วารสารเภสัชกรรมแพทย์ เชียงใหม่
Bulletin of Chiang Mai
Associated Medical Sciences

Vol. 8 No. 3
September 1975

THE CELLULAR IMMUNE ASPECTS OF FETAL LYMPHOCYTES
IV. Shift to the left phenomenon of PHA dose-response curve.

by

Werawan Ruangyuttikarn, B.Sc. (Med.Tech.)
Tawat Tositarat, B.Sc. (Med.Tech.)
Sanong Chaiyarasamee, B.Sc. (Med.Tech.), M.T. (ASCP.)
Panja Kulapongs, M.D., Dip. Am. Bd. of Ped.

INTRODUCTION :

Pregnancy has been regarded as a successful natural allograft. The resemblance of the usual mammalian pregnancy with its genetically dissimilar participants to a host with an allograft might lead one to expect its failure as a self-perpetuation mechanism. The mechanism whereby a histocompatible tissue can grow without evidence of immunological injury in the gravid female are still far from clear. Its success has led to several hypotheses usually centered on either defective or blocked cellular immune capabilities of the natural host, nonantigenicity of the fetal graft. or an immunologically neutral placental separation zone between host and graft (1-3). The cell-mediated immunocompetency of fetal lymphocytes is an interesting and unsettled topic. Morphologically, human neonatal blood lymphocytes are larger (4,5) and have higher nucleo-cytoplasmic ratios and more leptochromatic nuclei (6) than lymphocytes of adults. Both of the newborn and adult blood lymphocyte populations have similar numbers of B and T cells (7-9). Metabolically, neonatal lymphocytes demonstrate a higher in vitro labelling index for RNA (96) and DNA (4,5,10,11) than do similarly prepared adult cells, and evidence has been presentd for a high-labelling lymphoid cells in human neonatal lymphocytes (5,10). Functionally, neonatal lmphocytes react vigorously to PHA (12,13) and to certain antigens (14), and they are cytotoxic

for ^{51}Cr-labelled chicken erythrocytes (15). Fetal and neonatal lymphocytes manifest transplantation antigens (16-18) but these cells react less vigorously with maternal than with stranger lymphocytes in the mixed-lymphocyte-culture reaction (16).

The depression of the maternal cellular immunity may play a key role in the nonrejection of the fetus is suggested by the observations of depressed delayed cutaneous hypersensitivity (19) and in vitro lymphocyte response to PPD (20) and dalayed allograft rejection found in pregnant women (21). However, depression of cell-mediated immunity may be either generalized or specific, the latter being a form of immunological tolerance (22,23). On the other hand, generalized depression of cellular immunity is seen in a variety of congenital or acquired immune deficiency disorders and is characterized among other parameters, by an impaired or absent response of the blood lymphocytes to plant mitogen, PHA (9,24-27). If generalized depression of gravida's cellular immune system is a biologically significant explanation for the survival of pregnancy, one would expect lymphocytes obtained from pregnant women to show a clearly depressed or absent response to PHA stimulation. In the recent study by Carr and associates (28) the reactivity to PHA by peripheral blood lymphocytes taken from women at different stages of pregnancy was compared to that of lymphocytes of nonpregnant women. The results clearly showed that optimal PHA responsiveness as gauged by DNA synthesis was not consistently depressed in gravida lymphocytes, and therefore the results do not support the thesis of generalized depression of maternal cellular immunity as the explanation for nonrejection of the fetus during human gestation. It is interesting to note that, while the DNA synthesis of lymphocytes from pregnant women on the average was not significantly depressed in their response to optimal PHA doses when compared to control women, the shapes of the mean dose-response curves suggest that gravida lymphocytes required lower PHA than control lymphocytes for optimal stimulation (28).

The importance of testing PHA response at more than one dose level was stressed by Fitzgerald (29) who demonstrated that person with deficient cellular immune capacity may show a depressed response to a low dose of PHA, while responding mormally to

a higher dose. Peripheral blood lymphocytes acquire PHA responsiveness at 14 weeks of gestation [30]. Blood lymphocytes of full-term newborn infant [6,11,31] and of premature newborn infants [31] react more vigorously than adult's lymphocytes to stimulation by PHA and anti-human lymphocyte globulin (ALG) [32]. Other workers have seen no differences in response to PHA stimulation between newborn and adult's lymphocytes [13,33-36] or even a diminished response of new born lymphocytes [37-39]. These differences may be explained, in part, by differences in PHA dosages used.

Since both the gravida and newborn lymphocytes have in vitro a higher spontaneous DNA synthesis rate [6,11,28,40-42] than control non-pregnant adult lymphocytes [12,13,43] and variable results of response to a single dose PHA stimulation [6,11,28-31,33-39,44-47], it is of particular interest to investigate whether the "shift to the left" phenomenon of PHA dose-response curve does occur in neonatal lymphocyte culture similar to those of gravid lymphocytes [28]. In the present study, for maximal sensitivity, a dose-responses relationship with 8 PHA concentrations has been determined and responses are measured by 3 H-TdR uptake.

MATERIAL AND METHOD:

Ten normal newborns and 8 healthy adults were studied. Cord blood samples were collected in the delivery room within 5 minutes of birth from the umbilical vein. Adult blood samples were obtained from cubital vein. The blood specimen 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 lymphocyte rich plasma sample was obtained by gravity sedimentation and cultures at 37°C for 96 hours with autologous plasma (6.7% V/V) and varying doses of PHA ranging from 5 ul to 70 ul/1.5 ml. of total culture volume. One microcurie of tritiated thymidine solution was added into each culture tubes during the last 18 hours of experiment. All cultures were carried out in triplicates. Results are expressed as the proliferation index (P.I.).

$$P.I. = \frac{\text{average cpm. of stimulated cells}}{\text{average cpm. of unstimulated cells}}$$

The detail of the microtechnique employed in this study is being described in this issue of the journal.

RESULTS:

As shown in the Figure I., all healthy adult and cord blood lymphocytes demonstrate a linear increase of ^{3}H-TdR incorporation related to the doses of PHA then declined forming a sha-

rply peaked dose-response curves. The lower peak response of cord blood lymphocytes is misleading since the unstimulated cells incorporated ^{3}H-TdR higher than those of lymphocytes from adult controls. At any rate, it is clearly shown that cord blood lymphocytes respond to lower PHA doses better than adult's lymphocytes with a peak response at 10 ul PHA/1.5 ml culture compared to the peak at 30 ul PHA/1.5 ml. culture of the latter.

DISCUSSION :

Several investigators reported a normal distribution of proliferation rate of lymphocytes in response to PHA stimulation [48,49] but this is not the case with the others [50-52]. Our observation and those of others[53-56] have suggested a log-normal distribution of responses, and semi-log scale for presentation of the results has therefore been adopted [50,53,54,57-60].

In considering the events in an individual culture, it has been suggested that a logarithmic increase in the rate of DNA synthesis in response to PHA occurs with time [61]. Cells transforming in response to PHA release blastogenic factor [62], and thus each transforming cell is capable of recruiting other cell into mitosis. It has been suggested that 2 to 3 generations occur in culture over a 3-day period. The log dose-response curves from concanavalin A (Con. A), PHA, PWM (pokeweed mitogen) and LPS (Lipopolysaccharide) show approximately linear responses dose levels below the optimum [63]. The dose-response curve for PHA and Con. A were sharply peaked, whereas PWM and LPS produced broad dose - response curve [63]. The decrease of incorporation above the optimum dose is thought to be due to toxicity of mitogen [54]. For Con. A and PHA, excess mitogen appears to be tolerogenic, since elution of the mitogen can enable recovery of the full response [63, 64]. Both LPS and PWM show a marked lack of either tolerogenicity or toxicity as shown by the very broad log-dose response curves [63].

The reasons for the non-normal distribution of incorporation of ^{3}H-TdR into DNA could be accounted for in 2 ways : the PHA response might be dependent on a property of lymphocytes which is not normally distributed. For example, the proportion of T cells in peripheral blood lymphocytes, (and therefore in cultured lymphocytes) may be such a parameter. Alternatively, a property of normal lymphocytes which is normally distributed might be amplified non-linearly by PHA stimulation. Intrace-

llular events could augment incorporation of 3 H-TdR. There is a short in in vitro half-life of ^{3}H-TdR (65), and transformation of lymphocytes in response to PHA augments the thymidine salvage pathway (66). Since uptake of exogenous thymidine is inhibited by endogenous thymidine, utilization of the latter in response to an increase in DNA synthesis would potentiate the incorporation of the exogenous (labeled) nucleotide. These factors could result in a non-linear relationship before DNA synthesis and 3 H-TdR uptake and may account for the fact that the results do not follows a normal distribution.

Significant increases in the spontaneous DNA synthesis by neonatal lymphocyte was again observed in this study. We have earlier made this observation (42) as have others (67-69). Although heterologous serum and 20% autologous serum have been observed to cause this phenomenon (70), it also occured when neither one of them were employed (28). Autologous serum was used throughout this study because: (i) fetal calf serum contain factors inhibiting the lymphocyte transformation by up to 90% in comparison with autologous serum; (ii) of difficulties in obtaining fresh AB serum; (iii) although the use of serum-free media been reported (C_1, C_2) the problem in such assays is the large number of lymphocytes (more than 2×10^6 per culture) required for the expression of transformation. In clinical conditions serum factors which block or enhance are encountered. In normal subjects it seem likely that the optimum expression of transformation depends on a balance between such factors. Among many cancer patients with blocking factors at 20% autologous serum, it was found that when 10% autologous serum is used the inhibition is often lost (54).

The process of peripheralization of lymphocytes to peripheral lymphatic tissue may occur in human at about the time of birth, and this would also be accompanied by a release of metabolically active cells into the blood.

Part of the labeling index for lymphoid cells in neonatal blood may be due to the presence of transitional lymphocytes. Adults and neonates have approximately the same number of B and T cell in their peripheral circulation as judged by membrane immunofluorescence and rosette formation technique (7-9). Thus the high labeling index for neonatal lymphocytes may reflect the presence of more immature (ie. medium, large) lymphocytes in newborn.

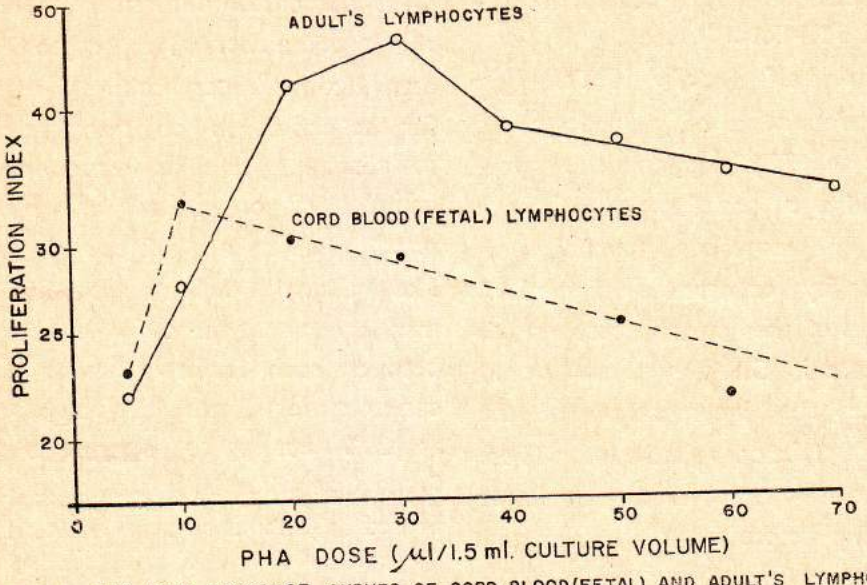

FIGURE 1 : PHA DOSE-RESPONSE CURVES OF CORD BLOOD(FETAL) AND ADULT'S LYMPHOCYTES

Additional findings are that neonatal lymphocytes not only seem to possess a greater response than control lymphocytes at suboptimal doses of PHA, but also respond optimally at lower dose of PHA (shift to the left) than control lymphocytes. An experimental model exists which may explain these findings. That is the observation that the triggering of immunocytes is a quantitative rather than a qualitative phenomenon, such that lymphocytes already exposed to small amount of antigens or mitogen require a smaller dose of another antigen or mitogen that otherwise needed to achieve a peak response [71]. In this situation, the peak response is seen at a lower dose of mitogen, significantly higher response are also seen at lower dose, while the amplitude of the peak response is not necessarily greater than a normal peak response. In an attempt to induce immunological tolerance to a highly purified protein phytohemagglutinin (PPHA) in newborn mice, Panzetta and associates observed the significantly higher in vitro response of lymphocytes from PPHA – immune mice than that of lymphocytes from untreated normal controls and from PPHA-tolerant mice, at PPHA doses around the optimum [72]. Further more, lymphocytes from PPHA-immune mice differ from control lymphocytes in that their PPHA

dose-response curve is shift to the left (72). In the light of the above studies (28,67,72) our results could be interpreted as indicating low level stimulation of neonatal lymphocytes exist in utero.

Lymphocyte activation in vitro initiated by the interaction of mitogens with receptors on the cell surface (73-76). Recently it has been suggested that the mitogen is required during certain critical steps in the cell cycle (61,77,78). The kinetics of the reactions between plant mitogens and cell receptors has been studied in a number of investigations (74,79-81). The reaction conform to the law of mass action and thermodynamics and appear to be completely reversible(80,81). The following formula should then be valid

$$Re + Mi \rightleftarrows Re\ Mi$$

Where Re is lymphocyte receptor, Ni, mitogen and ReMi, mitogen-receptor complex. Changing the concentrations of the reactants results in corresponding changes in the equilibrium. Apparently the mitogens are required at the beginning of the cell cycle in order to make the cell enter the G_1 phase from the resting phase, G_0 This event is designated preactivation. In previous reports it was shown that lymphocytes during a short contact with Con. A. in vitro, in some way, became stimulated although not sufficiently to enter the DNA synthetic S phase of the cell cycle (61,77,82,). Such cells were termed preactivated lymphocytes.

The mechanism of preactivation was studied by measuring the incorporation of radioactive uridine into RNA. It seems that a certain degree of RNA synthesis is required for the cells to reach a preactivated state (82). However, it is by no means clear whether the new synthesis of RNA is the limiting step or if this occurs in some other metabolic reactions. The RNA extracted from preactivated cells did not differ qualitatively from non-activated cells, but the quantitative differences, especially with respect to labeling were highly significant. Lymphocytes from peripheral blood of adults are normally in a resting state. Upon stimulation with PHA, the cells go through the different phase of cell cycle. Before division they pass through the G_1 period preceding the DNA synthetic phase, S, and the lag period G_2. Bender and Prescott (83) have determined these periods in lymphocytes of the adults after PHA stimulation and the G_1 period of these cells were at least 24 hours. Other investigators

have studied the time course of the macromolecule synthesis in lymphocytes after stimulation in vitro and have arrived at similar conclusion. The DNA synthesis starts at 20–24 hours after stimulation [41] and then rises linearly for at least 48 hours [84,85].

Weber and associates [41] have found a small number of preactivated cells, which started their DNA synthesis very soon (10–16 hours) after the addition of PHA, in the cord blood. The reason for these findings may be that the cells have been prestimulated in vitro by cells or their antigens from the mothers. A similar hypothesis had recently been forwarded by Carr and associates [67] and it has also been shown that lymphocytes can be preactivated in vitro to a state in which the subsequent addition of an adequate stimulant pushes them into the S phase [61]. The question has been raised to what an extent the admixture of maternal cells to cord blood lymphocytes may play a role in studying the latter in vitro. The passage of fetal lymphocytes through the placenta into the maternal circulation is a phenomenon occurring in most, if not all, pregnancies [86-89]. The evidences indicating the passage of maternal cells into fetal circulation are accummulating. Sensitization of fetal lymphocytes by the mother against PPD [90,91] and E. Coli [92] has been observed. Patients with congenital isolated IgA deficiency can synthesize in utero specific IgM and IgG antibodies against maternal IgA [93]. Desai and Creger [94] injected quinacrine-labeled leukocytes to 9 pregnant women and found stained granulocytes and lymphocytes in 6 of fetuses. Turner and associates [95] found occasional xx mitosis after PHA stimulation in the cord blood of 2 out of 183 newborn boys. The xx/xy chimerism in the lymphopoietic tissue of fetus have also been reported by others, but generally in connection with severe immunological disease [96,97]. Recently Schroder [98] demonstrated that even is normal pregnancies maternal lymphocytes capable of PHA transformation and mitotic division may occasionally pass the placenta and appear in the fetal circulation.

ABSTRACT:

The reactivity of neonatal (cord blood) lymphocytes and adult's peripheral lymphocytes in response to stimulation by different doses of phytohemagglutinin (PHA) is evaluated. The degree of responsiveness is quantitated by the ratio of triated thymidine (^{3}H-TdR) incorporation into

the DNA portion of the stimulated to the nonstimulated cells. The method employed is the new micromethod being described in detail elsewhere in this issue. The results demonstrated that in addition to higher spontaneous proliferative capacity, neonatal lymphocytes have a greater response than adult's lymphocytes at suboptimal doses of PHA and respond optimally at lower doses of PHA as shown by a "shift to the left phenomenon" of its PHA dose-response curve. These findings indicated that neonatal lymphocytes are actually the preactivated cells. The possible explanation for the intrauterine preactivation of these cells is the low dose stimulation by maternal lymphocytes passed through placenta into the fetal circulation during gestation.

ย่อเรื่อง

คณะผู้วิจัยได้รายงานผลการศึกษาเกี่ยวกับปฏิกริยาของ lymphocytes ใน neonatal (cord blood) และในเลือดผู้ใหญ่ปกติที่โต้ตอบต่อการกระตุ้นด้วย Phytohemagglutinin (PHA) ในขนาดต่าง ๆ กัน ระดับของปฏิกริยาโต้ตอบวัดได้จากอัตราส่วนของ Tritiated thymidine (^{3}H-TdR) ที่ถูก incorporated เข้าไปใน DNA ระหว่างเซลล์ที่ถูกกระตุ้นและไม่ได้ถูกกระตุ้นวิธีที่ใช้ตรวจเป็น micromethod และผลที่ได้รับแสดงให้เห็นว่า Cord blood lymphocytes จะมีปฏิกริยาโต้ตอบต่อ PHA ได้ดีที่ขนาดความเข้มข้นต่ำกว่า lymphocytes ของผู้ใหญ่ปกติ และโต้ตอบได้ optimal dose ต่ำกว่าด้วย ซึ่งได้แสดงไว้ใน "Shift to the left Phenomenon" ของ PHA dose-response curve แล้ว

จากการค้นพบนี้ เป็นที่ประจักษ์ชัดว่า neonatal lymphocytes ถูก preactivate มาก่อนใน intrauterine preactivation ซึ่งอธิบายได้ว่าเซลล์เหล่านี้ถูกกระตุ้นด้วย dose ต่ำๆ จาก maternal lymphocytes ผ่านทาง placenta เข้าไปในวงจรโลหิตของลูกขณะอยู่ในครรภ์มารดา.

REFERENCES

1. Medawar, P.B. : In "Society for Experimental Biology, Evolution, Symposium No 11, pp. 320, Academic Press, New York, 1953.
2. Billingham; R.E. ; Transplantation immunity and the maternal-fetal relation. New Eng. J. Med 270: 667, 1964.

3. Hellstrom, K.E., Hellstrom, I., and Brawn, J.; Abrogation of cellular immunity to antigenically for eign mouse embryonic cells by a serum factor. Nature (London) 224: 915, 1969.

4. Playfair, J.H.L., Wolfendale, M.R., and Kay, H.E.M.: The leukocytes of peripheral blood in the human foetus. Brit. J. Haemat. 9:336, 1963.

5. Faulk, W.P., Wang, A.C., Goodman, J.R., and Fudenberg, H.H.: Immunologic parameters unique for the placento fetal unit. Pediat. Res. 3:499, 1970.

6 Winter, G.C.B., Byles, A.B., and Yoffey, J.M. Blood lymphocytes in newborn and adults. Lancet 2:932, 1965

7. Papamichail, M., Brown, J.C., and Holborow, E.J.: Immunoglobulins on the surface of human lymphocytes. Lancet 2:850, 1971.

8, Froland, S.S., and Natvig, J.B.: Lymphocytes with membrane-bound immunoglobulin (B-lymphocytes) in newborn fetus. Clin. Exp. Immunol. 11:495, 1972.

9. Sanyanusin, P., Chaiyarasamee, S., Tositarat, T., and Kulapongs, P.: The cellular immune aspects offetal lymphocytes. I. Distribution of B and T lymphocyte subpopulations in cord blood. Bull. C.M. Ass. Med. Sci. 7: 59, 1974.

10. Faulk, W.P. : In "Sudden and Unexpected Death in Infancy (Cot Death)". Camps, F.E., and Carpenter, R.G., eds., p.82. John Wright and Sons, Bristol, 1972.

11. Stites, D P., Wybran, J., Carr, M.C., and Fudenberg, H.H.: In "Ontogeny of Acquired Immunity." Porter, R., and Knight, J., eds., p. 113. Associated Scientific Publishers, Amsterdam. 1972.

12. Pulvertaft, R., and Pulvertaft. L.: Spontaneous transformation of lymphocytes from the umbilical cord vein. Lancet 2 : 892, 1966.

13. Leikin, S., Mochir-Fatemi, F., and Park, K: Blast transformation of lymphocytes from newborn infants. J. Pediat. 72: 510, 1968.

14. Hayward, A.R., and Soothill. J.F.: In "Ontogeny of Acquired Immunity". Porter, R., and Knight, J., eds., p. 261. Associated Scientific Publishers, Amsterdam, 1972.

15. Carr, M.C., Lieber E., and Fudenberg, H.H. : In vitro cytolysis by human fetal lymphocytes. Cell. Immunol. 1 : 455, 1970.

16. Ceppellini, R., Bonnard, G.D., Coops, F., Miggians, V.C., Pospi-

sil, M., curtoni, E.S., and Pellegrino, M. : Mixed leukocyte cultures and HL-A antigens. I. Reactivity of young fetuses, new borns and mothers at delivery. Transplant. Proc. 3 : 58. 1971.

17. Seigler, H.F. and Metzgar, R.S.: Embryonic development of human transplantation antigens. Transplantation 9 : 478, 1970.
18. Ohama, K., and Kajii. T. ; Mixed cultures of fetal and adult lymphocytes. Amer. J. Obstet. Gynecol. 119 : 552, 1974.
19. Lichtenstein, M.R. : Amer. Rev. Tuberc. Pulm. Dis. 46 : 89, 1942.
20. Smith, J.K.; Caspary, E.A., and Field, E.J. : Immune response in pregnancy. Lancet 1 : 96, 1972.
21. Andresen, R H., and Monroe, C W. : Experimental study of the behavior of adult human skin homografts during pregnancy. Amer. J. Obstet. Gynecol. 84 : 1096, 1962.
22. Shwartz, M.R. : The mixed lymphocyte reaction . an in vitro test for tolerance. J. Exp. Med. 127 : 879, 1968.
23. Silvers, W.K., Lubaroff, D.M., Wilson, D.B., and Fox D . Mixed lymphocyte reactions and tissue transplantation tolerance. Science 167 : 1264, 1970.
24. Levey R.H., Klemperer, M.R., Gelfand, E.W., Sanderson, A.R., Batchelor, J.R., Berkel, A.I., and Rosen, F.S. : Bone-marrow transplantation in severe combined immunodeficiency syndrome. Lancet 2 : 571, 1971.
25. Rubinstein, A., Speck, B, and Jeannet, M. : Successful bone-marrow transplantation in a lymphopenic immunologic deficiency syndrome. New Eng. J. Med. 285 ; 1399, 1971.
26. August, C.S., Levey, R H. Berkel, A.I. Rosen, F.S., and Kay, H.E.M. : Establishment of immunological competence in a child with congenital thymic aplasia by a graft of fetal thymus. Lancet 1 : 1080, 1970.
27. Hong. R , Gatti, R., Rathbun, J.C., and Goo dR.A. , Thymic hypoplasis and thyroid dysfunction. New Eng. J. Med. 282 : 470, 1970.
28. Carr, M C., Stites D.P., and Fuden berg H.H.: Cellular immune aspects of the human fetal maternal relationship. II. In vitro response of gravida lymphocytes to phytohemagglutinin. Cell Immunol. 8 : 448, 1973.
29. Fitzgerald, M.G., : A satisfactory quantitative test of lymphocyte response to phytohaemagglutinin

for the definition of normal control values and recognition of immunological defects. J. Clin. Path. 25 : 163, 1972.

30. Carr M.C., Stites, D.P., and Fudenberg. H.H.,Dissociation of responses to phytohaemagglutinin and adult allogeneic lymphocytes in human foetal lymphoid tissues. Nature 241 : 279, 1973.

31. Prindull, G. : An in-vitro quantititative study of phytohaemagglutinin (PHA) induced transformation of lymphocytes from premature newborn infants, from older premature infants, and from full-term newborn infants. Blut 23 : 7, 1961.

32. Prindull, G. : Anti-human lymphocyte globulin-(ALG-) sensitive lymphocyte populations in the blood of newborn premature infants, of older premature infants and of full-term newborn infants. Blut. 23 : 320, 1971.

33. Lindahl-Kiessling, K., and Book, J.A. : Effects of phytohaemagglutinin on leukocytes Lancet 2 : 591, 1964.

34. Marshall, W.C., Cope, W.A., Soothill, J.F., and Dudgeon, J.A., :In vitro lymphocyte response in some immune deficiency diseases and in intrauterine virus infections Proc. Roy. Soc. Med. 63 : 351, 1970.

35. Pentycross, C.R. : Lymphocyte transformation in young people. Clin. Exp. Immunol. 5 : 213, 1969.

36. Vongyutidham M., Chaiyarasamee, O., Tositarat, T., and Kulapongs, P. ; The cellular immune aspects of fetal lymphocytes. III. In vitro response of fetal lymphocytes to phytohemagglutinin stimulation. Bull. C.. A.Mss. Med Sci. 7 :77, 1974.

37. Jones. W.R. : In vitro transformation of fetal lymphocytes. Amer. J. Obstet. Gynecol. 104 : 586, 1968.

38. Ayoub, J., and Kasakura, S. : In vitro response of fetal lymphocytes to PHA, and a plasma factor which suppresses the PHA response of adult lymphocytes. Clin. Exp. Immunol. 8 : 427, 1971.

39. Montgomery, J.R., Mason, E.O., and South M.A. : Mitogenic response differences between adult and newborn lymphoid cells. Pediat. Res. 7 : 365, 1973.

40. Papiernik, M. : Correlation of lymphocyte transformation and morphology in the human fetal thymus. Blood 36 : 470, 1970.

41. Weber, T.H., Santesson, B., and Skoog, V.T. : The activation of fetal lymphocytes. Scand. J. Haemat. 11 : 177, 1973.

42. Kulapongs, P. Tositarat. T., Chaiyarasamee. O. and Chaiyarasamee. S. : The cellular immune aspects of fetal lymphocytes. II. Spontaneous transformation and nucloside incorporation of human fetal lymphocytes. Bull. C.M. Ass. Med. Sci. 7 : 69, 1974.

43. Prindull, G. : Spontaneous DNA synthesis of blood lymphoid cells in premature newborn infants, in older premature infants, and in full-term newborn infants.1974

44. Comings, D.E. : Lymphocyte transformation in response to phytohemagglutinin during and following a pregnancy. Amer. J. Obstet. Gynecol. 97: 213, 1967.

45. Thiede, H.A., Choate, J.W., and Dyre, S. : Pregnancy and lymphocyte: Amer. J. Obstet. Gynecol. 102 : 642, 1968.

46. Morin, p., Alcalay, D., and Choukroun, J. : Gynecol. Obstet. (Paris) 70 ; 55. 1971.

47. Purtilo, D.T., Hallgren, H.M., and Yunis, E.J.: Depressed maternal lymphocyte response to phytohaemagglutinin in human pregnancy. Lancet 1 : 769, 1972.

48. Brown, R.S., Haynes, H A, Foley. H.T., Goodwin, H A., Berard, C.W., and Carbone, P.P. : Hodgkin's disease. Immunologic clinical, and histologic features of 50 untreated patients. Ann. Intern. Med. 67 : 291, 1967.

49. Han, T., and Sokal; J.E. : Lymphocyte response to phytohemagglutinin in Hodgkin's disease. Am. J. Med. 48 : 728, 1970.

50. Mac Kinney, A.A, Jr. : Dose-response curve of phytohaemagglutinin in tissue culture of normal human leucocytes. Nature 204 : 1002, 1964.

51. Hersh, E.M., and Oppenheim, J.J. : Impaired in vitro lymphocyte transformation in Hodgkin's disease. New Eng. J. Med. 273 : 1006, 1965.

52. Garth, R.A., Garrioch, D.R., and Good R A. : In "Proceedings of the Fifth Leukocyte Culture Conference". Harris J.E., ed,. p. 339, Academic Press. New York, 1970.

53. Ziegler, J.B., Hansen, P.J., Davies, W.A., and Penny, R. : The PHA dose-response curve :validation of the use of logarithmic graph paper by computer analysis results. J, Immunol. 113 : 2035, 1974.

54. Yamamura, M. : Standardization of lymphocyte transformation to

phytohaemagglutinin. Clin Exp. Immunol 14 : 457 1973

55. Fitzgerald, M.G. : The establishment of a normal human population dose-response curve for lymphocytes cultured with PHA (phytohemagglutinin). Clin. Exp. Immunol. 8 : 421, 1971.

56. Jones, L.H., Hardisty, R.M. Wells, D.G., and Kay, H.E.M. : Lymphocyte transformation in patients with acute lymphoblastic leukaemia. Brit. Med. J. IV. : 329, 1971.

57. Oppenheim, J.J., Blaese, R.M., and Waldmann, T.A, : Defective lymphocyte transformation and delayed hypersensitivity in Wishott-Aldrich syndrome. J. Immunol. 104 : 835, 1970.

58. Craig, A.W., Garrett, J.V., and Jackson, S.M. : Quantitation of lymphocyte transformtaion using radioactive iododeoxyuridine. J. Clin. Path. 22: 558. 1969.

59. Oppenheim, J.J : Relationship of in vitro lymphocyte transformation to delayed hypersensitivity in guinea pigs and man. Fed. Proc. 27 : 21, 1968.

60. Bradley, J., and Oppenheim, J.J. : The in vitro proliferation of lymphocytes from patients with hypozarnmaglobulinemia. Clin Exp. Immunol. 2 : 549, 1967.

61. Lindahl-Kiessling, K. : Mechanism of phytohemagglutinin (PHA) action. V. PHA compared with concanavalin A (Con. A). Exp. Cell Res. 70 : 17, 1972.

62. Jarvis, M., and Bach, F.H. : In "Proceedings of the Fourth Annual Leukocyte Culture Conference. edited by O.R. Mc Intyre, p. 335. Appleton-Century-Crofts, New York, 1971.

63. Thorpe, P.E., and Knight, S.C. : Microplate culture of mouse lymph node cells. I. Quantitation of responses to allogeneic lymphocytes endotoxin and phytomitogens. J. Immunol. Methods 5 : 387. 1974.

64. Andersson, J., Sjöberg, O., and Moller, G. : Reversibility of high dose unresponsiveness to concanavalin A in thymus lymphocytes. Immunology 23 : 637, 1972.

65. Sample, W.F. and Chretien, P.B. : Thymidine kinetics in human lymphocyte transformation: determinatoin of optimal labelling conditions. Clin. Exp. Immunol, 9 : 419, 1971.

66. Rabinowitz, Y., Wong, P., and Wilhite, B.A. : Effect of phytohemagglutinin on enzyme of thymidne salvage pathway of

cultured chronic lymphatic leukemia lymphocytes. Blood 35 : 236 1970.

67. Carr, M,C.. Stites, D.P., and Fudenberg, H.H.: Cellular immune aspects of the human fetal-maternal relationship, I. In vitro response of cord blood lymphocytes to phytohemagglutinin. Cell. Immunol. 5 : 21, 1972.

68. Leikin, S., and Oppenheim, J.J. : Differences in transformation of adult and newborn lymphocytes stimulated by antigen, antibody and antigen-antibody complexes. Cell. Immunol. 1 : 468, 1970.

69. Meuwissen, H.J., Bach, F.H., Hong, R., and Good, R.A. : Lympho cytes studies in congenital thymic dysplasia : The one way stimulation test. J. Pediat. 72 : 177, 1968.

70. Leikin, S., Whang-Peng, J.; and Oppenheim, J.J. : In "Proceedings of the Fifth Annual Leucocyte Culture Conference"; edited by J. Harris, p. 839, Academic Press, New York, 1970.

71. Moller, G. Induction of DNA synthesis in human lymphocytes: interaction between non-specific mitogens and antigens. Immunology 19 : 583, 1970.

72. Panzetta P., Pirofsky, B. and Rigas, D A.: Mechanism of the mitogenic action of the phytohemagglutinin. I. Induction of tolerance and lymphocyte transformation. J. Reticuloendo Soc. 13 : 298, 1973.

73. Nowell, Phytohemagglutinin : initiator of mi oses in cultures of normal human leukocytes. Cancer Res. 20 : 462, 1960.

74. Lindahl-Kiessling, K., and Mattsson, A. : Mechanism of phytohemagglutinin (PHA) action. IV. Effect of some metabolic inhibitors on binding of PHA to lymphocytes and the stimulation potential of PHA-pretreated cells Exp. Cell Res. 65 : 307, 1971.

75. Weber, T., Lindahl-Kiessling, K., Mattsson, A., and Alm, G.: Autoradiographic studies of lymphocytes stimulated in vitro with tritium labelled kidney bean leucoagglutinin. Life Sci. 11: 343,1972.

76. Skoog V.T., Weber,T., NordgrenH., Mattsson, A., and Lindahl-Kiessling. K. : In "Proceedings of the Eighth Annual Leucocyte Culture Conference, edited by K. Lindahl -Kiessling and D. Osoba, p. 45. Academic Press, New York,1974.

77. Lindahl-Kiessling, K., Mattsson,A., Skoog, V.T,, and Weber. T, : In "Proceedings of the Seventh

Annual Leucocyte Culture Conference, edited by F. Dagnillard, p. 163. Academic Press, New York 1972.

78. Skoog. V.T.. Weber; T., Mattsson, A., and Lindahl-Kiessling, K. : Scand. J. Immunol. 2 : 90, 1973.

79. Steck, T.L., and Hoelzl Wallach, D.F. : The binding of kidney-bean phytohemagglutinin by Ehrlich ascites cacrinoma. Biochom Biophys. Acta 97: 510, 1965.

80. Stein M.D., Sage, H.J. and Leon M.A.: Studies on a phytohemagglutinin from the lentil. V. Binding of Lens culinaris hemagglutinin to lymphocytes and oerythrocytes. Arch. Biochem. Biophys. 150 : 412, 1972.

81. Weber, T. : Klnetics of the reaction of Kidney-bean leucoagglutinin with human lymphocytes. Experimentia 29 : 863, 1973.

82. Weber. T, Skoog, V.T., Mattsson, A., and Lindahl Kiessling, K.: Kinetics of lymphocyte stimulation in vito by non-specific mitogens. Exp. Cell Res 85: 351, 1974

83: Bender. M.A: and Prescott, D.M, : DNA synthesis and mitosis in Cultures of human peripheral leukocytes. Exp, Cell Res. 27 : 221, 1962.

84. Kay, J.E. : RNA and protein synthesis in lymphocytes incubated with phytohaemagglutinin. In "The Biological Effects of Phytohaemagglutinin," edited by M.W. Elves, p. 37. Crane, Oswestry, England, 1966.

85. Rigas, D.A., Johnson, E.A., Jones, R. T., Mc Dermed, J.D. and Tisdale, V.V : The relationship of the molecular structure to the hemagglutinating and mitogenic activities of the phytohemagglutinin from Phaseolus vulgaris. In Proc. Journees Hellenes de separation immediate et de cnromatograsphie, G.Parissakis (ed)., p. 151; Athens, 1965.

86. Desai. R.G., Mc Cutcheon, E., Little, B: and Driscoll, S.G. : Fetomaternal passage of leukocytes and plateletes in erythroblastosis fetalis. Blood 27:858, 1966.

87. Walknowska, J Conte F.A, and Grumbach, M.M. : Practical and theoretical implications of fetal/maternal lymphocyte transfer. Lancet 1 :1119, 1969.

88. Schroder, J., and de la Chapelle, A. : Fetal lymphocytes in the maternal blood. Blood 39 : 153, 1972.

89. Schroder, J., Tiilikainen, A., and de la Chapelle, A. : Fetal leukocytes in the maternal circulation after delivery. I Cytological aspects. Transplantation. 17 : 346 1974.

90. Field, E.J., and Caspary, E.A. : Is maternal lymphocyte sensitization passed to the child? Lancet 2 : 337: 1971.

91. Astor, S.H., and Frick. O. : MIF or PNIF production indicates passage of cellular imunity from mother to fetus. Pediat. Res. 7 : 365, 1973.

92. Brody, J.J., Oski, F.A. and Wallach, E.E. : Neonatal lymphocyte reactivity as an indicator of intrauterine bacterial contact. Lancet 1 : 1396, 1968.

93. Vyas, G.N Levin. A.S. and Fudenberg, H.H. : Intrauterine immunization caused by maternal IgA crossing the placenta. Nature 225 : 275, 1970.

94. Desai, R.G., and Creger, W.P. : Maternofetal passage of leukocytes and platelets in man Blood 21 : 665, 1963.

95. Turner, J.H., Wald, N., and Quinlivan, M.D. : Cytogenetic evidence concerning possible transplacental transfer of leukocytes in pregnant women. Amer. J. Obstet. Gynecol. 95: 831, 1966.

96. Kadowaki, J., Thompson, R.I., Zuelzer, W.W., Woolley, P.V., Brough, A.J. and Gruber, D. : xx/xy lymphoid chimerism in congenital immunological deficiency syndrome with thymic alymphoplasia. Lancet 2 : 1152, 1965.

97. Taylor, A.I., and Polani, P.E. : xx/xy mosaicism in man. Lancet 1 : 1226, 1965.

98. Schroder J. : Passage of leukocytes from mother to fetus. Scand J. Immunol. 3 : 369, 1974.