MATERNAL FETAL IMMUNOLOGICAL RELATIONSHIP PARTICULARLY MYCOBACTERIAL IMMUNITY

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ABSTRACT

Thirty-nine paired maternal and cord blood from normal full term deliveries were tested for lymphocyte function by proliferative response to mitogens – Phytohemagluttin-P (PHA) and Poke weed mitogens (PWM). Monocyte function was assessed by the ability of the monocytes to release hydrogen peroxide (H_2O_2) in response to standard stimulus (PMA). Mycobacterial immunity was assessed by lymphocyte proliferative response to purified proteins derivative (PPD) and IgM and IgG antibody response to $H_{37}Rv$ and 5 atypical mycobacteria.

Lymphocyte functions were significantly lower in cord blood (PHA 20.6, PWM 21.2) as compared with maternal blood (PHA 65.8, PWM 37.8). The capacity of fetal monocytes to release H_2O_2 was comparable to maternal monocytes. The mean proliferative response of fetal lymphocytes to tubercular protein (PPD) was 0.67 as compared ($P< 0.01$) to maternal lymphocytes (3.79). Nearly 86% of the cord blood did not show any response to PPD. None of the cord blood showed IgM antibody response to $H_{37}Rv$ nor to any of the range of 5 atypical mycobacteria though maternal IgM and IgG response was present. There was only passive transfer of IgG antibody from mother to fetus.

Hence, though this is a highly endemic area for atypical mycobacteria and M. tuberculosis, there was apparently 170 transplacental transfer of antigen in normal sensitized mothers.

Key words: *Immunity, Mycobacteria, Matemofetal transfer.*

Conventional wisdom for many years indicated that the newborn had deficient cellular immunity. Reports have been variable regarding proliferative response of cord lymphocytes(1). Monocytes play a central role in host defence as circulating. phagocytes as well as precursors of macrophages. In an attempt to explain the enhanced susceptibility of neonates to systemic infections, certain aspects of lymphocytes and monocytes functions have been studied in cells from newborns and compared to the maternal cells.

Protective immunity to *Mycobacterium tuberculosis* in newborns is essential in our environment due to the high prevalence of tuberculosis. The immunity to tuberculosis conferred in the fetus from the mother was studied. This may be important from the view of timing of BCG vaccination.

One of the reasons for the failure of BCG vaccination as reported from Chingleput studies was ascribed to the interference with atypical mycobacteria(2). The third aspect of the study was therefore to assess if the fetus was sensitized to atypical mycobactcria.

Material and Methods

Thirty-nine paired maternal and cord bloods were studied. The newborns were full term, appropriate for gestational age babies born of normal deliveries. Umbilical cord blood was withdrawn into heparinized containers immediately after delivery. The

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mothers were healthy and blood from them was withdrawn immediately after procurement of cord blood.

The cord blood was centrifuged for 20 min at 200 g at room temperature and the buffy coat was removed. This was layered on to Ficoll hypaque and centrifuged to isolate peripheral blood mononuclear cells(3). The cells were adjusted to $4x10^6$ cells/ml in RPMI-1640 for monocyte function and $0.5x10^6$ cells/ml for lymphocyte transformation test.

Nonspecific esterase staining: Smear for non-specific esterase was prepared with the mononuclear cells (MNC) suspension for determining the percentage of monocyte population in each specimen(4).

H_20_2 assay: To a 96 well flat bottom plate (Falcon) 100 µl of MNC was added in each well. After incubating the plate for one hour at 37°C the nonadherent cells were removed by aspiration. The adherent cells were used for H_2O_2 assay. The assay is based on the peroxide mediated oxidation of phenol red to a product that is measured by increased absorbance at 610-610 nm(5).

0.5 X 10^6 per ml peripheral blood monocellular cells in RPMI-1640 supplemented with penicillin (100 µg/ml), streptomycin (100 µg/ml), streptomycin (100 µg/ml), glutamine (300 µg/ml) and 10% pooled AB serum were cultured in triplicate in 96 well (U bottom) tissue culture plates (Laxbro).

PHA was added to a final concentration of 1µg/ml, PWM 1µg/ml, PPD 50 µg/ml and BCG 50 µg/ml. Cultures were incubated at 37°C in 5% CO_2 for 96 hours, for mitogens and 144 hours for PPD and BCG. The cultures received 1µCi of ^{3}H-Thymidine (specific activity 13,000 µCi/mol, Bhabha Atomic Research Centre, Bombay) 16h before harvesting.

Cells were harvested with MASH-11 (Microbiological Associates, USA) and deposited on fibre glass filter paper. Paper discs were then transferred to bio-vials containing 1 ml scintillation fluid and counted in a B scintillation counter (Packed Tricarb 300). Stimulation index was calculated as *CPM in stimulated culture/CPM in control culture.*

Antibodies (IgM, IgG) to H_{37}Rv and 5 commonly encountered environmental mycobacteria *(M. avium intracellulare* (MAI), *M. cheloni, M. kansasi, M. scrofulaceum, M. fortuitum)* were assessed by the ELISA technique.

Results

There was significant decrease in the lymphocyte response of cord lymphocytes to mitogens PHA, PWM as compared to maternal lymphocytes *(Table I).*

The cord lymphocytes also showed significant decreased response to antigens PPD and BCG. A total of 86% of the fetal

TABLE I – *Lymphocyte Response to PHA, PWM (1 µg/ml)*

Group	PHA	PWM	PPD	BCG
Maternal	65.8 ± 40.2	37.8 ± 35.0	3.7 ± 2.1	3.4 ± 2.6
Cord	20.6 ± 15.8	21.2 ± 16.6	0.6 ± 0.2	1.1 ± 0.8
Significance	$p < 0.001$	$p < 0.013$	$p < 0.01$	$p < 0.03$

Values are mean ± SD.

TABLE II- H_20_2 *Release from Monocytes*

Group	H_2O_2 release	Significance
Materna1	7.5 ± 5.0	p > 0.510
Cord	6.4 ± 6.9	

lymphocytes showed no response to PPD though the maternal lymphocytes were sensitized (Table I).

Table II indicates that the fetal peripheral blood monocytes were comparable to maternal peripheral blood monocytes with respect to production of H_2O_2 *in vitro* .

Table III shows that there was no IgM antibody response to H_{37}Rv, *Mycobacterium avium intracellulare, M. cheloni, M. kansasi, M. scrofulaceum, M. fortuitum* in the fetus though there was maternal IgM antibody response. There was IgG antibody response to H_{37}Rv, M. *cheloni, M. scrofulaceum* and *M. fortuitum* in the fetus probably due to passive transfer from mother.

Discussion

Tuberculosis as a major health hazard is well recognised in developing countries like India. The newborn is, therefore, exposed to an highly infective environment. In tuberculosis the protective immunity is mainly cell mediated although macrophages play a significant role(6).

The present data indicates that the new born lymphocytes show significantly less proliferative response to mitogens compared to the mother. The monocytes capacity to release H_2O_2 on activation is however normal. This has also been reported by other authors(7).

There was no sensitization to *M. tuberculosis* conferred in the fetus from the mother as indicated by significantly low responses of fetal lymphocytes to PPD and BCG as also total lack of IgM antibody response of the fetal blood to H_{37}Rv. Therefore, there was apparently no transfer of sensitized lymphocytes from the mother nor was there transfer of tuberculosis antigen. There was also no IgM antibody response in the fetus to a range of commonly encountered atypical mycobacteria. This data indicates that the newborn may be vulnerable from birth to tuberculosis.

One of the reasons for the failure of BCG vaccination was thought to be due to interference with atypical mycobacteria(9). This study indicates that the newborn is not sensitized to the atypical mycobacteria and hence may be more receptive to BCG vaccination.

Raj Narain *et al.* (10) have demonstrated rather low levels of PVA after vaccination of newborn. The lower PVA could be attributed to the immune mechanism not being fully developed in the newborn.

The lower lymphocyte responsiveness of the newborn could be a disadvantage to the optimum response to the vaccination. But in a study of Kathipari *et al.* (11) where,

TABLE III – *IgM Response to* H_{37} *Rv and 5 Atypical Mycobacteria*

Group	H_{37}Rv	MAI	*M. cheloni*	*M. kansasi*	*M. scrof*	*M. fortuit*
Maternal	0.389	0.353	0.494	0.443	0.658	0.421
Cord	0.060	0.015	0.059	0.014	0.036	0.017

The differences between groups were highly significant ($p < 0.001$) for all.

cell mediated immune response (CMIR) was compared when BCG was given at newborn age to BCG given at 3 months age, the results were comparable indicating that newborns are capable of evoking CMIR at birth and the practice of giving BCG at birth could be continued.

There is a need for early protection as the newborn has apparently no conferred immunity from the mother as shown by present data. The CMIR to BCG is good(11) and as indicated by this study there is noninterference from atypical mycobacteria.

It may be advisable to give BCG vaccination in the newborn. However, follow up studies after newborn BCG vaccination are necessary in order to see when the child shows immunological responsiveness to tuberculo-proteins and if this is related to protection.

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