# Cellular Immune Aspects of the Human Fetal–Maternal Relationship

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ABSTRACT: In the present study the number and the function of T lymphocytes obtained from peripheral blood of 35 mothers immediately after delivery and from the cord blood of the respective newborns were tested. Furthermore, the effect of the newborns' lymphocytes on the immunological competence of the mothers' lymphocytes was investigated by means of graft-versus-host-reaction xenograft test. The blood of 14 of the mothers and their newborns was also examined by monoclonal antibodies for determination of T helper and T suppressor lymphocytes. A marked reduction in the absolute number of T helper cells was found in the blood of mothers after delivery. In addition, mothers' lymphocytes manifested a high suppressor activity when added to normal human lymphocytes. It was also found that the cord blood contains lower number and percentage of T suppressor cells. Yet the T cells of the cord blood exert a high suppressor activity on the mother as well as on normal donor lymphocytes. The impaired function of cord blood lymphocytes could be restored by in vitro addition of THF, a thymic hormone. (Am J Reprod Immunol Microbiol. 1986; 11:125–129.)

Key words: Graft versus host reaction, cord blood, T suppressor lymphocytes, fetal–maternal relationship.

## INTRODUCTION

In previous reports an exchange of antigenic material between mother and fetus during normal pregnancy has been found.[1] Regardless of this, pregnancy usually progresses unaffected and the fetus is normally not rejected. The available data suggests that the prevention of immune rejection may be attributed to alterations in maternal immunocompetence.[2–5] It has been shown that lymphoid cells from human newborns exert a strong mitotic inhibition on lymphocytes from their mothers.[2,3] The cell inducing this suppressor effect was found in the T-cell-enriched fraction of cord blood lymphocytes.[4] It has been demonstrated that untreated OKT4$^+$ or OKT8$^+$ lymphocytes from cord blood do not manifest helper activity; on the contrary, cord OKT4 cells possess a marked suppressive activity.[5]

In previous publications we have shown that the local xenogeneic graft-versus-host reaction (GVHR) is a sensitive test for measuring human T lymphocyte immune competence, as well as T suppressor and T helper activities.[6,7] In the present study, fetal–maternal relationship was investigated by testing the T cell number and function of the peripheral blood of 35 mothers immediately after delivery, and of the cord blood of their respective normal newborns using the GVHR technic. In addition, the effect of THF, a thymic hormone on the cord blood lymphocytes was tested.

## MATERIALS AND METHODS
### Patients

We included 35 women aged 22–34 years after delivery and their respective newborn healthy babies in this study. All babies were born of healthy mothers with normal and uncomplicated pregnancies and deliveries (27 of 35 mothers had only 1–2 pregnancies).

### Lymphocytes

Cord blood was sampled from 35 newborn babies and peripheral vein blood was sampled from their mothers immediately after delivery but before the expulsion of the placenta. Informed consent was obtained upon admission to the hospital for blood testing. Mononuclear cells obtained from the heparinized peripheral blood were separated by the Ficoll–Hypaque sedimentation technic and finally resuspended in phosphate-buffered saline (PBS). E rosettes were determined according to the technic of Jondal et al.[8] The theophylline sensitivity method as described by Shore et al.[9] was used for determination of T suppressor blood lymphocytes of mothers and their respective newborns. This method was also used in order to separate the theophylline-sensitive T suppressor lymphocytes from the theophylline resistant T-helper lymphocytes. Both populations were tested for suppressor or helper activities by means of GVHR. Lymphocyte subsets were also tested by the OKT4 and OKT8 monoclonal antibodies.[10]

### Graft-Versus-Host Reaction (GVHR)

We injected 0.1 ml of the mononuclear cells obtained either from cord blood or mothers' peripheral blood (containing 20 × 10$^6$ cells) intradermally into the closely shaven abdominal skin of inbred Lewis rats weighing 100–180 gm pretreated 24 hr previously with 100 mg/kg cytoxan administered intravenously. On the third day, the rats were injected intravenously with 0.4 ml of 1% Evans blue. The entire abdominal skin was excised 5 hr later and the blue stain measured with calipers. A three-dimensional measurement was made by taking the length (a), width (b), and thickness (c) of the lesion and its volume was calculated by the formula $4/3 \, \pi \, (a/2) \times (b/2) \times (c/2)$. A volume of 10 mm$^3$ was considered to be a negative reaction, from 10–15 mm$^3$ a weak reaction, and greater than 15 mm$^3$, a strongly positive reaction.[6]

### Determination of Suppressor Activity of Cord Blood Lymphocytes on Mothers' Lymphocytes

The suppressor activity of a newborn's cord blood lymphocytes on mothers' lymphocytes was tested by the use of the same xenogeneic GVHR. For this purpose, 1 × 10$^6$ mononuclear cells from the cord blood were mixed with 20 × 10$^6$ (1:20) lymphocytes obtained from the mother. Following the technic described previously,[7] this mixture was injected into the abdominal skin of an
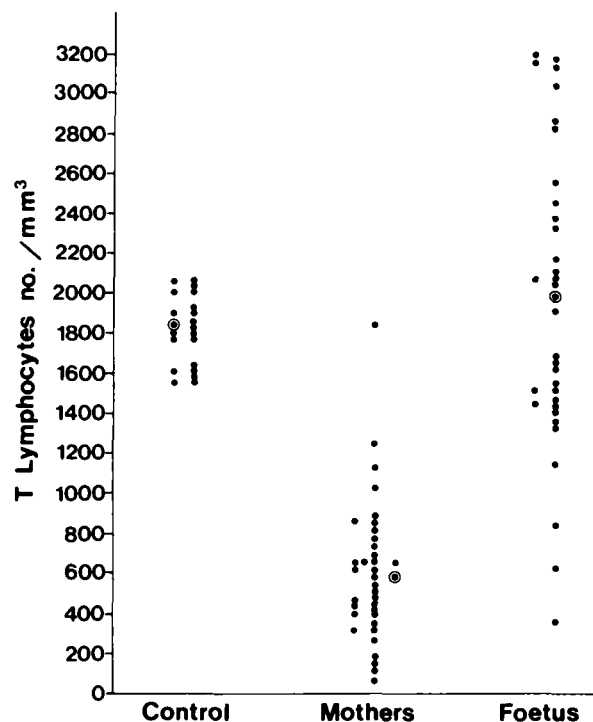
Fig. 1. Absolute number of control, mother, and newborn T lymphocytes.

immune suppressed rat, while the contralateral site was injected with an equivalent sample of the mother lymphocytes only. Inhibition of a positive control reaction was considered to be the result of the presence of active suppressor cells in the experimental sample.[7] Statistical analysis of data using Student's t-test was performed. The same method was used for testing the activity of cord blood and mothers' lymphocytes on normal donor's lymphocytes obtained from age–sex matched blood bank donors.

### Effect of Thymic Humoral Factor (THF) on Mononuclear Cord Blood Cells

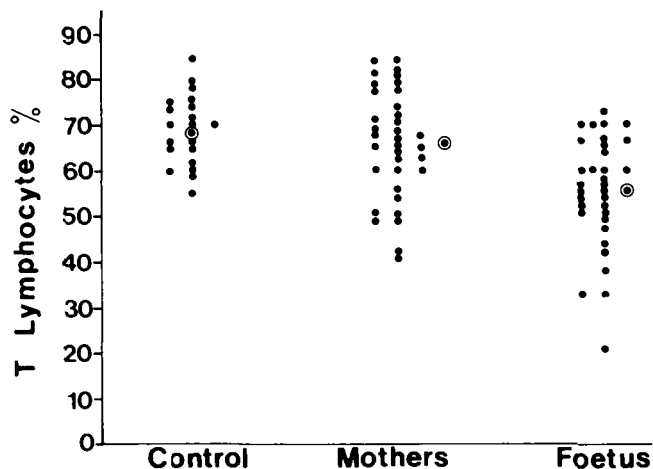We incubated twenty million mononuclear cells obtained from cord blood for 60 min at 37°C with 100 $\mu$g



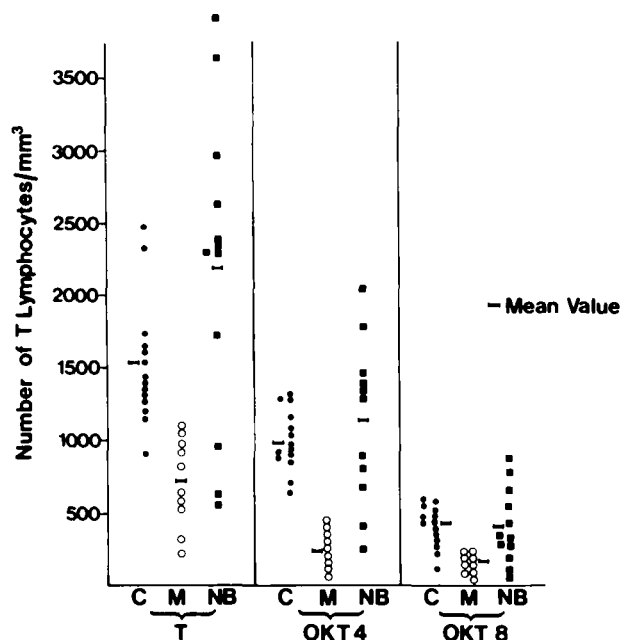Fig. 2. Relative number of T lymphocytes as above.



Fig. 3. Absolute number of total T cells, T helper (OKT4$^+$), and T suppressor (OKT8$^+$) lymphocytes in normal controls (C) as compared to mothers (M) after delivery and their newborns (NB).

THF. Control cord blood cells were incubated in PBS alone. Following incubation the cells were washed three times in PBS and inoculated into rats as described above for induction of a local xenogeneic GVHR. A calf thymus hormone, THF, was prepared by a method previously described.[11]

### RESULTS

The absolute and relative numbers of mother and newborn T cell lymphocytes as tested by the E rosette method in 35 mothers and respective newborns are shown in Figures 1 and 2. Although the relative numbers of T lymphocytes of mothers and newborns were within the normal range (69 and 62%, respectively), the absolute number of T lymphocytes in the mothers' blood was significantly diminished (600 T cells/mm$^3$; $p \leqslant 0.001$). The number of T lymphocytes in newborns manifested a wider scattering with a nonsignificant trend to higher than adult control numbers (1950 T cells/ mm$^3$; $p < 0.05$). These results were confirmed by the use of OKT3$^+$ monoclonal antibodies, as seen in Figure 3 (first column). The absolute T cell number of controls was 1500 T cells/mm$^3$; for mothers, 711 cells/mm$^3$ and for newborns, 2090 cells/mm$^3$. The absolute and relative numbers of helper and suppressor T cells determined by OKT4$^+$ and OKT8$^+$ monoclonal antibodies, respectively, were lower than normal in the mothers' blood; the number of T helper cells (272 cells/mm$^3$) was more severely impaired (Figs. 3 and 4) while in the newborns the absolute number of the T helper cells was higher: 1125 cells/mm$^3$ and the number of T suppressor cells was only 431 cells/mm$^3$. The number of parity was not found to affect the cellular immunity of the mother.

The functional activity of peripheral blood lymphocytes of mothers tested immediately after delivery by the GVHR indicated some impairment as compared to normals (size of skin reaction mean value, 19 mm$^3$; the
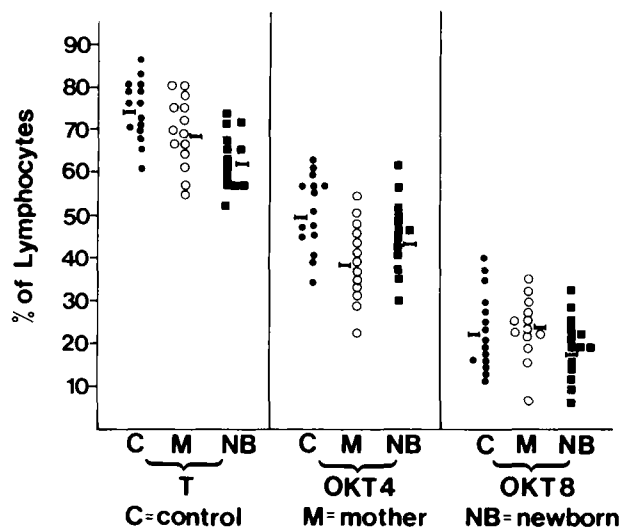
Fig. 4. Percentages of total T, T helper (OKT4⁺), and T suppressor (OKT8⁺) lymphocytes in normal controls as compared to mothers after delivery and their newborns.

normal value, 35 mm³). Moreover, the addition of newborn lymphocytes to their respective mothers' lymphocytes in a proportion of 1:20 induced a further significant suppression of this reaction (Fig. 5, $p < 0.05$). Lymphocytes obtained from cord blood were incompetent to induce a GVHR in 9 out of 16 cases tested (Fig. 6).

The addition of cord blood or mothers' lymphocytes to lymphocytes obtained from normal donors in a ratio of 1:20 provoked a high suppressive effect on the ability of the normal cells to induce a local GVHR (Fig. 7). This suppressive effect was also manifested when sera from
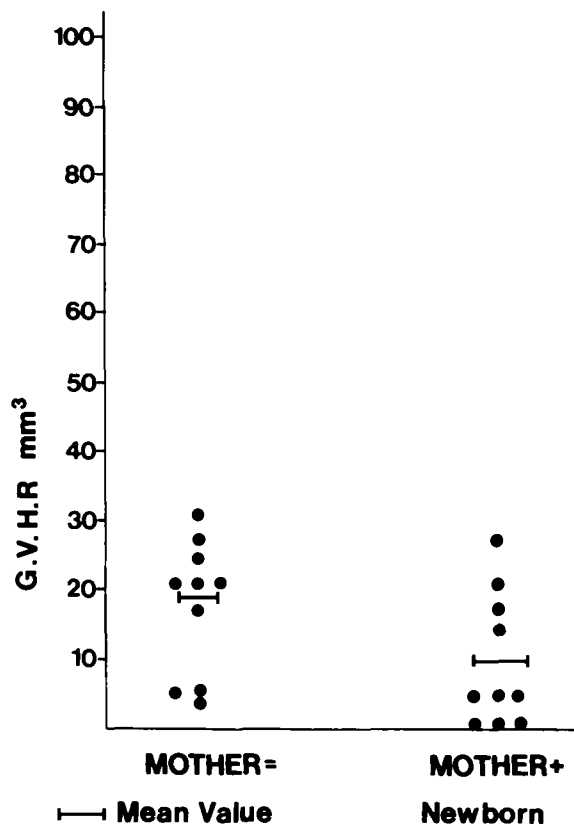


Fig. 6. Local xenogeneic GVHR obtained with newborn lymphocytes.



Fig. 5. Effects of newborn lymphocytes on the ability of mothers' lymphocytes to induce a GVHR.
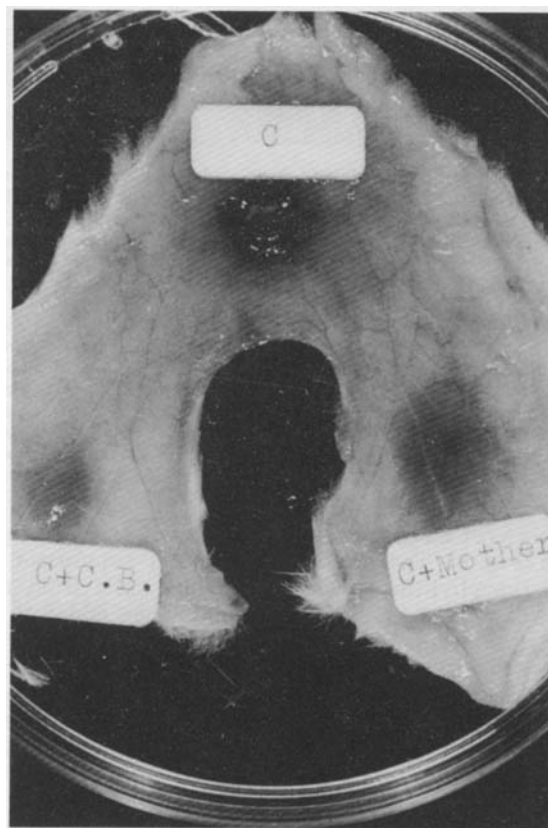


Fig. 7. Effect of cord blood (C.B.) lymphocytes and mothers' lymphocytes on the ability of 20 × 10⁶ normal control lymphocytes (C) to induce a local xenogeneic graft-versus-host-reaction. A striking inhibition is observed. C, Reaction induced by normal control lymphocytes (37 mm³); C + Mother, reaction induced by normal control and mother lymphocytes (1:40, 12 mm³); C + C.B., reaction induced by normal control and cord blood lymphocytes (1:40, 9 mm³).

which only relative numbers of the different T lymphocyte subsets were presented, while absolute numbers were missing. In the present investigation, when absolute numbers of the T cells and T-cell subsets were compared, a significant decrease in total T lymphocytes as well as in the helper and suppressor subsets was found in mothers immediately after delivery as compared to those of the newborn and of the normal controls. A small but significant reduction in circulating T lymphocytes and the T-helper subset was already described during pregnancy by Vanderbeeken et al.[13] Furthermore, we found that in spite of low relative numbers of T suppressor cells, the cord blood manifested a high suppressor activity on the mothers' lymphocytes (Fig. 5) as well as on normal donors' lymphocytes (Fig. 7), and that the theophylline resistant T helper lymphocytes of cord blood did not show any helper activity (Fig. 9). Jacoby et al.[12] have shown that the OKT4$^+$ cord lymphocytes do contain a radioresistant subset of T helper cells. Since we have not irradiated theophylline-resistant T-helper subset, we cannot conclude whether the theophylline resistant inactive T helper cells of the cord blood would have turned to helper activity after radiation. From experiments with the whole population of lymphocytes, we can conclude that the suppressor activity of cord blood overwhelms this helper activity.

Our results of high suppressor activity of newborns' lymphocytes conform with those of others, [2,3,12,16] suggesting a general phenomenon in biology because fetal and newborn cells obtained from other species, such as mouse, were also found to suppress the response of the parental lymphocytes in a local GVHR.[17] In addition, we confirmed that the reduced immune competence of
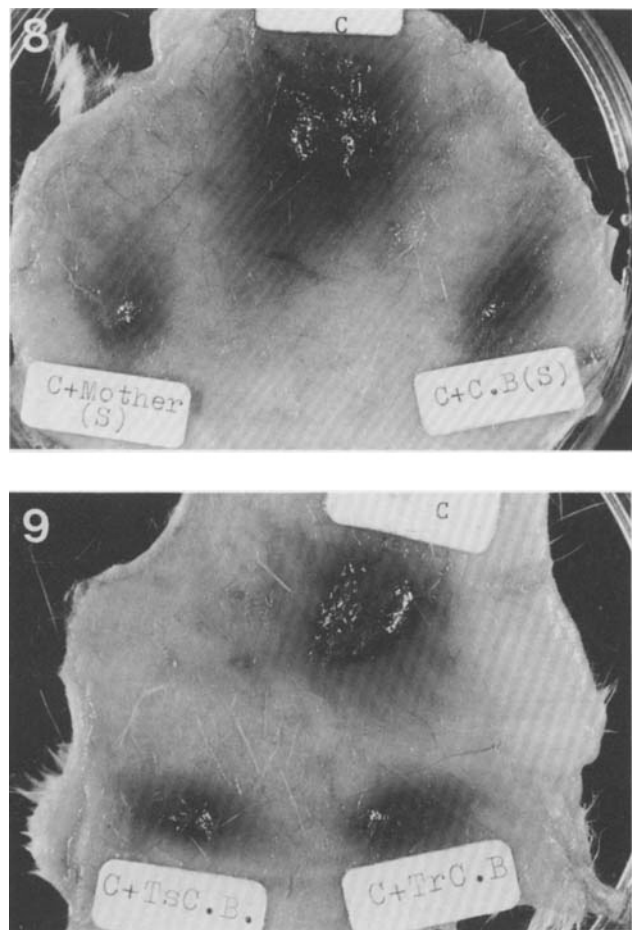


Fig. 8. Effect of serum obtained from cord blood and mother on the ability of normal control lymphocytes to induce a local GVHR. C, Reaction induced by normal control lymphocytes (94 mm³); C + C.B.(S), reaction induced by normal control and serum from cord blood (16 mm³); C + Mother(S), reaction induced by normal control and serum from mothers (16 mm³).

Fig. 9. Inhibition of the local xenogeneic GVHR induced by control normal lymphocytes by addition of theophylline-sensitive suppressor (T$_s$) cells or/and theophylline-resistant (T$_r$) cells from cord blood. C, Reaction induced by 20 × 10⁶ normal control lymphocytes (45, 5 mm³); C + T$_s$ C.B., reaction induced by 20 × 10⁶ normal lymphocytes T-suppressor theophylline-sensitive lymphocytes of cord blood (1:40, 13 mm³); C + T$_r$ C.B., reaction induced by 20 × 10⁶ normal lymphocytes T-resistant theophylline-resistant T helper cells (1:40, 9, 8 mm³).

newborns or mothers were used instead of using lymphocytes (Fig. 8). Theophylline-sensitive T suppressor and even theophylline-resistant T helper cells from newborns displayed a suppressive effect when added to normal lymphocytes in the same GVHR model (Fig. 9). The inability of cord blood lymphocytes to induce a normal GVHR was reversed after THF treatment. Indeed, eight out of nine cord blood samples responded normally after incubation with THF at a concentration of 100 μg/ml for 1 hr at 37°C (p < 0.01) (Fig. 10).

## DISCUSSION

No significant differences between the percentages of T cell or the T helper subsets in the blood of mothers, newborns, and controls were found in this study. These results are in agreement with those of Jacoby et al.[12] in
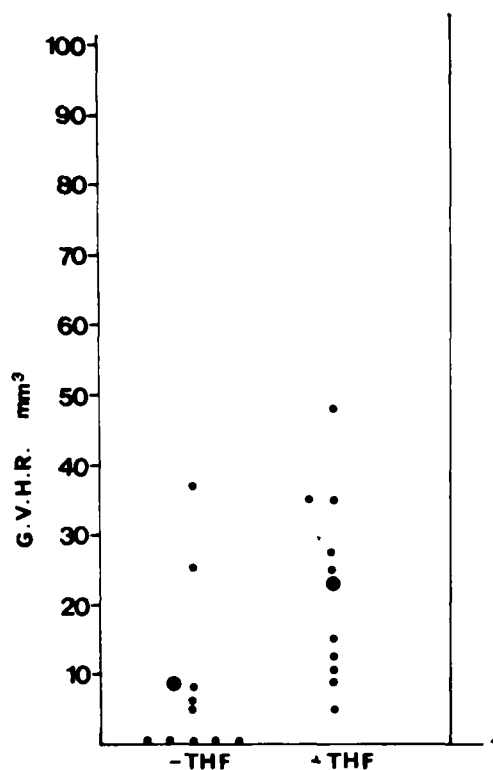


Fig. 10. Effect of THF on the ability of cord blood lymphocytes to induce a GVHR.

cord blood lymphocytes could be improved by THF.[18] Similar results on the effect of thymic hormones on lymphocytes were shown in asthmatics.[19] The results presented here and in other studies [2-5,12] indicate the existence of T-cell populations in cord blood that differ from those found in normal adult blood. Finally, the soluble suppressor activity found by us in the serum of cord blood confirms the concept that immunological suppression is one of the ways to avoid fetus rejection.

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