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The comparison of cytokine production in cord and adult blood cell cultures

Porównanie wytwarzania cytokin w hodowli komórek krwi pępowinowej i krwi osób dorosłych

SUMMARY

Production of some cytokines by whole cord blood cell cultures following induction *in vitro* by different inducers was assessed and compared with that found in adult blood cell cultures. The cord blood cell cultures produced significantly less IL-1β and IL-10 after induction with lipopolysaccharide (LPS) and considerably less IL-4, IFN-γ and IL-2 after phytohemagglutinin (PHA) administration (but only when IL-2 level was examined 72 hrs after induction with 50 μg/ml of PHA). However, the level of IL-2 following stimulation with PHA at a dose of 10 μg/ml and IL-1α and IL-8 after LPS induction was comparable with the cytokine level generated by cells of adult persons.

In conclusion, the study provides evidence that neonatal blood cells differ from adult blood cells in respect to the capacity of cytokines production by monocytic cells (IL-1β and IL-10) and those of T-cell-derived (IL-4, IFN-γ, IL-2). However, these differences may, to some extent, be caused by various sensitivity of the blood cells to the mitogens or bacterial products used in experiments.

STRESZCZENIE

Porównano ilości cytokin wytwarzanych w hodowli komórek krwi pępowinowej oraz komórek krwi osób dorosłych po indukcji *in vitro* różnymi induktorami. Hodowle komórek krwi pępowinowej wytwarzały znacząco mniej IL-1β i IL-10 po indukcji lipopolisacharydem (LPS), a także

wyraźnie mniej IL-4, IFN-γ oraz IL-2 indukowanej fitohemaglutyniną (PHA) (lecz tylko wtedy gdy poziom IL-2 badano po 72 godzinach od momentu indukcji 50 μg/ml PHA). Natomiast poziom IL-2 po stymulacji PHA w dawce 10 μg/ml oraz IL-1α i IL-8 indukowanych LPS, był porównywalny ze stężeniem wytwarzanym przez hodowle komórek krwi osób dorosłych. Wyniki badań świadczą o tym, że komórki krwi noworodków różnią się od komórek krwi dorosłych pod względem zdolności do wytwarzania cytokin przez komórki monocytarne (IL-1β i IL-10) oraz komórki T (IL-4, IFN-γ, IL-2). Różnice te mogą być, przynajmniej częściowo, uwarunkowane inną wrażliwością komórek krwi noworodków na mitogeny i substancje bakteryjne.

Key words: cord blood cells, IL-1α, IL-1β, IL-2, IL-4, IL-8, IL-10, IFN-γ.

ABBREVIATIONS

ELISA — enzyme linked immunoabsorbent assay, IL-1α— interleukin 1α, IL-1β — interleukin 1β, IL-2 — interleukin 2, IL-4 — interleukin 4, IL-8 — interleukin 8, IL-10 — interleukin 10, IFN-γ — interferon gamma, LPS — lipopolysaccharide, MEM — Minimal Essential Medium, NK — natural killer, PHA — phytohemagglutinin, TNF-α — tumor necrosis factor.

INTRODUCTION

Several developmental deficiencies of the neonatal-host defense system were described, including a defect in NK cells activity and monocyte/macrophage system (6, 7). However, literature data concerning the cytokine production ability of cord blood cells is inconsistent. A variety of different experimental techniques, including enzyme-linked immunoabsorbent assay (ELISA), flow cytometry or bioassays have been used to measure cytokine production by cord blood cells leading to inconclusive results even in clinically healthy neonates. Moreover, several inducers of cytokines were used: bacteria (Listeria monocytogenes), viruses (Newcastle disease, Sendai, respiratory syncytial), mitogens (phytohemagglutinin, lipopolysaccharide, enterotoxins) and phorbol esters with ionomycin. In addition cytokine production was examined in mononuclear blood cells or in separated populations of lymphocytes and monocytes. Generally, in most experiments cord blood cells produced less IL-4, IFN-γ, IL-10 and TNF-α than adult peripheral blood cells, and amounts of IL-2 and IL-6 comparable to adults (1–3, 5, 8–11, 13, 14, 16, 18, 19, 21–24).

As the whole blood cultures were shown to be a feasible method of assessing the immune status of organism (22, 23), we chose this method to measure cytokine production by cord blood cell after induction with typical and well known good inducers of cytokines, phytohemagglutinin (PHA) as inducer of IL-2, IL-4 and IFN-γ (in T lymphocytes) and lipopolysaccharide (LPS) as inducer of IL-1α, IL-1β, IL-8 and IL-10 (which after LPS induction are mainly produced by monocytes and B cells) (4, 8, 15).

MATERIALS AND METHODS

Samples

Adult blood was obtained from 14 healthy non-pregnant volunteer women, 23–33 years old. Cord blood samples were obtained from 23 healthy, full-term (38–42 weeks) babies after

normal vaginal delivery. Blood was taken from placental end of the cord and collected into tubes with heparin (20 U/ml of Heparinum, Polfa). Criteria of exclusion were: complicated pregnancy, prematurity, infections and congenital abnormalities (following ethical committee approval). All the samples were processed directly after arriving at the laboratory (1–2 h after collection). The whole cord and adult blood was diluted in Eagle's Minimal Essential Medium (MEM) supplemented with 2 mM L-glutamine, 100 U/ml of penicillin and 100 μg/ml of streptomycin to obtain a density of leukocytes of 1×10^6 cells/ml and the autologous serum was added to 10%.

Cell cultures

Cell suspensions were distributed (2 ml/well) into 24-well plastic plates (Falcon, Bedfors MA) and induced to cytokine production with PHA (Sigma, St. Louis MO) 50 μg/ml or 10 μg/ml, LPS from *E. coli* 0111: B4 (Sigma,) 10 μg/ml and incubated at 37°C in 5% CO_2 in air. Supernatants were collected after 24 h of incubation (samples induced with LPS) or after 72 h (after induction with PHA) and stored at –20°C until cytokine assay.

Cytokine assays

Concentrations of IL-1β, IL-2, IL-4, IL-8, IL-10 and IFN-γ were measured in supernatants using specific ELISA assays (Predicta, Genzyme, Cambridge MA). IL-1α amounts were also measured in ELISA (Endogen Inc., Woburn MA) according to the manufacturer's instructions. Minimal detectable levels of cytokines were IL-1α 2 pg/ml, IL-1β 3 pg/ml, IL-2 4 pg/ml, IL-4 6 pg/ml, IL-8 1 pg/ml, IL-10 5 pg/ml, IFN-γ 3 pg/ml. All results were expressed as pg of cytokine per 1 ml of culture medium (1 million of leukocytes).

Statistics

The Mann-Whitney test was used for comparison of cytokine data from cord blood and adult blood cells. The level of significance was chosen as $p<0.05$.

RESULTS

Following stimulation with LPS, production of IL-1α, IL-1β IL-10 and IL-8 was measured in supernatants from cord and adult blood cell cultures (Fig. 1A, B, C and D, respectively). Cord blood cells produced less IL-1α, IL-1β, as well as lower levels of IL-10 than adult blood cells, however, the difference in IL-1α production was not statistically significant. IL-8 level produced by cord blood cell cultures was comparable to that produced by adult blood cells. When PHA 50 μg/ml was used as the inducer and the levels of cytokines were estimated after 72 h of incubation, the levels of IL-4, IFN-γ and IL-2, which are lymphokines produced mainly by lymphocytes T, were significantly lower in cultures of cord blood cells than in blood cells of adults (Fig. 1E, F and G, respectively). However, when a lower dose of PHA (10 μg/ml) was used and the IL-2 production in cord

and adult blood cells was measured, cord blood cells generated IL-2 in amounts comparable to adult blood cells (Fig. 2).

DISCUSSION

In our experiments we detected that cord blood cells stimulated with LPS produce slightly less IL-1α and significantly less IL-1β than adult blood cells. These data are in agreement with the results obtained by Han and Hodge (9), who also used LPS as inducer in whole blood cell cultures but they differ from those obtained by Sautois et al. (16) who, after stimulation with LPS (25 μg/ml) together with PHA (5 μg/ml), observed that mononuclear cells isolated from cord blood produced IL-1β levels comparable to blood cells of adults. We can only speculate that LPS alone without PHA was not an appropriate stimulator of cord blood cells. However, we suppose that LPS used alone mimicked more accurately the infection with Gram negative bacteria.

Such discrepancies in the literature concern not only proinflammatory monokines but cytokines produced by T lymphocytes as well. In most papers and also in our experiments, IL-4 and IFN-γ production by cells of neonates was detected to be lower in comparison to adults after induction with phorbol esters, ionomycin, enterotoxin B (SEB), PHA together with LPS or PHA alone (2, 11, 16). However, when IL-12 was used as IFN-γ inducer or allogenic cells were inducers of IFN-γ and IL-4, cytokine production was similar in cord and adult blood cells (12, 20). The differences in the results obtained by several authors concern also IL-2 production by cord blood leukocytes, but in this case they can be associated rather with the sensitivity of neonatal blood cells to different concentrations of PHA and differences in the kinetics of IL-2 production. It was shown in the paper of Han and Hodge (9) that IL-2 was produced in response to PHA earlier in cord blood cells than in adult blood, especially 24 h after induction. When samples were collected 72 hrs after induction a significant drop in IL-2 activity was observed in cord blood, and a significant increase in IL-2 level in adult blood cell cultures leading to a statistically significant difference in IL-2 production by cord and adult blood cells. In our study we detected that PHA at a dose of 10 μg/ml induced comparable amounts of IL-2 in cord and adult blood cells. However, when used at a high 50 μg/ml dose, PHA was a poor inducer of IL-2 in cord blood cells. We cannot exclude that the high dose of PHA was toxic for neonatal blood cells.

We also measured the levels of IL-10 produced in cord blood cells in response to LPS. The production of this cytokine was significantly lower than that by cells of adults. Also other authors (3, 10) described a low IL-10 production in cord

Fig. 1. Cytokine production in whole cord and adult blood cell cultures. Whole blood cell cultures were incubated with LPS 10 μg/ml (IL-1α, IL-1β, IL-10, IL-8), with PHA 50 μg/ml (IL-2, IL-4, IFN-γ) and in appropriate time cytokine level was estimated by ELISA. The results are expressed as mean ± standard deviation. The differences between cytokine levels in cord and adult blood cells significant in Mann-Whitney test are marked by asterisks

Fig. 2. IL-2 production in cord and adult blood cell cultures after induction with low dose of PHA. IL-2 was induced with PHA (10 μg/ml) and the level of cytokine present in supernatants of blood cell cultures was estimated 24 and 72 hrs later

blood cells after LPS stimulation, but when allogenic cells were inducers cord leukocytes produced similar amounts of this cytokine as leukocytes of adults (1, 10). This low IL-10 production may, in part, be secondary to the low TNF-α production because TNF-α is known to enhance IL-10 secretion and significant defect in TNF-α production by cord blood cells was described (3, 18).

In our experiments we showed that cord blood cells can produce IL-8 level comparable to adult blood cells in response to LPS. These results are in agreement with Seghaye et al. (18), who also used LPS as inducer of IL-8.

In conclusion, we confirmed the observations of other authors indicating that blood cells of neonates exhibit significant differences in some cytokine production in comparison to blood cells of adults, which may be, to some extent, related to differences in the expression of cell surface molecules which are targets for mitogens or bacterial products (17). However, IL-2 production kinetics indicate that also mechanisms regulating cytokine expression may be involved in these differences.

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