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Lymphocyte blastogenesis in normal and low birth weight infants and the effect of monocyte depletion on it

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A response of lymphocytes in the neonatal period is different from that in later stages, and a capacity for cellular immunity is reduced in infants with intrauterine growth retardation (IUGR) [6]. Although immune response of newborn infants is largely dependent on the intrauterine life, the relation between gestational age and lymphocyte activity has not yet been well established. To elucidate how the intrauterine life affects the immune response of neonates and at which moment those aberrant responses return to normal, we have studied spontaneous and phytohaemagglutinin (PHA)-induced blastogenesis of circulating lymphocytes from neonates and adults. (Experiment 1)

Spontaneous blastogenesis of lymphocytes (SBL) from neonates was always greater than that of adults and the addition of autologous or homologous plasma did not affect their blastogenesis, suggesting that the greater responsibility of lymphocytes is an intrinsic characteristic [12]. However, the effect of monocytes could not be excluded, since monocytes are present in the lymphocyte culture. Then we depleted monocytes from mononuclear leucocytes (MNL) fraction and tested their effect on the lymphocyte blastogenesis. (Experiment 2)

1 Material and methods

Experiment 1. A follow-up study was done with a total of 63 neonates. Twenty-three full-term healthy newborn infants were of 38 to 42 weeks' gestational age and mean birth weight was 3189 gm (range 2845 to 3980 gm). Eighteen out of 40 low birth weight infants (LBW) were below the third percentile of weight for age (IUGR), whose gestational age ranged from 35 to 42 weeks and mean birth weight was 1913 gm (range 1430 to 2425 gm). The other 22 were appropriate for date infants (AFD) of 26 to 37 weeks of gestational age and mean birth weight was 1896 gm (range 876 to 2480 gm). Gestational age was assessed by the last menstruation and verified by DUBOWITZ score [4]. Blood samples were taken at birth, within 24 hours after delivery (cord blood from full-term infants and capillary blood from LBW), and on the 7th and 30th postnatal days (capillary blood). All infants were clinically well at the time of the test. Eight adults, 25 to 35 years-old, were healthy volunteers of our medical staff. Capillary blood was used for total leucocyte counts and differential counts. Lymphocyte blastogenesis was assessed

Curriculum vitae

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by our modified whole blood method [17], which is reproduced here briefly. Seventy-five μl of blood was taken in duplicate into a heparinized capillary tube, which was centrifuged at 5000 rpm for 5 minutes and cut off, just below the buffy coat. Then, the buffy-coat and plasma (buffy-coat fraction) were blown out to 1 ml of RPMI 1640 medium containing 30% of heat-inactivated fetal calf serum (FCS) with or without PHA-P (Difco) to a final concentration of 15 μl/ml. This method has an advantage to avoid the contamination by red blood cell ghosts interfering the estimation of thymidine uptake. After incubation for 48 hours at 37 °C under 5% CO_2, ^{3}H-thymidine (The Radio-chemical Center, Amersham, Sp. Act. 5 μCi/μM) was added and additional incubation was done for 24 hours, then the radio-activity was counted. Statistical analysis was carried out by t-test. Correlation between spontaneous blastogenesis (y dpm) and gestational age (x weeks) was calculated from the results of AFD at birth.

Experiment 2. Thirteen full-term healthy neonates and 5 healthy adults were examined. MNL were obtained from 15 ml sample of cord blood and 20 ml sample of adult blood by Ficoll-Hypaque density gradient centrifugation. Interphase cells were utilized as MNL fraction or further separation, for which the cell concentration was adjusted to 5×10^6/ml with RPMI 1640 medium containing 30% FCS. Six ml of aliquots were incubated in 90 × 15 mm plastic culture dishes (TERUMO, Tokyo) for 1 hour at 37 °C under 5% CO_2. Non-adherent cells in the suspension were used for further depletion of monocytes by the column method of JULIUS [8]. Approximately 50% of the cells were recovered in the eluate and the monocyte were identified by peroxidase and Giemsa staining. Mononuclear cell count in each fraction was adjusted to 2.5×10^5/ml, then they were incubated with or without PHA as mentioned above.

2 Results

Experiment 1. 1) SBL from all neonates was much greater (Fig. 1) than that from adults (615 ± 225 dpm, mean ± SE). 2) SBL from AFD at birth

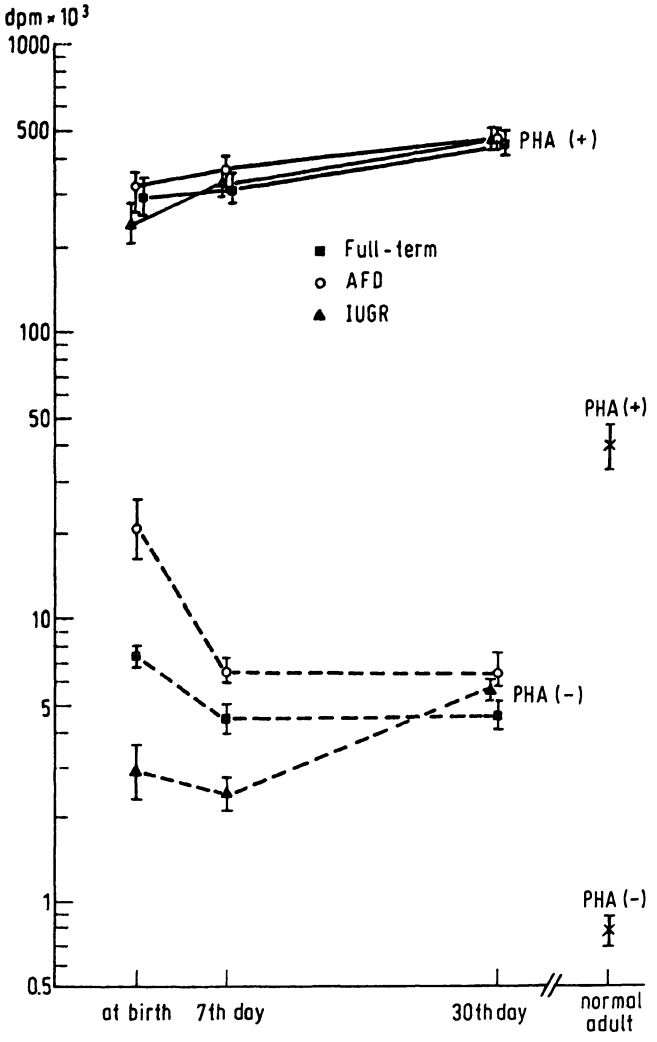

Fig. 1. Spontaneous and PHA-induced blastogenesis of lymphocytes in relation to postnatal age.

(20779 ± 4233 dpm, $p < 0.005$) and on 7th day of age (6504 ± 671 dpm, $p < 0.025$) was significantly greater than that from normal full-term neonates (7370 ± 1219 dpm at birth, 4526 ± 419 dpm on 7th day). 3) In neonates with IUGR, SBL was significantly less (2991 ± 731 dpm, $p < 0.025$, at birth and 2462 ± 329 dpm, $p < 0.001$, on 7th day) than in normal full-term neonates. 4) On 30th day of age, however, no significant difference was seen among the full-term, AFD and IUGR neonates. 5) SBL from AFD at birth was reversely correlated to the gestational age ($r = -0.76$, the regression equation: $y = -4115x + 156341$, Fig. 2). 6) There was no significant difference in lymphocyte counts among full-term, AFD and IUGR infants at the corresponding postnatal ages. Average lymphocyte counts were 3400/mm^3 at birth, 4900

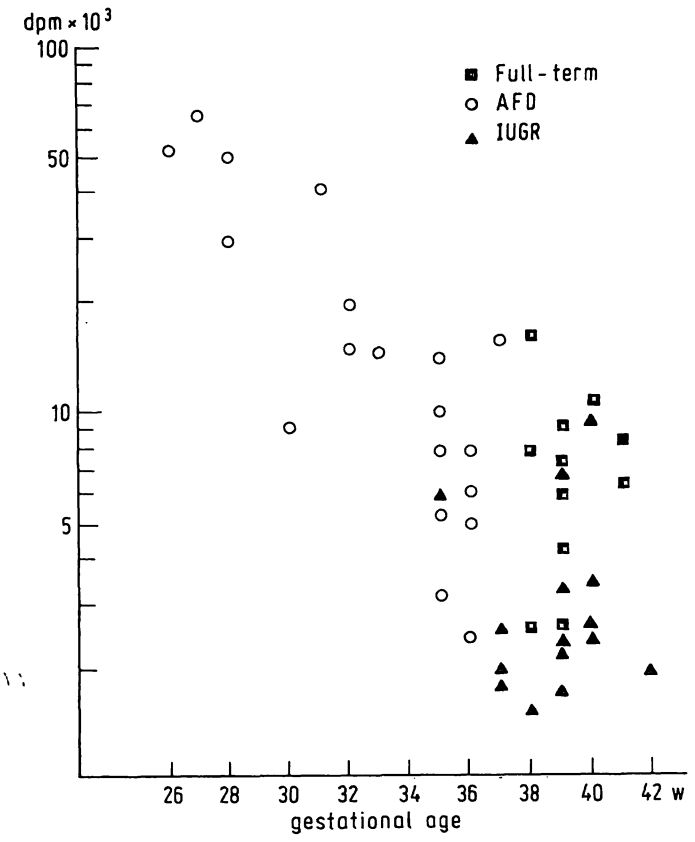

Fig. 2. Spontaneous blastogenesis of lymphocytes at birth in relation to gestational age.

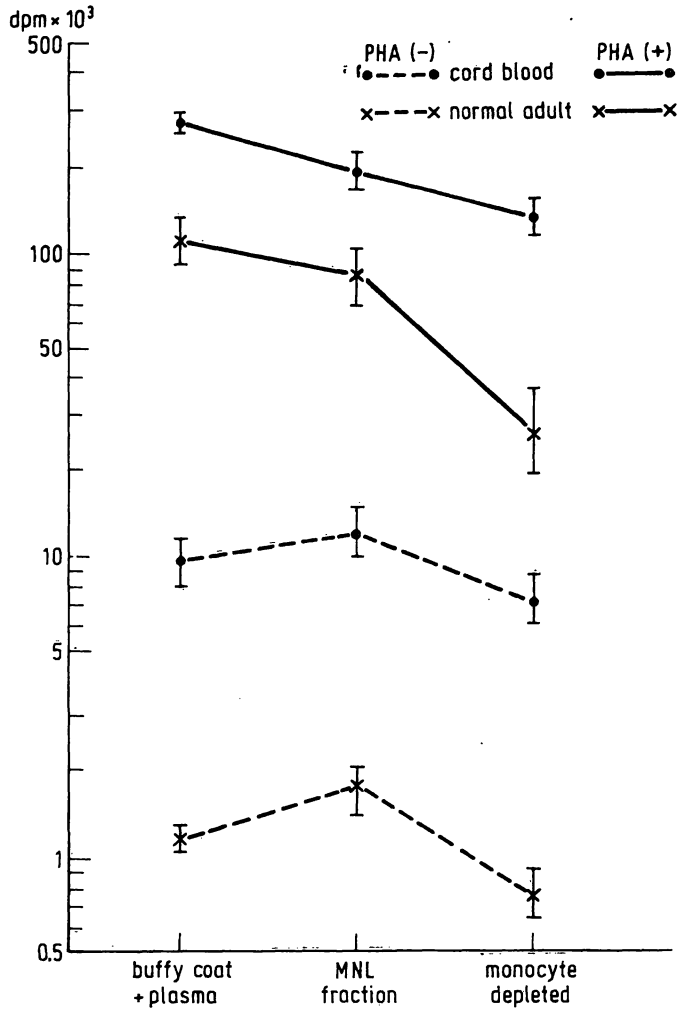

Fig. 3. Effect of monocyte-depletion on spontaneous and PHA-induced blastogenesis of lymphocytes from cord blood and adult blood.

on 7th day, 5800 on 30th day, but 2900 in adults. PHA-induced blastogeneses showed no significant difference among full-term, AFD and IUGR neonates at any time, but they were all significantly greater than that of normal adults (Fig. 1), and increased with postnatal age.

Experiment 2. The number of monocytes were 4–8% (adult), 5–15% (cord blood) in buffy-coat fraction, 18–29% (adult), 25–35% (cord blood) in MNL fraction and 0.3–1.0% (adult), 0.5–2.0% (cord blood) in monocyte-depleted fraction. Spontaneous uptake of ^{3}H-thymidine by the buffy-coat fraction, MNL fraction and monocyte-depleted fraction of cord blood from full-term neonates was 9781 ± 1740, 12010 ± 2278 and 7231 ± 1452 dpm, respectively (Fig. 3). They were all significantly greater than those from adults' blood (1181 ± 105, 1715 ± 327 and 774 ± 147 dpm, respectively). SBL from cord and adult blood was not significantly reduced by monocyte depletion. Lymphocytes in the buffy-coat fraction proliferated less than in the MNL fraction.

PHA-induced ^{3}H-thymidine uptake by the three fractions from cord blood was always greater than that from adult blood (Fig. 3). PHA-induced blastogenesis of monocyte-depleted fraction from cord blood was reduced only by 16.2 ± 14.1% as compared with MNL fraction, but that of adult lymphocytes was decreased significantly (−68.3 ± 5.8%, $p > 0.025$). Buffy-coat fraction showed the greatest response to PHA stimulation both in cord and adult blood.

3 Discussion

Lymphocyte blastogenesis in normal newborn infants is always greater than in later stage or adults [12, 15], resulting from immaturity of immune

and haematopoietic systems [11, 15]. On the other hand, no consistent results were reported in LBW. PRINDULL [14] found a greater spontaneous and PHA-induced blastogenesis of lymphocytes from neonates 28–34 weeks of gestational age than from full-term neonates, while WIERSBITZKY et al. [18] and JONES [9] found no significant difference in blastogenesis among infants with different gestational ages. However, the present experiment revealed the reverse correlation between SBL and gestational age in AFD at birth. Such inconsistency could be derived partly from the fact that in the experiments other than ours they did not classify LBW into AFD and IUGR.

As shown in Fig. 1, the greater SBL in AFD at birth became markedly reduced on the 7th day but still higher than that from full-term infants. Then, on the 30th day there was no significant difference between AFD and full-term infant. At this time of age, the lymphocytes from LBW and full-term infants proliferated to the same extent as those from adults when expressed as a stimulation index (PHA-induced/spontaneous blastogenesis). As one of the reasons for the greater SBL in less matured infants, we anticipated the effect of monocytes, but the depletion of monocytes did not affect the spontaneous blastogenesis of either neonatal or adult lymphocytes much.

Recently, many colony forming cells were found in cord blood [7, 11]. These stem cells might be attributed to a higher response in less matured infants and the cells might decrease in number and/or cease their proliferation during maturation. On the other hand, impaired cellular immunity associated with IUGR was widely recognized [1, 3, 6] and attributed to the reduced number of T-cells in peripheral blood [1, 2, 5] due to foetal malnutrition. FERGUSON [6] reported in his transverse study the reduced proliferative capacity of lymphocytes with PHA upto 5 years of age. In our longitudinal study, however, no impairment of lymphocyte response to PHA was found in IUGR infants either at birth or 30th day of age. On the contrary, SBL was significantly reduced in IUGR infants at birth but returned to normal on 30th day, suggesting that enhanced or reduced SBL in neonatal period might depend on the number of colony forming cells, which might be reduced (AFD) or increased (IUGR) in number by the 30th day of age, then SBL converged to the same level as in full-term infants.

In Experiment 2, the greatest response of lymphocytes to PHA was obtained by buffy-coat fraction from both neonates and adults. This could be ascribed to the contamination of autologous serum [16], erythrocytes and/or granulocytes other than monocytes [10]. WILSON et al. [19] stated that depletion or addition of monocytes had similar effects on the response to Concanavalin A (Con A) of lymphocytes from either neonates or adults. In our study, however, PHA-induced blastogenesis was significantly reduced in adult blood when monocytes were depleted, but not so in cord blood. POTTER et al. [13] described that PHA response of lymphocyte was greatly reduced when monocytes were depleted to $1.4 \pm 0.4\%$, and the optimal response was obtained in the presence of 2–5% monocytes. In our experiments, monocytes remained as much as 0.3–1% in the monocyte-depleted adult blood and 0.5–2% in the cord blood. Even such slight difference in number of monocytes might have an effect on PHA stimulation. On the other hand, WOLF et al. [20] reported the inhibition of PHA stimulation on adult lymphocytes with the supernatant of cultured media of monocytes from cord blood. At present, the effect of monocytes on lymphocyte blastogenesis is controversial and remains to be studied further.

Summary

To elucidate the effect of intrauterine life on the immune response of newborn infants, we have studied spontaneous and phytohaemagglutinine (PHA)-induced blastogenesis of lymphocytes from 23 normal full-term neonates, 40 low birth weight infants (LBW), of which 18 were intrauterine growth retarded (IUGR), and 22 were appropriate for date (AFD), and 8 adults. As a follow-up study of infants blood samples were taken at birth (cord

blood or capillary blood), on the 7th and the 30th day (capillary blood) into heparinized capillary tubes, which were centifuged and cut off just below the buffy-coat portion. The buffy-coat and plasma was blown out to RPMI 1640 medium with or without PHA (15 μg/ml). After preincubation period, ^{3}H-thymidine (Sp. Act. 5 μCi/μM) was added and additional incubation was done at 37 °C for 24 hours under 5% CO_2.

Thirteen full-term healthy neonates and 5 healthy adults were examined to elucidate the effect of monocyte on the lymphocyte blastogenesis. Mononuclear leucocytes (MNL) fraction was obtained from cord blood or venous blood (adults) by Ficoll-Hypaque density gradient centrifugation, then this MNL fraction was purified further by adhesion and column chromatography.

Spontaneous blastogenesis of lymphocytes from all neonates was much greater than that from adults (Fig. 1). Spontaneous blastogenesis in AFD at birth and on the 7th day of age was significantly greater than in normal full-term neonates, but significantly less in neonates with IUGR than in normal full term neonates (Fig. 1). On the 30th day of age, however, no significant difference was seen in full-term, AFD and IUGR infants. Spontaneous blastogenesis was reversely correlated to the gestational age ($r = -0.76$) in AFD and birth (Fig. 2). PHA-induced blastogenesis showed no significant difference between full-term, AFD and IUGR neonates at any age, but they were all significantly greater than that in adults (Fig. 1). PHA-induced blastogenesis in all infants was increased with postnatal age.

Spontaneous blastogenesis of lymphocytes from cord and adult blood was not significantly reduced by the monocyte depletion. In contrast, PHA-induced blastogenesis of monocyte-depleted fraction from adult blood was significantly reduced, but that from cord blood was not reduced so much (Fig. 3). The enhanced spontaneous blastogenesis in AFD newborn infants and the reduced one in neonates with IUGR might be due to the number and function of colony forming cells and may return to normal level by 30 postnatal days. At present, the effect of monocyte on lymphocyte blastogenesis is controversial and remains to be studied further.

Keywords: Appropriate for date (AFD) infant, gestational age, intrauterine growth retardation (IUGR), lymphocyte blastogenesis, monocyte depletion, newborn infant, phytohaemagglutinin (PHA).

Zusammenfassung

Zur Lymphoblastengenese bei Kindern mit normalem bzw. niedrigem Geburtsgewicht und ihrer Beeinflussung durch Monozyten

Uns interessierte der Zusammenhang zwischen intrauterinem Wachstum und der Immunantwort des Neugeborenen. Hierzu untersuchten wir die spontane bzw. phytohämagglutinin-induzierte Lymphoblastengenese bei 23 normalgewichtigen Neugeborenen am Termin und 40 Kindern mit zu niedrigem Geburtsgewicht. Von diesen 40 Kindern zeigten 18 eine intrauterine Wachstumsverzögerung und 22 waren normalgewichtig bezogen auf das Schwangerschaftsalter. Weiterhin untersuchten wir 8 Erwachsene. Die Blutproben wurden jeweils bei der Geburt (Nabel- oder Kapillarblut), am 7. und am 30. Tag (Kapillarblut) in heparinisierten Röhrchen gesammelt, die zentrifugiert und gerade unterhalb der Buffy-Coat-Schicht (= Leukokrit) abgetrennt wurden. Leukokrit und Plasma wurden in ein bestimmtes Medium (RPMI 1640) gegeben. Einer Serie wurde in einer Konzentration von 15 μg/ml Phytohämagglutinin (PHA) zugesetzt. Nach einer Präinkubationszeit applizierten wir ^{3}H-Thymidin (Aktivität: 5 μCi/μmol) und inkubierten unter 5%iger CO_2-Begasung 24 Stunden bei 37 °C.

13 Neugeborene am Termin und 5 Erwachsene dienten als Versuchspersonen, um den Einfluß von Monocyten auf die Lymphoblastengenese zu prüfen. Wir erhielten die mononukleären Leukozyten aus dem Nabel- bzw. Venenblut durch Dichtegradientenzentrifugation (Ficoll-Hypaque); anschließend wurde diese Fraktion über Säulenchromatographie gereinigt.

Die spontane Lymphoblastengenese war bei allen Neugeborenen größer als bei Erwachsenen (Abb. 1). Vergleicht man die Untergruppen bei den Neugeborenen miteinander, so ergibt sich folgendes Bild: spontane Lymphoblastengenese bei Unter-, aber altersentsprechendem Geburtsgewicht signifikant höher als bei Neugeborenen am Termin; spontane Lymphoblastengenese bei Neugeborenen mit intrauteriner Wachstumsretardierung signifikant niedriger als bei Neugeborenen am Termin. Diese Ergebnisse zeigten sich bei den Proben von der Geburt bzw. dem 7. Tag. Am 30. Tag entnommene Proben lieferten jedoch keine signifikanten Unterschiede mehr. Die spontane Blastengenese korrelierte negativ zum Gestationsalter ($r = -0.76$) innerhalb der Neugeborenengruppe mit niedrigem, aber altersentsprechendem Geburtsgewicht (Abb. 2). PHA-induziertes Wachstum war wiederum bei allen Neugeborenen größer als bei Erwachsenen. Hier zeigten sich jedoch zu keinem Zeitpunkt Unterschiede innerhalb der Untergruppen (Abb. 1). Die PHA-induzierte Wachstumsrate stieg mit dem postnatalen Alter an. Die spontane Lymphoblastengenese wurde nicht signifikant reduziert nach Entfernung der Monozyten. Dies galt für Nabelschnurblut und die Blutproben von Erwachsenen. Im Gegensatz hierzu wurde die PHA-induzierte Blastengenese in den Proben ohne Monozyten erheblich reduziert, und zwar im Erwachsenenblut deutlicher als im Nabelschnurblut (Abb. 3).

Die verstärkte Blastengenese bei Neugeborenen mit niedrigem, aber dem Schwangerschaftsalter entsprechendem Geburtsgewicht und die reduzierte Blastengenese bei Neu-

geborenen mit intrauteriner Wachstumsretardierung könnte auf die Zahl und Funktion von koloniebildenden Zellen zurückgeführt werden. Dieses unterschiedliche Verhalten zeigt sich jedoch nicht mehr am 30. Tag nach der Geburt. Der Einfluß von Monozyten auf die Lymphoblastengenese ist ungeklärt. Diese Frage bleibt Gegenstand weiterer Untersuchungen.

Schlüsselwörter: Intrauterine Wachstumsretardierung, Lymphoblastengenese, Monozytenabtrennung, Neugeborenes, niedriges, aber dem Schwangerschaftsalter entsprechendes Geburtsgewicht, Phytohämagglutinin (PHA), Schwangerschaftsalter.

Résumé

Blastogenèse lymphocytaire chez les enfants à poids de naissance normal ou faible et effet de la déplétion monocytaire sur celle-ci

Afin d'elucider l'effet de la vie intrautérine sur la réponse immunologique des nouveaux-nés, nous avons étudié la blastogenèse spontanée et induite par la phytohemagglutinine (PHA) sur les lymphocytes de 23 nouveaux-nés normaux à terme, de 40 enfants à faible poids de naissance (LBW) parmi lesquels 18 accusaient un retard de croissance intrautérin (IUGR) et 22 correspondaient à l'âge gravidique (AFD) ainsi que de 8 adultes. Comme étude complémentaire, nous avons prélevé des échantillons de sang foetal à la naissance (sang ombilical ou capillaire) les 7ème et 30ème jours (sang capillaire) dans des tubes capillaires héparines qui ont été centrifugés et coupés juste au dessous de la portion sèdimentée. Le sédiment et la plasma ont été déposés sur un milieu RPMI 1640 avec ou sans PHA (15 microg/ml).

Après une période de préincubation, il a été ajouté de la ^{3}H-thymidine (Sp. Act. 5 microCi/microM) et une incubation supplémentaire de 24 heures à 37° dans une atmosphère de 5% de CO_2 a été entreprise.

Afin d'elucider l'effet du monocyte sur la blastogenèse lymphocytaire nous avons examiné 30 nouveaux-nés sains et à terme et 5 adultes en bonne santé. Nous avons obtenu la fraction des leucocytes mononucléaires (MNL) à partir du sang ombilical ou du sang veineux (chez les adultes) à l'aide de centrifugation selon le gradient de densité Ficoll-Hypaque, puis cette MNL – fraction a été purifiée par adhésion et chromatographie sur colonne.

La blastogenèse spontanée de lymphocytes était nettement supérieure chez l'ensemble des nouveaux-nés par rapport à clle des adultes (fig. 1). La blastogenège spontanée chez les enfants AFD à la naissance et âges de 7 jours était significativement supérieure à celle chez les nouveaux-nes normaux à terme, mais significativement inférieure chez les nouveaux-nés avec IUGR par rapport aux nouveaux-nés normaux à terme (fig. 1). Le 30ème jour cependant, il n'apparaissait plus de différence significative entre les enfants à terme, les AFD et les IUGR. La blastogenèse spontanée était inversement proportionnelle à l'âge gravidique ($r = 0.76$) chez les AFD à la naissance (fig. 2). La blastogenèse induite par PHA ne montrait pas de différence significative entre les enfants à terme, les AFD et les IUGR quelque soit l'âge, mais elle était significativement supérieure par rapport à celle des adultes (fig. 1). La blastogenèse induite par PHA augmantait avec l'âge postnatal chez tous les enfants.

La blastogenèse spontanée de lymphocytes dans le sang ombilical ou adulte n'était pas significativement réduite par la péplétion monocytaire. Au contraire, la blastogenèse induite par la PHA dans la fraction sans monocytees du sang adulte était significativement réduite, alors que celle du sang ombilical ne l'était pas autant (fig. 3).

La blastogenèse spontanée augmentée chez les nouveaux-nés AFD et celle réduite chez les nouveaux-nés à IUGR est probablement liée au nombre et la fonction des cellules formant des colonies et peut retourner à la normale au 30ème jour postnatal. L'effet de la blastogenèse lymphocytaire sur la monocytaire est controversé et reste à étudier.

Mots-clés: Age gestationnel, blastogenèse lymphocytaire, déplétion monocytaire, enfants correspondant au terme (AFD), nouveau-né, Phytohemagglutinine (PHA), retard de croissance intrautérin (IUGR).

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