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THE CELLULAR IMMUNE ASPECTS OF FETAL LYMPHOCYTES. III. IN VITRO RESPONSE OF FETAL LYMPHOCYTES TO PHYTOHEMAGGLUTININ STIMULATION.

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ABSTRACT

Immunocompetency of fetal lymphocytes was evaluated in vitro by determination of their response to phytohemagglutinin(PHA) stimulation. Fetal lymphocytes from cord blood of 14 newborns were stimulated with PHA and the degrees of response were determined by both the percentages of lymphoblastic transformation and rate of tritriated thymidine incorporation. Lymphocytes from 14 healthy adults were used as controls. There was no statistical significant difference in PHA responsiveness between these 2 groups of lymphocytes studied with suggest that the immunocompetency of fetal lymphocytes is comparable to those of adults.

INTRODUCTION.

The cell-mediated immunocompetency of fetal lymphocytes is still the interesting and unsettled subject. Limited numbers of the in vivo studies including the DNFB sensitization (1), skin grafting (2), and

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chimerism following intrauterine transfusion[3] suggest a depressed or diminished cellular immunity in newborn infants. However, a completely different conclusiou could be drawn from many in vitro studies such as the normal or better than normal response to stimulation with PHA [4–9], pokeweed mitogen and staphylococcal filtrate [4], adequate response to foreign histocompatibility loci on adult lymphocytes [10-12], and the recent study of the distribution of peripheral lymphocytes which are responsible for the body cellular immune competeney has shown that it was normal in newborns [13]. Unfortunately, these in vitro results are far from uniform due to many variations including the different PHA doses used [8] and the presence of inhibitor in cord sera [14, 15].

Our earlier report has shown that fetal lymphocytes are metabolically active presumably due to sensitizatien in utero [16]. We are now reporting our findiugs which further indicated the normal immune competency of fetal lymphocytes in cord blood.

MATERIAL AND METHOD.

Lymphocytes isolated from 14 cord blood samples and 14 healthy normal adults were studied concomittantly. Cord blood samples were collected in the delivery room within 5 minutes of birth from the umbilical vein. Blood sample was drawn into a 12 ml. sterile plastic syringe containing heparin (50 units/ml. of blood) and immediately mixed with one fifth volume of sterile 6% dextran solution. The mixture was allowed to stand in the upright syringe for 60 minutes at 37° C. The washed leukocytes were finally resuspended in Hank's–Hepes solution(pH 7.4). The leukocyte suspension was incubated with PHA (0.05 ml/1.5 ml of culture volume) for 72 hours at 37° C. Leukocytes from cord blood were cultured in autologous or adult plasma and, conversely, adult's leukocytes were cultured in autologous or cord blood plasma. At the end of incubation period, cultures were centrifuged and smears were made from the cell buttons and stained with Wright's-Giemsa. The percentages of blastic transformed lymphocytes were determined. The DNA synthesis rate was determined by adding tritriated thymidine solution 1 uC/1.5 ml. of culture volume/10^6 lymphocytes) into the incubating vessels at 54 hours of incubation and harvesting the leukocytes 18 hrs.

later. The radioactivity of the perchloric acid-precipitated nuclear material were expressed as cpm/10^6 lymphocytes in each cultures (in triplicate).

RESULTS.

There is no statistical significant difference noted between the fetal and adults' lymphocytes in both the percent of blastic transformation and degrees of tritriated thymidine incorporation. There is no evidence of the presence of inhibitor in cord blood and adult's plasmas.

TABLE I.: BLASTIC TRANFORMATION RATES OF FETAL AND ADULT'S LYMPHOCYTES AFTER IN VITRO PHA STIMULATION*

	FETAL PLASMA	ADULT PLASMA
FETAL CELLS	67.64 ± 10.53	68.85 ± 13.72
ADULT'S CELLS	69.69 ± 10.14	75.53 ± 6.54

* expressed as Mean ± 1 S.D. values in percent.

TABLE II.: TRITRIATED-THYMIDINE INCORPORATION RATES OF FETAL AND ADULT'S LYMPHOCYTES AFTER IN VITRO PHA STIMULATION *

	FETAL PLASMA	ADULT PLASMA
FETAL CELLS	34,842.78 ± 24,930.65	34.850.11 ± 23,920.32
ADULT'S CELL	56,159.5 ± 23,189.39	57.014.22 ± 18,546.08

* expressed as Mean ± 1 S.D. values of nine samples in each groups as cpm/10^6 lymphocytes.

COMMENTS

Our results are in agreement with those of Carr and associates(8) that the response of fetal lymphocytes to phytohemagglutinin stimulation is comparable to those of adult's lymphocytes. We have confirmed earlier impression that both the fetal lymphocytes (4, 8, 16) and maternal lymphocytes are regulary undergoing

low level stimulation (17). Thus the hypotheses previously postulated th explain how the mammalian fetus avoids immunologic rejection by the mother has to be re-examined. Previous hypotheses usually centered on either defective or blocked cellular immune capability of the maternal host, nonantigenicity of the fetal graft, or a immunologically neutral placental separation zone between host and graft (18-20). Experimental evidence indicates that fetal protection against maternal immunologic attack is dependent on a placental barrier requiring at least 2 specific properties: (1) a trophoblastic layer low or lacking in transplantation antigens and (2) the ability to limit the exchange of cells, especially leukocytes, between mother and fetus.

The frequent observations of donor cells (3) and persistence of donor lymphocytes after intrauterine transfusion (21) have been interpreted as the indications that the average fetal response to donor cells in terms of rejection is minimal, and probably resulting from the variable immunologic maturity (3). Graft-versus-host (G.V.H.) disease, a fatal complication of the treatment of severe hemolytic anemia by intrauterine transfusion, are surprisingly rarely observed (22). This disease results from reaction by lymphocytes in donor blood to histocompatibility antigens of the recipient resembling those by which healthy individuals reject foreign grafts. The lymphocytes responsible for G.V.H. disease are T-cells and they can survive in bank-blood for up to 3 weeks. It is interesting to note that in a few cases observed recently (23, 24) the foreign lymphocytes were derived from the postnatal exchange transfusion rather than the original intrauterine transfusion donor. Parkman et al (24) speculated that the intrauterine transfusion may have induced tolerance in the fetus to certain histocompatibility antigens which were shared by the exchange transfusion donors. This might have allowed the donor's lymphocytes to colonise the infant instead of being rejected. Special mechanisms must account for the G.V.H. disease in these few cases since most intrauterine transfusions do not result in total G.V.H. disease. Tolerance to foreign lymphocytes encountered during gestation has not been demonstrated in human fetus. On the other

hand, human fetal lymphocytes and fetal thymocytes as young as 12–14 weeks gestation can respond to foreign cells in mixed lymphocyte culture (10–12, 25). Susceptibility to attack by transfused incompatible lymphocytes is greatest in immunodeficient individuals (26, 27). Thus, the rarity of G.V.H. disease in the recipients of intrauterine transfusion presumably reflects the effectiveness of the fetus's immune system

ย่อเรื่อง

ได้ทำการศึกษาและทดสอบทางห้องปฏิบัติการเกี่ยวกับปฏิกิริยาโต้ตอบต่อการกระตุ้นด้วยสาร PHA ของ fetal lymphocytes ใน vitro โดยใช้ lymphocytes จากเลือดสายสะดือของทารกปกติจำนวน 14 ราย การตรวจหาอัตราปฏิกริยาโต้ตอบ ทำโดยวิธีหาเปอร์เซนต์ของ lymphoblastic transformation และอัตรา incorporation กับ tritiated thymidine โดยใช้ Lymphocytes จากเลือดของผู้ใหญ่ปกติจำนวน 14 รายเป็น Control

ผลการตรวจพบว่าไม่มีความแตกต่างให้เห็นอย่างชัดเจนในปฏิกริยา โต้ ตอบ ต่อ สาร PHA ระหว่าง lymphocytes จากเลือดของทารกและจากเลือดผู้ใหญ่ปกติ.

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