Analysis of Decreased Autologous Mixed Lymphocyte Reaction of Cord Blood Lymphocytes: With Special Reference to Production of and Response to Interleukin-2 (IL-2)*

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SUMMARY **In cord blood lymphocytes, the autologous mixed lymphocyte reaction (AMLR) was decreased. In AMLR, cord blood T cells did not produce IL-2 nor respond to IL-2. This failure to respond seemed to be caused by the failure of expression of Tac antigens (IL-2 receptors) and thus of absorption of IL-2. The deletion of OKT_8^+ cells could not improve these IL-2 related abnormalities, suggesting that the activation of OKT_8^+ suppressor T cells was not the cause. The cord blood T cells seemed to have the potential to produce or respond to IL-2, just as adult T cells after stimulation with PHA. Phenotypic characterisation of the stimulator cells, non-T cells, displayed no remarkable difference between cord blood and adult T cells. Therefore, decreased AMLR was thought to be related to the defects of either the stimulatory function of non-T cells or the recognising mechanism of responder T cells.**

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Autologous mixed lymphocyte reaction (AMLR)[1] reflects a mechanism by which T lymphocytes regulate lymphocyte function[2] and exhibit two classical attributes of immune phenomena: memory and specificity.[3] The T cells activated by AMLR have been shown to have helper,[4,5] suppressor[6-8] and cytotoxic[9-11] activities. Interleukin-2 (IL-2)[12] has recently been found to be closely related to the induction of these effector cells, and the relationship between IL-2 and the T-cell subsets defined by monoclonal antibodies, such as OKT_4 and OKT_8[13-17] in AMLR, has been disclosed.

Huber *et al*[18] suggested the possibility that AMLR may represent the response to xeroprotein determinants adsorbed to antigen-presenting cells during rosette formation with sheep erythrocytes (SRBC). Considering this, we used T cells and non-T cells separated by a polystyrene resin column[19] instead of by the SRBC rosetting method; we analysed the proliferative response of T cells, production of IL-2 and response to IL-2 in AMLR of cord blood lymphocytes in order to disclose the immune status of human neonates.

MATERIALS AND METHODS

Isolation of mononuclear cells

Cord blood was collected immediately after uncomplicated birth. Adult venous blood was obtained from healthy adult volunteers. Mononuclear cells were isolated from heparinised whole blood by Ficoll-Hypaque density gradient centrifugation.[20]

Separation of T cells and non-T cells

Mononuclear cells ($1x10^7$) suspended in 0.5 ml foetal calf serum (FCS, Gibco) were incubated for one hour on a 2-ml polystyrene resin particle column (Asahi Chem. Inc., Japan)[19] at 37°C. A T-cell-rich fraction was eluted by adding to the column 5 ml of warm (37°C) phosphate buffered saline (PBS) containing 10% FCS. The remaining cells were recovered by pipetting the resin particles and washing out the cells; they were used as non-T cells.[19]

Non-T cells (as the stimulators of AMLR) were treated with mitomycin-C (MMC, Sigma Chemical Co.) 100 μg/ml for 30 minutes at 37°C and washed three times with PBS.

Isolation of T-cell subsets

T cells ($5x10^6$ to $1x10^7$) were suspended in 0.5 ml of medium RPMI 1640 supplemented with 5 μl of OKT_4 or OKT_8 monoclonal antibodies (Ortho Pharmaceutical Corporation) and incubated for one hour at room temperature. After incubation, 0.1 ml of rabbit complement was added to the cell suspension and the cells were incubated for another hour at 37°C with occasional shaking. At the end of the incubation period, they

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were washed three times with RPMI 1640 with 5% FCS. OKT_8-treated T cells contained 80-90% OKT_4^+ cells and less than 3% OKT_8^+ cells, while the OKT_4-treated population contained 80-90% OKT_8^+ cells and less than 3% OKT_4^+ cells. Thus, we named the former as OKT_4^+ cells and the latter as OKT_8^+ cells.

Proliferative response of T cells stimulated with autologous non-T cells

T cells ($5x10^4$) and MMC-treated autologous non-T cells ($5x10^4$) were suspended in 200 μl of RPMI 1640 supplemented with 10% FCS in each well of 96-well flat-bottom microplates (Nunc) and incubated at 37°C in a 5% CO_2 atmosphere for six days. The cultures were pulsed with 1 μCi of tritiated thymidine (^{3}H-TdR) for the final 20 hours and harvested by filtration through glass filters, followed by determination of ^{3}H-TdR uptake using a liquid scintillation counter. The data were expressed as the difference of the counts per minute of cultures containing T cells and non-T cells from those of cultures containing T cells alone (Δcpm).

IL-2 production

T cells or T-cell subsets ($2x10^6$) and autologous non-T cells ($2x10^6$) treated with MMC were suspended in 1 ml of RPMI 1640 supplemented with 5% FCS in 5 ml plastic culture tubes (Falcon 2054), and cultured at 37°C for two days in a 5% CO_2 atmosphere. At the end of the culture period, the cells were removed by centrifugation and the supernatants were collected and stored at -20°C until calculation of IL-2 activity.[21]

As positive controls, $2x10^6$ mononuclear cells were suspended in 1 ml of RPMI 1640 supplemented with 5% FCS and 0.25% PHA-P (Difco) in 5 ml plastic culture tubes (Falcon 2054) and IL-2 samples were obtained by the same procedure as described above.

Preparation of IL-2 dependent cultured T cells

Peripheral blood mononuclear cells ($2.5x10^7$) from healthy adult volunteers were suspended in 50ml RPMI 1640 supplemented with 10% FCS and 0.13% PHA-P in 75 cm^2-style flasks (Falcon 3024 F) and incubated at 37°C for five days in a 5% CO_2 atmosphere. The culture medium was replenished with RPMI 1640 containing 10% FCS and an optimal concentration (5%) of IL-2.[22] Subcultures were prepared every four days. The propagated cells were maintained for over two weeks and used as IL-2-dependent T cells.[22,23]

Assay for IL-2 activity

IL-2-dependent cultured T cells (CTC) were maintained over two weeks in IL-2-dependent proliferative cultures, then washed free of growth medium and resuspended in RPMI 1640 medium with 5% FCS. Added to 100 μl of the culture supernatant to be tested were $1x10^5$ cells (100 μl). The supernatant had been diluted serially (50%, 25%, 12.5%). Assays were carried out in 96-well flat-bottom microplates (Nunc). The cells were incubated at 37°C in a 5% CO_2 atmosphere for three days. The viable cells of each well counted with the trypan blue dye exclusion method were compared with those of wells free of culture supernatant, and the ratio was expressed as a stimulation index (S.I.).

Besides the trypan blue dye exclusion method, these cultures were pulsed with 0.5 μCi of ^{3}H-TdR after 36 hours and harvested by filtration through glass-fibre filters, followed by determination of ^{3}H-TdR uptake after an additional 24 24 hours' incubation.[21-23] The counts of the cells cultured with the supernatant and those without were compared.

Estimation of responsiveness to IL-2

T cells or T-cell-subsets stimulated with autologous non-T cells were cultured for five days at 37°C in a 5% CO_2 atmosphere. Exogenous IL-2 obtained from culture supernatant of AMLR of adult lymphocytes (100 μl) serially diluted (50%, 25%, 12.5%) was added to $1x10^5$ viable cells suspended in 100 μl of the fresh medium. Assays were carried out in 96-well flat-bottom microplates (Nunc). The cells were incubated at 37°C in a 5% CO_2 atmosphere for three days. At the end of the culture period, the number of viable cells counted with the trypan blue dye exclusion method was compared with those of the cultures with IL-2 and those free of IL-2. The ratios were expressed as S.I.

At the same time, these cultures were pulsed with 0.5 μCi ^{3}H-TdR after 36 hours of co-cultures with exogenous IL-2 and then assayed for ^{3}H-TdR uptake after an additional 24 hours' incubation.

As positive controls, the T cells stimulated with 0.13% PHA-P and cultured for five days were also put through the same assay system described above and their response to IL-2 was also measured.

Absorption of IL-2

T cells ($2x10^6$) stimulated with autologous non-T cells and cultured for six days were washed three times with PBS and resuspended in medium containing IL-2 (culture supernatant of AMLR of adult lymphocytes) and incubated at 37°C for three hours. After incubation, the cells were removed by centrifugation, and IL-2 activity remaining in the supernatant was measured by adding the supernatant to CTC. The IL-2 activity was compared with that of the control supernatant without absorption treatment.

The absorption ability of the T cells stimulated with PHA and cultured for six days was also measured for comparison.

Analysis of Tac (IL-2 receptor) expression

T cells ($5x10^5$) stimulated with

autologous non-T cells or PHA and cultured for five days were washed twice with PBS and pelletted. The pellet was supplied with 25 μl of anti-Tac monoclonal antibody (a gift from Dr. Takashi Uchiyama, Kyoto University), diluted to 1: 1,000 with PBS and incubated at room temperature for one hour. Then they were washed with PBS and incubated with fluorescence-labelled rabbit anti-mouse IgG (Cappel) at room temperature for one hour and washed again.[24-26] These cells were analysed on a fluorescence-activated cell sorter(FACS, Becton Dickinson).

Characterisation of surface phenotypes of non-T cells

In order to characterise non-T cells separated by the polystyrene resin column, we examined the percentage of peroxidase positive, $OKM1^+$, $OKM5^+$, surface membrane immunoglobulin $(SmIg)^+$ and Ia^+ cells. Non-T cells ($5x10^5$) separated by the polystyrene resin column were washed twice with PBS and pelleted. The cell pellet was supplied with 2.5 μl of monoclonal antibodies (OKM1, OKM5 and OKIa1) and incubated with 25 μl of PBS with 0.01% sodium-azide at 4°C for 30 minutes. Then it was washed with PBS containing 0.01% sodium-azide and incubated with 50 μl of fluorescence-labelled rabbit anti-mouse IgG (Cappel) at a dilution of 1:10 at 4°C for 30 minutes. After washing twice with PBS containing sodium-azide, the cells were resuspended in PBS with 50% glycerol. $SmIg^+$ cells were assessed by direct immunofluorescence with a fluorescein-conjugated F $(Ab')_2$ rabbit anti-human Ig. The percentage of positive cells was determined under a fluorescence microscope.

RESULTS

Proliferative response of cord blood T cells stimulated with non-T cells

As shown in Table 1, AMLR was significantly decreased in cord blood lymphocytes as compared with adult cells ($P<0.01$).

Production of IL-2 by cord blood lymphocytes in AMLR

The culture supernatants of adult T cells stimulated with autologous non-T cells gave a proliferation of CTC in proportion to their concentration, whereas those of cord blood T cells usually did not, suggesting the decreased production of IL-2 ($P<0.01$) (Fig. 1-a). Further analysis of IL-2 production showed that adult OKT_4^+ cells produced IL-2 but not cord blood OKT_4^+ cells ($P< 0.01$) (Fig. 1-b). Neither adult nor cord blood OKT_8^+ cells produced IL-2 (Fig. 1-c). The activation of OKT_8^+ suppressor T cells was not considered to be the cause of the impaired production of IL-2 by cord blood T cells because production was not recovered by the deletion of OKT_8^+ cells.

The cord blood T cells stimulated with PHA produced IL-2 to the same extent as adult T cells (data not shown).

These results were compatible with those obtained with regard to ^{3}H-TdR uptake (data not shown).

Table 1 Thymidine incorporation by adult T cells or cord blood T cells stimulated with autologous non-T cells.

	△cpm
Adult (n=6)	1383.3 ± 409.4
Cord blood (n=6)	420.0 ± 385.8

T cells ($5x10^4$) were incubated with MMC-treated autologous non-T cells ($5x10^4$) in 0.2 ml RPMI 1640 with 10% FCS for six days. Tritiated tymidine incorporation was measured during the last 20 hours of incubation. The △counts per minute are the mean ± S.D. of six experiments.

Response to IL-2 by cord blood lymphocytes

Adult T cells stimulated with autologous non-T cells proliferated in proportion to the concentration of exogenous IL-2 added, while cord blood T cells did not, indicating the failure of response to IL-2 in cord blood lymphocytes ($P<0.01$) (Fig. 2-a). Assessment of response to IL-2 by T-cell subsets revealed that neither OKT_4^+ cells nor OKT_8^+ cells responded to IL-2 in cord blood lymphocytes ($P<0.01$) (Figs. 2-b,c).

Both adult T cells and cord blood T cells stimulated with PHA were reactive to IL-2 to the same extent (data not shown).

These results were compatible with those involving ^{3}H-TdR incorporation (data not shown).

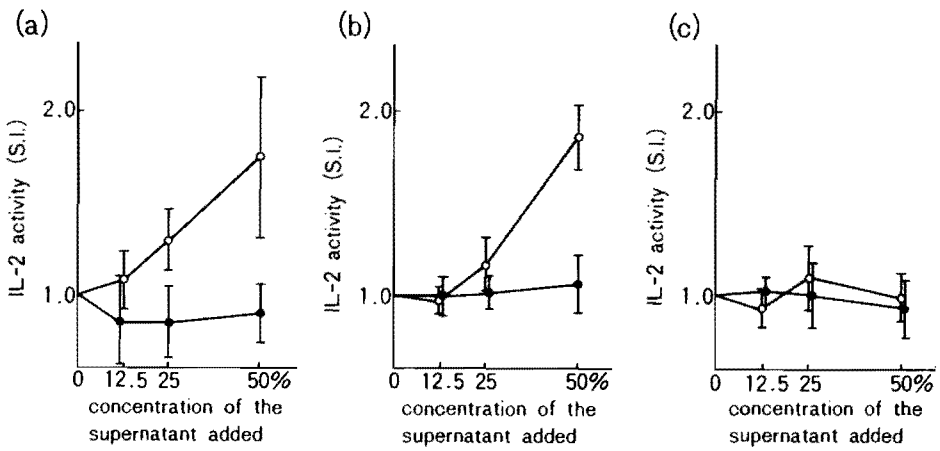

Fig. 1 IL-2 activity of culture supernatant from T cells (a), OKT_4^+ cells (b) and OKT_8^+ cells (c) stimulated with autologous non-T cells.
○——○ : IL-2 activity of culture supernatant from adult T cells.
●——● : IL-2 activity of culture supernatant from cord blood T cells.
The results represent the mean ± S.D. of at least five experiments.

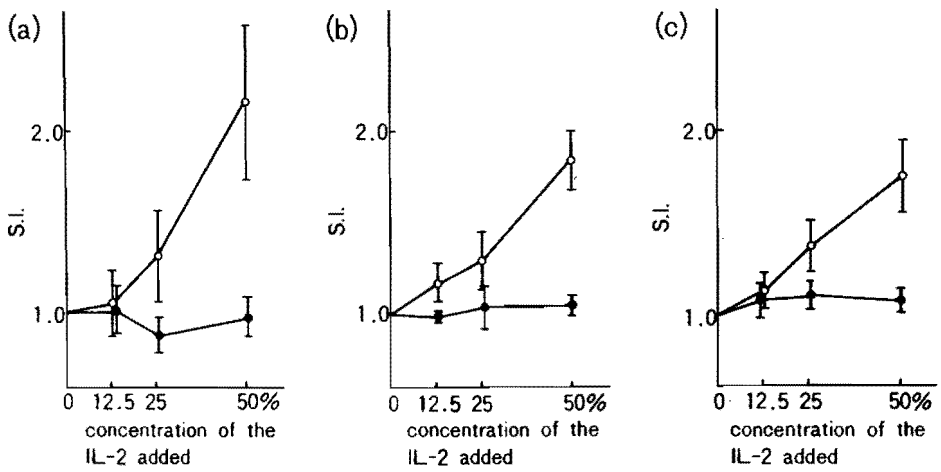

Fig. 2 Response to IL-2 by T cells (a), OKT_4^+ cells (b) and OKT_8^+ cells (c) stimulated with autologous non-T cells.
○——○ : response to IL-2 by adult T cells.
●——● : response to IL-2 by cord blood T cells.
The results represent the mean ± S.D. of at least five experiments.

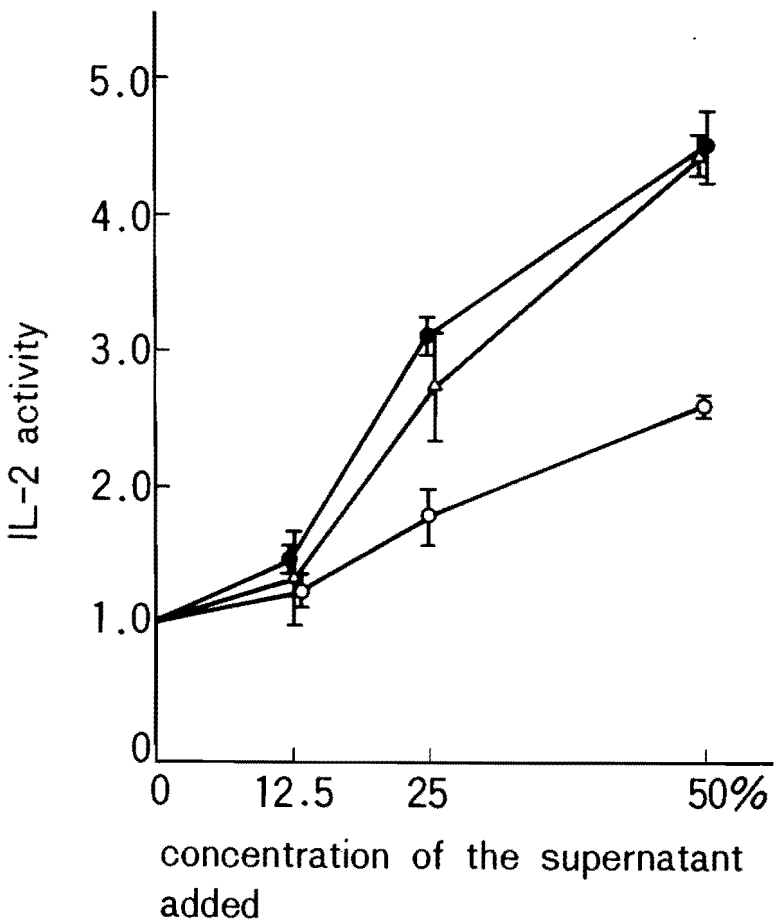

Fig. 3 IL-2 activity remaining in the supernatant absorbed by adult T cells or cord blood T cells activated by AMLR.
●——● : IL-2 activity of control supernatant.
○——○ : IL-2 activity of supernatant absorbed by adult T cells.
△——△ : IL-2 activity of supernatant absorbed by cord blood T cells.
The results indicate the mean ± S.D. of one representative experiment out of five.

Absorption of IL-2 by cord blood lymphocytes

In order to disclose whether the function of IL-2 receptors is related to the decreased response to IL-2 by cord blood lymphocytes, we measured the absorption of IL-2 by cord blood lymphocytes.

IL-2 activity remaining in the supernatant absorbed by adult T cells activated in AMLR was significantly lower than that of the control supernatant ($P<0.01$), whereas that absorbed by cord blood T cells was almost the same as that of the control supernatant, which suggested that IL-2 was not absorbed by cord blood T cells in AMLR (Fig. 3).

In contrast, the cord blood T cells stimulated with PHA absorbed IL-2 as well as adult T cells (data not shown).

Tac expression of cultured cord blood T cells

It has been reported that the anti-Tac monoclonal antibody recognises the antigen which is closely related to the receptor for human T-cell growth factor (IL-2)[24,27] and Tac antigen is expressed on activated T cells.[25,26] Therefore, we intended to confirm whether the decreased response to and adsorption of IL-2 by cord blood T cells was the result of the decreased expression of IL-2 receptors.

In AMLR, Tac^+ cells appeared in certain numbers among adult T cells (Fig. 4-a), while they did not among cord blood T cells (Fig. 4-b). PHA-stimulated adult T cells (Fig. 4-c) and PHA-stimulated cord blood T cells (Fig. 4-d) displayed the appearance of the same number of Tac^+ cells with the same fluorescence intensity.

Phenotypic characterisation of cord blood non-T cells

In order to know the difference of cellular composition of non-T cells as the stimulator of AMLR betweeen cord blood and adult T cells, we examined the phenotypic

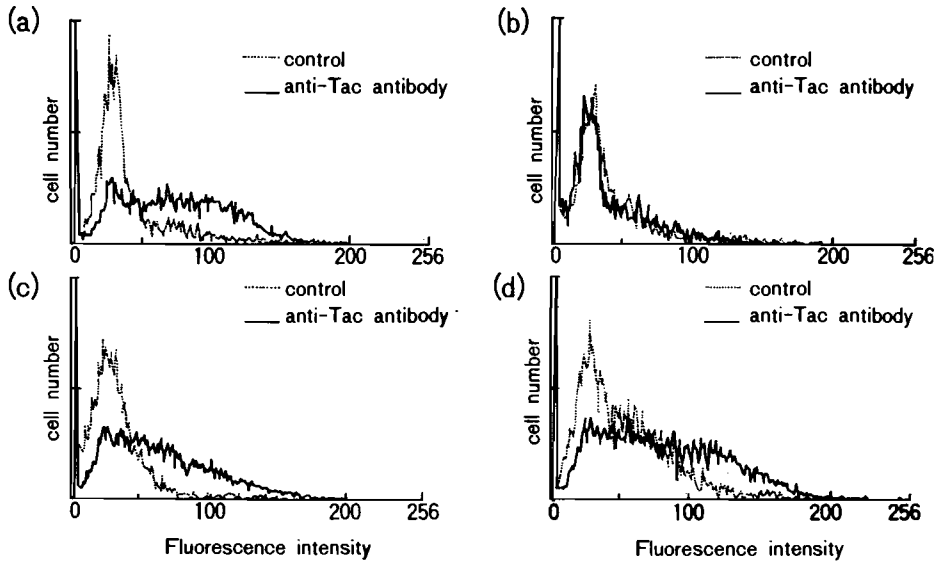

Fig. 4 Fluorescence profiles of Tac expression of five-day-cultured adult and cord blood T cells stimulated with autologous non-T cells or PHA.
(a) adult T cells stimulated with autologous non-T cells.
(b) cord blood T cells stimulated with autologous non-T cells.
(c) adult T cells stimulated with PHA.
(d) cord blood T cells stimulated with PHA.

Table 2 Comparison of phenotypes of adult and cord blood non-T cells separated by a polystyrene resin column.

T cells	Peroxidase staining	OKM1	OKM5	SmIg	Ia
Adult	31.5 ± 6.6	44.6 ± 4.5	34.4 ± 6.9	33.1 ± 4.6	43.8 ± 6.0
Cord blood	39.7 ± 6.6	50.6 ± 4.5	36.7 ± 5.6	28.6 ± 2.4	46.2 ± 10.1

The results represent the mean ± S.D. percentage of positive cells in five experiments.

characterisation of cord blood non-T cells. As depicted in Table 2, the proportion of peroxidase$^+$ cells ($P<0.05$) and OKM1$^+$ cells ($P<0.01$) was somehow higher in cord blood non-T cells whereas we found no difference in the proportion of OKM5$^+$ cells, SmIg$^+$ cells and Ia$^+$ cells with regard to adult and cord blood non-T cells.

DISCUSSION

AMLR is the proliferative response of T cells against autologous non-T cells. It has been reported to be decreased in various diseases including malignancy and autoimmunity.[28-32]

This study was undertaken to investigate the immunological background of the heightened susceptibility of the newborn to severe infections. Our data indicated that AMLR was impaired in cord blood lymphocytes, and we analysed the reason for this phenomenon.

T-cell-rich fraction separated by a polystyrene resin column contained SRBC rosette-forming cells 93.1 per cent ± 3.1 (SD), SmIg$^+$ cells fewer than 1 per cent, peroxidase$^+$ cells fewer than 1 per cent, OKT_3^+ cells 90.0 per cent ± 2.6 (SD), OKT_4^+ cells 61.8 per cent ± 3.5 (SD) and OKT_8 cells 31.3 per cent ± 4.9 (SD); there was no significant difference between the T-cell subsets separated by this method and those by the SRBC rosetting method. The recovery of T cells was 77.4 per cent ± 10.4 (SD). Additionally, PHA-stimulated blastogenesis, helper and suppressor activities directed at pokeweed mitogen-stimulated immunoglobulin secreting cells and NK activity of T cells separated by this method exhibited almost the same response as those of T cells separated by the SRBC rosetting method. On the other hand, the T-cell-rich fraction separated by the nylon wool column method contained 85.6 per cent ± 2.6 (SD) SRBC rosette-forming cells, 2.8 per cent ± 1.1 (SD) SmIg$^+$ cells and 3.4 per cent ± 2.9 (SD) peroxidase$^+$ cells. The recovery of T cells was 71.6 per cent ± 27.2 (SD). Therefore, we concluded that separation by this new method was superior to the nylon wool column method with regard to reproducibility of recovery and purity of T cells and contamination of SmIg$^+$ cells and peroxydase$^+$ cells. Furthermore, the culture supernatant of AMLR by T cells and non-T cells separated by the SRBC rosetting method induced proliferation even of resting T cells bearing no IL-2 receptors, which suggested the presence of stimulatory factors other than IL-2. In contrast, the culture supernatant of AMLR by T cells and non-T cells separated by the polystyrene resin column did not stimulate the resting T cells, suggesting that it contained no mitogenic factors other than IL-2 and it was suitable for exogenous IL-2 on the assay for responsiveness to IL-2.

It is known that HLA-DR antigens in AMLR render resting T cells sensitive to IL-2 and enable OKT_4^+ lymphocytes to respond to IL-1 and, subsequently, to produce IL-2.[13-17] Therefore, to explain the decreased AMLR of cord blood lymphocytes, the analysis was undertaken to determine where the abnormalities resided: in defective production of IL-2 or failure of response to IL-2.

The production of IL-2 from cord blood lymphocytes in AMLR

was impaired and it was not recovered by the deletion treatment of OKT_8^+ cells which might work as suppressor cells.

The cord blood T cells in AMLR responded poorly to IL-2. This seemed to be due to the failure of expression of IL-2 receptors and the inability to absorb IL-2. Neither OKT_4^+ cells nor OKT_8^+ cells responded to exogenous IL-2, suggesting that neither types of cells were activated in AMLR.

Previous reports[33,34] and our data suggested that PHA-stimulated cord blood T cells were good producers of IL-2. PHA-stimulated cord blood T cells also responded to IL-2 and absorbed IL-2 well in our experiments, which is compatible with the finding of a previous report that the proportion of Tac^+ T cells in a PHA-stimulated culture was nearly the same for cord blood and adult T cells.[35]

Therefore, cord blood T cells have the potential to produce and respond to IL-2 and it was suggested that the reason for the abnormalities could be traced to either the non-T cells as stimulator for AMLR or the recognition mechanisms of responder T cells.

No definite remarks have been obtained concerning the identification of stimulator cell populations. B cells,[1,36] null cells[37] and macrophages[37] have been found capable of triggering AMLR. Recent analysis utilising monoclonal antibodies indicated that $OKM1^-$, $OKM5^+$ cells[38] or Mac 120^+ macrophages[39] comprised the predominant subset of stimulator populations. It was reported that HLA-DR antigens activate both IL-2-producer T cells and IL-2-responder T cells in AMLR.[13-17]

Based on these remarks we investigated the phenotypes of cord blood non-T cells such as cytoplasmic peroxidase activity, OKM1, OKM5 and Ia antigens. The proportions of $OKM5^+$, $SmIg^+$ and Ia^+ cells in cord blood non-T cells and adult non-T cells were not significantly different, although the proportions of $peroxidase^+$ cells and $OKM1^+$ cells in cord blood non-T cells were slightly higher than in adult non-T cells. Monoclonal antibody OKM1 recognises monocytes, null cells and granulocytes, whereas OKM5 reacts only with monocytes. Therefore, OKM5 is more specific for the cells of monocyte lineage than OKM1.[38,40,41] Consequently, it was suggested that monocytes or null cells may be slightly increased in cord blood non-T cells. A reduction by adherence of $peroxidase^+$ cells or $OKM1^+$ cells to the proportion identical to that of adult non-T cells did not improve AMLR in cord blood lymphocytes. Therefore, it was unlikely that the difference in the constitution of the cells caused the defective AMLR of cord blood lymphocytes.

AMLR is a process in which T cells recognise self and display various functions. The decreased AMLR of cord blood lymphocytes, therefore, may reflect immunological status peculiar to the perinatal period.

Although Huber *et al*[18] suggested that AMLR may represent the response to xeroprotein determinants adsorbed to antigen-presenting cells during rosette formation with sheep erythrocytes, we excluded the possibility that SRBC might be picked up during the separation procedure by adopting an alternate procedure for the isolation of T cells. Nevertheless, FCS used in the culture remains an unresolved issue in this study.

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REFERENCES

1. Operz G, Kiuchi M, Takasugi M, Terasaki PI. Autologous stimulation of human lymphocyte subpopulations. J Exp Med 1975; 142: 1323-33.
2. Kuntz MM, Innes JB, Weksler ME. Lymphocyte transformation induced by autologous cells. IV. Human T-lymphocyte proliferation induced by autologous or allogeneic non-T lymphocytes. J Exp Med 1976; 143: 1042-54.
3. Weksler ME, Kozac R. Lymphocyte transformation induced by autologous cells. V. Generation of immunologic memory and specificity during the autologous mixed lymphocyte reaction. J Exp Med 1977; 146: 1833-8.
4. Hausman PB, Stobo JD. Specificity and function of a human autologous reactive T cell. J Exp Med 1979; 149: 1543-8.
5. Chiorazzi N, Fu SM, Kunkel HG. Induction of polyclonal antibody synthesis by human allogeneic and autologous helper factors. J Exp Med 1979; 149: 1543-8.
6. Smith TB, Knowlton RP. Activation of suppressor T cells in human autologous mixed lymphocyte culture. J Immunol 1979; 123: 419-22.
7. Sakane T, Green I. Specificity and suppressor function of human T cells responsive to autologous non-T cells. J Immunol 1979; 123: 584-9.
8. Innes JB, Kuntz MM, Kim YT, Weksler ME. Induction of suppressor activity in the autologous mixed lymphocyte reaction and in cultures with Concanavalin A. J Clin Invest 1979; 64: 1608-13.
9. Miller RA, Kaplan HS. Generation of cytotoxic lymphocytes in the autologous mixed lymphocyte culture. J Immunol 1978; 121: 2165-7.
10. Weksler ME, Moody CE, Ostry PF, Casazza BA. Lymphocyte transformation induced by autologous cells. J Exp Med 1980; 152: 284s-91s.
11. Tomonari K. Cytotoxic T cells generated in the autologous mixed lymphocyte reaction. J Immunol 1980; 124: 1111-21.
12. Aarden LA, Brunner TK, Cerottini JC, et al. Revised nomenclature for antigen-nonspecific T cell proliferation and helper factors. J Immunol 1979; 123: 2928-9.
13. Palacios R, Moeller G. Cyclosporin A blocks receptors for HLA-DR antigens on T cells. Nature 1981; 290: 792-4.
14. Palacios R, Moeller G. HLA-DR antigens render resting T cells sensitive to Interleukin-2 and induce production of the growth factor in the autologous mixed lymphocyte reaction. Cell Immunol 1981; 63: 143-53.
15. Palacios R. HLA-DR antigens render Interleukin-2-producer T lymphocytes sensitive to Interleukin-1. Scand J Immunol 1981; 14: 321-6.
16. Palacios R. Mechanism of T cell activation: Role and functional relationship of HLA-DR antigen and Interleukins. Immunol Rev 1982; 63: 73-110.
17. Palacios R, Guy K, Heyningen VV. Monoclonal antibodies against HLA-DR antigens acting on stimulator cells prevent OKT_8^+ T lymphocytes from acquiring

sensitivity to Interleukin-2 and expressing supressor function. Eur J Immunol 1983; 13:64-72.
18. Huber C, Merkenschlager M, Gattringer C, Royston I, Fink U, Braunsteiner H. Human autologous mixed lymphocyte reactivity is primarily specific for xeroprotein determinants adsorbed to antigen-presenting cells during rosette formation with sheep erythrocytes. J Exp Med 1982; 155:1222-7.
19. Noma T, Yata J, Shishiba Y, Inatsuki B. *in vitro* detection of anti-thyroglobulin antibody forming cells from the lymphocytes of chronic thyroiditis patients and analysis of their regulation. Clin Exp Immunol 1982; 49:565-71.
20. Boyum A. Isolation of mononuclear cells and granulocytes from human blood. Scand J Clin Lab Invest 1968; 21:77-90.
21. Alvarez JM, Silva A, Landazuri MO. Human T cell growth factor. I. Optimal conditions for its production. J Immunol 1979; 123:977-83.
22. Pauly JL, Russel CW, Planinsek JA, Minowada J. Studies of cultured human T lymphocytes. I. Production of the T cell growth-promoting lymphokine Interleukin-2. J Immunol Methods 1982; 50:173-86.
23. Ruscetti FW, Morgan DA, Gallo RC. Functional and morphologic characterization of human T cell continuously grown *in vitro*. J Immunol 1977; 119:131-8.
24. Leonald WJ, Depper JM, Uchiyama T, Smith KA, Waldmann TA, Greene WC. A monoclonal antibody that appears to recognize the receptor for human T-cell growth factor; partial characterization of the receptor. Nature 1982; 300:267-9.
25. Uchiyama T, Broder S, Waidmann TA. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. I. Production of anti-Tac monoclonal antibody and distribution of Tac (+) cells. J Immunol 1981; 126:1393-7.
26. Uchiyama T, Nelson DL, Fleisher TA, Waldmann TA. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. II. Expression of Tac antigen on activated cytotoxic killer T cells, suppressor cells, and one of two types of helper T cells. J Immunol 1981; 126:1398-403.
27. Miyawaki T, Yachie A, Uwadana N, Ohzeki S, Nagaoki T, Taniguchi N. Functional significance of Tac antigen expressed on activated human T lymphocytes: Tac antigen interacts with T cell growth factor in cellular proliferation. J Immunol 1982; 129:2474-8.
28. Smith JB, Knowlton RP, Koons LS. Immunological studies in chronic lymphocytic leukemia: defective stimulation of T cell proliferation in autologous mixed lymphocyte culture. J Natl Cancer Inst 1977; 58:579-84.
29. Engleman EC, Benike CJ, Hoppe RT, Kaplan HS, Berberich FR. Autologous mixed lymphocyte reaction in patients with Hodgkin's disease. Evidence for T cell defect. J Clin Invest 1980; 66:149-58.
30. Sakane T, Steinberg AD, Green I. Failure of autologous mixed lymphocyte reactions between T and non-T cells in patients with systemic lupus erythematosus. Proc Natl Acad Sci (USA) 1978; 75:3464-8.
31. Kuntz MM, Innes JB, Weksler ME. The cellular basis of the impaired autologous mixed lymphocyte reaction in patients with systemic lupus erythemetosus. J Clin Invest 1979; 63:151-3.
32. Miyasaka N, Sauvezie B, Pierce DA, Daniels TE, Talal N. Decreased autologous mixed lymphocyte reaction in Sjogren Syndrome. J Clin Invest 1980; 66:928-33.
33. Hayward AR, Kurnick J. Newborn T cell suppression: early appearance, maintenance in culture, and lack of growth factor suppression. J Immunol 1981; 126: 50-3.
34. Paganelli R. Spontaneous suppressor cells for mitogen responsiveness of cord blood lymphocytes. Clin Immunol Immunopathol 1981; 21:295-300.
35. Yokoi T, Miyawaki T, Yachie A, Ohzeki S, Taniguchi N. Discrepancy in expression ability of Tac antigen and Ia determinants defined by monoclonal antibodies on activated or cultured cord blood T lymphocytes. J Immunol 1982; 129:1441-5.
36. Smith JB. Stimulation of autologous and allogeneic human T cells by B cells occurs through separate B-cell antigen systems. Cell Immunol 1978; 36:203-9.
37. Beale MG, McDermott RP, Stacey MC, et al. Stimulating cell types in the autologous mixed leukocyte reaction in man. J Immunol 1980; 124:227-32.
38. Shen HH, Talle MA, Goldstein G, Chess L. Functional subsets of human monocytes defined by monoclonal antibodies: a distinct subset of monocytes contains the cells capable of inducing the autologous mixed lymphocyte culture. J Immunol 1983; 130:698-705.
39. Raff HV, Picker LJ, Stobo JD. Macrophage heterogeneity in man: a subpopulation of HLA-DR-bearing macrophages required for antigen-induced T cell activation also contains stimulators for autologous-reactive T cells. J Exp Med 1980; 152:581-93.
40. Breard J, Reinhertz EL, Kung PC, Goldstein G, Shlossman SF. A monoclonal antibody reactive with human peripheral blood monocytes. J Immunol 1980; 124: 1943-8.
41. Talle MA, Rao PE, Westberg E, et al. Patterns of antigenic expression on human monocytes as defined by monoclonal antibodies. Cell Immunol 1983; 78:83-99.