h on the sixth day of culture before harvesting on a Skatron cell harvester (Flow Laboratories). Glassfibre filter discs with entrapped cells were evaluated by liquid scintillation counting. Results were expressed as stimulation index (SI) = counts per minute (ct/min) of cultures with antigens divided by ct/min of cultures without added antigens.

LIF assay. For LIF generation 1×10^6 PBL from cord blood samples were incubated for 48 h in the presence (test) or absence (controls) of 1·6 μg PPD or 300 μg of phytohaemagglutinin (Burroughs Welcome) (positive control), in 1 ml of RPMI media with 10% pooled heat inactivated human serum (Winter *et al.*, 1983). Further controls consisted of antigen in media without cells. Ten microlitres of the supernate (S) was used for incubation of 2×10^6 indicator leucocytes (IL) in the migration assay. IL were collected after dextran sedimentation of venous blood from healthy volunteers. Triplicates of 2×10^6 IL incubated in S for 1 h were placed into 4 mm wells in 1·8% agarose in RPMI and allowed to migrate for 48 h at 37°C. After fixing the migrated cells with 7% glutaraldehyde the diameter of the migration area was measured with callipers. Percent migration inhibition

$$(\mathrm{MI}) = \frac{\text{average of migration areas of IL in test S}}{\text{average of migration areas of IL in control S}} \times 100$$

Karyotyping. Cord blood from boys was cultured in RPMI with 10% FCS in the presence of 12 μg PPD/ml. Cultures were stopped at 5 days with colcemid. After centrifugation, cells were placed on glass slides, fixed with 25% glacial acetic acid in methanol, and stained with 3% Giemsa in phosphate buffer. Slides were read for cells in metaphase for presence of Y chromosomes by a technician blinded to the project.

Statistical analyses were done by χ^2 with Yates correction for continuity.

RESULTS

Blastogenesis by specific antigens. Stimulation indices of PBL in response to PPD, *Candida*, and SK from 67 consecutive mother-infant pairs are summarized in Fig. 1. Only the highest response to any antigen concentration is shown in each baby or mother. In both infants and mothers, cultures in AB serum usually yielded higher SI than in autologous plasma. In homologous serum cultures SI > 2·0 was found in 54%, 18% and 23% of newborns responding to PPD, *Candida* or SK respectively (Table 1). There was a significant agreement between mother and infant SI results for PPD cultures in homologous serum ($P < 0{\cdot}05$). Mothers with SI greater and equal to 2 or less than 2 are likely to have infants with SI greater and equal to 2 or less than 2 respectively. For cultures in autologous plasma there is a significant agreement between mother and infant SI results for PPD ($P < 0{\cdot}05$) and *Candida* ($P < 0{\cdot}001$).

There was no significant agreement between mother and infant SI results for *Candida* and SK cultures in homologous serum or for SK cultures done in autologous plasma. In two babies, the SI to PPD was > 2·0 at birth but their mothers' was < 2·0. In these two mothers PBL from two repeat blood samples during the next 6 months had SI values > 3·0. This observation agrees with previous studies on the waning of specific CMI before and immediately after parturition (Covelli & Wilson, 1978; Brunham *et al.*, 1983). In five infants PBL from repeated blood samples were obtained up to 7 months of age. The progressive diminution of their SI after PPD stimulation is represented in Fig. 2. In all infants tested sequentially, the SI dropped to < 2·0 by 5 months of age.

Leukocyte inhibition factor. The results of the LIF assay on cord blood of 8 consecutive

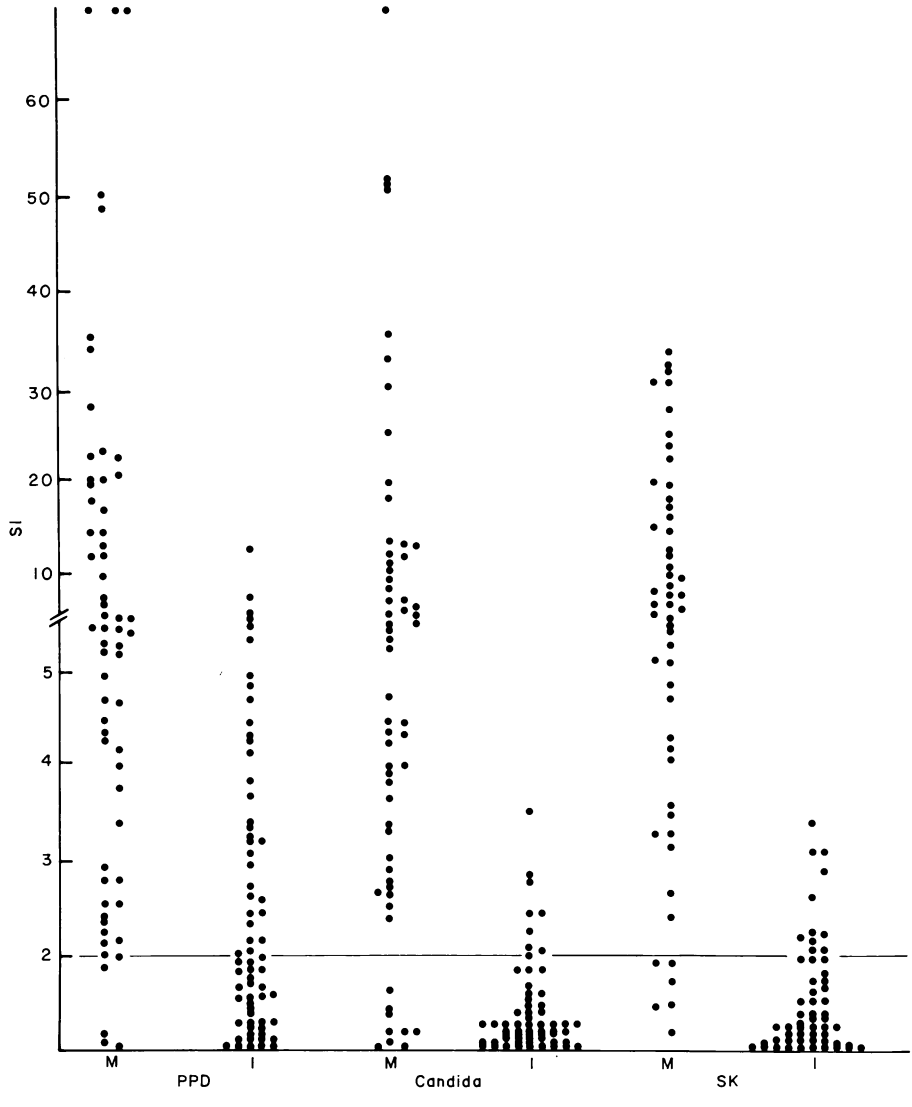

Fig. 1. Stimulation indices (SI) for mothers (M) and infants (I) of PBL or cord lymphocytes, using PPD, *Candida*, or streptokinase as antigens.

newborns are summarized in Table 2. Progressive diminution of migration inhibition was found with reduction of lymphocyte blast transformation by PPD.

Karyotyping. A search in cord blood was made for the frequency with which maternal cells would respond to PPD by blastogenesis. Mitoses from nine consecutive baby boys were analysed. All 68 mitoses examined were XY karyotype (Table 3). Their SI in response to PPD did not correlate with the number of mitoses which could be reliably counted. This may be due to different culture conditions in the two methods, due to the difficulty in some cultures to obtain satisfactory chromosomal spreads, or other factors.

DISCUSSION

Our results add strong support to the concept of specific CMI passage from the mother to the fetus during pregnancy. Blast transformation by antigen of cord lymphocytes has been used by others as

Table 1. Mother and infant cultures grouped by stimulation index category for homologous serum and autologous plasma

		Homologous serum infant SI		Autologous plasma infant SI	
Antigen		≧2	<2	≧2	<2
PPD					
Mother SI	≧2	32	27	9	1
	<2	0	7	0	18
Candida					
Mother SI	≧2	9	42	5	8
	<2	0	9	0	15
Streptokinase					
Mother SI	≧2	11	36	6	12
	<2	0	9	0	7

NB. Not all results on mothers had an accompanying result on an infant (see Methods).

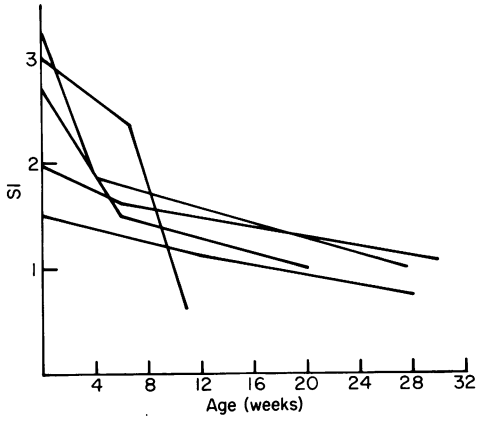

Fig. 2. Change of stimulation index to PPD with increasing age of infants.

Table 2. Migration inhibition by supernates from cord lymphocytes stimulated with PPD

Infant	Stimulation index	Migration inhibition (%)
1	3·3	71
2	2·8	64
3	2·6	68
4	2·5	55
5	2·1	44
6	1·7	32
7	1·5	30
8	1·4	35

Table 3. Karyotypes of PPD-stimulated cord lymphocytes

Baby boy	SI Mother/infant	Karyotype	Mitoses counted
1	50/4	46XY	10
2	38/1	46XY	10
3	5/4	46XY	3
4	8/5	46XY	10
5	10/7	46XY	6
6	4/3	46XY	4
7	50/2	46XY	5
8	ND	46XY	10
9	5/1	46XY	10

ND, not determined.

a measure of specific CMI in T cell separation and cell suicide experiments (Shiratsuchi & Tsuyuguchi, 1981). Although the sensitivity of this assay is limited, additional evidence for the transplacental transfer of specific CMI was obtained by the generation of PPD induced LIF (Tager *et al.*, 1985).

The mechanism of transplacental CMI transfer to the fetus is presumably serological. Although maternal cells are occasionally found in the newborn and could therefore respond to specific antigen in cord blood, this is the exception rather than the rule (Schroder, 1975). Our limited karyotyping results are in keeping with this finding. Breast feeding also appears to provide infants with specific CMI (Mohr, 1973; Schlesinger & Covelli, 1977; Ogra, Weintraub & Ogra, 1977) but we found this established before the first feeding. We therefore believe that humoral factors produced by the mother's immune system and reaching the fetus transplacentally must be responsible for the newborn's specific CMI. Transfer factor (Lawrence, 1969) could fit these requirements. Its capacity to provide specific CMI to the recipient is well established *in vivo* (Pabst & Swanson, 1972; Wilson & Fudenberg, 1983) and by specificity experiments *in vitro* (Borkowsky & Lawrence, 1981). The lack of correlation of the level of sensitization between mothers and infants in our experiments may be a consequence of the marked reduction of specific CMI during the later stages of pregnancy and shortly after parturition (Covelli & Wilson, 1978; Brunham *et al.*, 1983) when our CMI measurements were made.

Since the duration of specific CMI in our infants appears limited, it is not likely to be associated with immunological memory. Rather, it is akin to passive specific humoral immunity which also wanes during the first few months of life. Can this passively acquired CMI interfere with active immunization as preexisting humoral immunity does? This question must have considerable relevance to immunization efficacy with vaccines such as BCG (Grindulis *et al.*, 1984; Narain *et al.*, 1977). Theoretically therefore, preexisting passive CMI to specific antigen may interfere with the establishment of active immunity by altering the host response to active immunization to that antigen. Babies with passive PPD cellular immunity will respond differently to BCG vaccine to babies without this passive specific CMI. At least part of the widely varying efficacy of BCG immunization may be related to this passive form of preexisting immunity.

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REFERENCES

BORKOWSKY, W. & LAWRENCE, H.S. (1981) Deletion of antigen-specific activity from leukocyte dialysate containing transfer factor by antigen-coated polystyrene. *J. Immunol.* **126,** 486.

BOWEN, T. & PABST, H.F. (1976) Immunologic response *in vitro* after thymectomy in patients with myasthenia gravis. *Can. med. Assoc. J.* **115,** 619.

BRUNHAM, R.C., MARTIN, D.H., HUBBARD, T.W., KUO, C.C., CRITCHLOW, C.W., CLES, L.D., ESCHENBACH, D.A. & HOLMES, K.K. (1983) Depression of the lymphocyte transformation response to microbial antigens and phytohemagglutinin during pregnancy. *J. clin. Invest.* **72,** 1629.

COVELLI, H.D. & WILSON, R.T. (1978) Immunologic and medical considerations in tuberculin-sensitized pregnant patients. *Am. J. Obstet. Gynecol.* **132,** 256.

CUTLER, R. & PABST, H.F. (1972) Materno-fetal transfer of cellular immunity. *Clin. Res.* **20,** 937.

FIELD, E.J. & CASPARY, E.A. (1971) Is maternal lymphocyte sensitization passed to the child? *Lancet* **i,** 337.

GALLAGHER, M.R., WELLIVER, R., YAMANAKO, T., EISENBERG, B., SUN, M. & OGRA, P.L. (1981) Cell-mediated immune responsiveness to measles. *Am. J. Dis. Child.* **135,** 48.

GRINDULIS, H., BAYNBAM, M.D., SCOTT, P.H., THOMPSON, R.A. & WHARTON, B.A. (1984) Tuberculin response two years after BCG vaccination. *Arch. Dis. Child.* **59,** 614.

LAWRENCE, H.S. (1969) Transfer factor. In: *Advances in Immunology* (ed. by F.J. Dixon & Henry G. Kunkel) Vol. 11, p. 195. Academic Press, New York.

LEIKEN, S., WHANG-PEN, J. & OPPENHEIM, J.J. (1974) *In vitro* transformation of human cord blood lymphocytes by antigens. In: *Proceedings of the Fifth Leukocyte Culture Conference* (ed. by J.E. Harris) p. 389. Academic Press, New York.

MOHR, J.A. (1973) The possible introduction and/or acquisition of cellular hypersensitivity associated with ingestion of colostrum *J. Pediatr.* **82,** 1062.

NARAIN, R., KRISHNASWAMY, K.V., VALLISHAYEE, R.S., NARMADA, R., RABIN, M.A. & RADHAMANI, M.P. (1977) Assessment of BCG vaccination in newborn babies. *Ind. J. med. Res.* **68,** 403.

OGRA, S.S., WEINTRAUB, D. & OGRA, P.L. (1977) Immunologic aspects of human colostrum and milk. III. Fate and absorption of cellular and soluble components in the gastrointestinal tract of the newborn. *J. Immunol.* **119,** 245.

PABST, H.F. & SWANSON, R. (1972) Successful treatment of mucocutaneous candidiasis with transfer factor. *Brit. med. J.* **3,** 442.

SCHLESINGER, J.J. & COVELLI, M.D. (1977) Evidence for transmission of lymphocyte responses to tuberculin by breast feeding. *Lancet* **ii,** 529.

SCHRODER, J. (1975) Transplacental passage of blood cells. *J. med. Genet.* **12,** 230.

SCOTT, R., PULLAN, C.R., SCOTT, M. & MCQUILLAN, J. (1981) Cell mediated immunity in respiratory syncitial virus disease. *J. med. Virol.* **13,** 105.

SHIRATSUCHI, H. & TSUYUGUCHI, I. (1981) Tuberculin purified protein derivative-reactive T cells in cord blood lymphocytes. *Infect. Imm.* **33,** 651.

TAGER, I.B., KALAIDJIAN, R., BOLDINI, L. & ROCKLIN, R.E. (1985) Variability in the intradermal and *in vitro* lymphocyte responses to PPD in patients receiving isoniazid chemoprophylaxis. *Am. Rev. respir. Dis.* **131,** 214.

WILSON, G.B. & FUDENBERG, H.H. (1983) Is controversy about 'transfer factor therapy' nearing an end? *Immunol. Today* **4,** 157.

WINTER, H.S., GARD, S.E., FISCHER, T.J., BRYSON, Y.J. & STIEHM, E.R. (1983) Deficient lymphokine production of newborn lymphocytes. *Ped. Res.* **17,** 573.