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The Effect of Route of Delivery on Neonatal Natural Killer Cytotoxicity and Antibody-Dependent Cellular Cytotoxicity to Herpes Simplex Virus-Infected Cells

JOHNNIE P. FRAZIER, STEVE KOHL,[13] LARRY K. PICKERING, AND LIAN-SIM LOO

Program in Infectious Diseases and Clinical Microbiology and Department of Pediatrics, The University of Texas Health Science Center, Houston, Texas, USA

Summary

The ability of human neonatal and adult Ficoll-hypaque purified mononuclear cells to mediate natural killer cytotoxicity (NKC) and antibody-dependent cellular-cytotoxicity (ADCC) against ^{51}Cr labeled *herpes simplex* virus-infected (HSV-infected) and uninfected cells was evaluated in healthy term infants delivered vaginally or by Cesarean (C)-section without labor, and in healthy adult controls. Cord blood NKC to HSV-infected cells (12.5 ± 7.0) was lower ($P < 0.01$) than adult controls NKC (29.5 ± 7.0). NKC to HSV-infected cells of babies delivered vaginally (16.6 ± 3.4) was lower ($P < 0.05$) than adult controls (28.4 ± 4.2). NKC to HSV-infected cells of neonates delivered by C-section without labor (7.6 ± 2.8) was also lower ($P < 0.001$) than adult controls (30.7 ± 4.0) and lower ($P < 0.05$) than that of neonates delivered vaginally. Cord blood ADCC (43.1 ± 9.0) was lower ($P < 0.05$) than ADCC of adult controls (58 ± 10). ADCC of neonates delivered vaginally (50 ± 5.9) was similar to ADCC of adult controls (57.4 ± 6.9). ADCC of neonates delivered by C-section without labor (30.4 ± 7.2) was lower than ADCC of adult (58.5 ± 7.4) and was lower ($P < 0.05$) than ADCC of neonates delivered vaginally.

These findings demonstrate that the method of delivery influences subsequent neonatal leukocyte NKC and ADCC. Further experiments will delineate the cause of these variations, which probably include labor or stress related hormonal changes in the mother or neonate.

Speculation

Low natural killer cytotoxicity (NKC) and antibody-dependent cellular-cytotoxicity (ADCC) have been suggested to be associated with serious HSV infections in neonates. Past studies have revealed conflicting data concerning levels of NKC and ADCC in the newborn. The present data may help explain disparities of past studies but raise serious questions regarding the use of cord blood ADCC studies to determine the subsequent immunologic competency of newborn infants. Studies on peripheral blood of newborns must be undertaken before the role of ADCC and NKC can be evaluated.

Defects in host defense mechanisms in newborn infants responsible for their susceptibility to *Herpes simplex* virus (HSV) dissemination are not fully understood (10, 12). Defects in the ability of neonatal leukocytes to kill HSV-infected cells in the presence of antibody (antibody-dependent cellular-cytotoxicity, ADCC) and in the absence of antibody (natural killer cytotoxicity, NKC) have been described (2, 5, 7, 8, 11). We now report the effects of methods and route of delivery on subsequent cord blood mononuclear cell ADCC and NKC to HSV-infected cells. The lower ADCC and NKC of leukocytes from infants born by C-section without labor compared to that of infants who were delivered after labor demonstrates that the method and route of delivery affects these functions. These data help explain disparities in previous studies, but raise questions regarding the use of cord blood experiments to determine the immunocompetence of the older neonates.

MATERIALS AND METHODS

Target cells were Chang liver cells, infected with HSV type I (HE strain) 24 h before use, and labeled with radioactive chromium the day of use as previously described (5–7). Blood was obtained from the placental portion of the umbilical vein after delivery of full term infants, and from healthy adult controls, after signed informed consent from patients or parents, as appropriate. Heparinized blood (10 units/ml) was processed by sequential dextran sedimentation and Ficoll-Hypaque centrifugation to isolate purified mononuclear cells (MC) which were a combination of lymphocytes and monocyte-macrophages (5–7). Target cells and MC were incubated at an effector to target cell ratio of 30:1 in HSV immune (neutralizing titer 1:32) or nonimmune sera for 18 h to determine the ADCC and NKC activity of neonatal and adult control MC (5–7).

Natural killer cytotoxicity (% NKC) = [% chromium release of target cells + effector cells + media) − (% chromium release of target cells + media] × 100 divided by (100 − % chromium release of target cells + media). Antibody-dependent cellular-cytotoxicity (% ADCC) = [% chromium release of target cells + effector cells + immune sera] − (% chromium release of target cells + effector cells + nonimmune sera) × 100 divided by (100 − % chromium release of target cells + effector cells + nonimmune sera).

Spontaneous release of HSV-infected cells was 18.1 ± 3.0%, and of uninfected cells 33.7 ± 3.8% in 18 h. These values were not effected by sera alone. All assays were performed in triplicate with S.D. of less than 5%. Data are expressed as the mean ± S.E of experiments. The significance of differences was determined by Student's unpaired two-tail t test.

RESULTS

Natural killer cytotoxicity to uninfected target cells. The NKC of MC from twenty adults (29 ± 6.5) was higher ($P < 0.05$) when compared to NKC of MC from twenty neonates (13 ± 6.0). Table 1 shows NKC to HSV-uninfected cells of adults and neonates when grouped according to method of delivery. MC-NKC of neonates delivered vaginally (8.8 ± 2.7) remained significantly lower ($P < 0.05$) than MC-NKC of matched adult controls (25.4 ± 7.2) (Table 1) MC-NKC of neonates delivered by C-section without labor (10.5 ± 2.7) was also lower when compared to MC-NKC of matched adult controls (32.0 ± 10.1). MC-NKC of

neonates delivered vaginally (8.8 ± 2.7) was not significantly different from MC-NKC of neonates delivered by C-section without labor (10.5 ± 2.7).

Natural killer cytotoxicity to HSV-infected target cells. Examining twenty pairs of adult and neonates' MC, neonates MC-NKC (12.5 ± 7.0) was significantly lower ($P < 0.01$) when compared to MC-NKC of adult controls (29.5 ± 7.0). Table 1 shows NKC to HSV-infected target cells of adults and neonates delivered either vaginally or by C-section without labor. Grouped by method of delivery, MC-NKC of neonates delivered vaginally (16.6 ± 3.4) was significantly lower ($P < 0.05$) when compared to MC-NKC of adult controls (28.4 ± 4.2). MC-NKC of neonates delivered by C-section without labor (7.6 ± 2.8) was also significantly lower ($P < 0.001$) when compared to MC-NKC of adult controls (30.7 ± 4.0). The MC-NKC of neonates delivered vaginally (16.6 ± 3.4) was significantly higher ($P < 0.05$) than MC-NKC of neonates delivered by C-section without labor (7.6 ± 2.8).

Antibody-dependent cellular-cytotoxicity. ADCC activity of MC from 17 neonates (43.1 ± 9.0) was significantly lower ($P < 0.05$) when compared to MC from 17-adult controls (58 ± 10). When grouped by method of delivery, the MC-ADCC of neonates delivered vaginally (50 ± 5.9) was not significantly different than MC-ADCC of adult controls (57.4 ± 6.9) (Table 2). The MC-ADCC of neonates delivered by C-section without labor (30.4 ± 7.2) was lower but not significantly different than MC-ADCC of adult controls (58.5 ± 7.4). MC-ADCC of neonates delivered vaginally (50 ± 5.9) was significantly higher ($P < 0.05$) when compared to MC-ADCC of neonates delivered by C-section without labor (30.4 ± 7.2).

DISCUSSION

Low ADCC or NKC may partially explain the susceptibility of new born infants to severe HSV infections (2, 5, 7, 8, 11). Several studies have shown defects in both ADCC and NKC of HSV-infected cells using cord blood leukocytes from human neonates (2, 5, 7, 8, 11). In contrast, normal lymphocyte, monocyte-macrophage and polymorphonuclear leukocyte (PMNL)-ADCC of cord blood to HSV-infected cells has been reported (6, 7). As described in previous reports of ADCC and NKC abnormalities in human neonates, metabolic-oxidative PMNL dysfunction in the ill or stressed neonate has been demonstrated (1). Our recent work has revealed depressed metabolic oxidative function of leukocytes from normal neonates to depend on the method of delivery alone. Vaginally delivered, presumably stressed, neonates' PMNL demonstrated significantly poorer function than those from neonates delivered by elective C-section without labor (4). A recent study has demonstrated that leukocytes of neonates born before labor, unlike those born after labor, were poor producers of leukocyte pryogen (3).

In evaluating ADCC and NKC of cord blood MC according to route of delivery, we have shown that NKC of MC from neonates delivered by C-section without labor was significantly lower than MC-NKC from neonates delivered vaginally. The MC from both groups of newborn infants had significantly lower NKC than adults. The ADCC of MC from infants delivered by C-section without labor was also significantly lower than MC from neonates delivered vaginally. The ADCC of each group of newborn infants was lower but not significantly different from that of adults. This is the first time that the route of delivery has been shown to influence MC mediation of ADCC or NKC. This may explain previous discrepancies in the literature, in which this factor has not been assessed (2, 5, 7, 8, 11). None of the mothers in the present study received general anesthesia. Possible mechanisms to explain these findings include stress or labor related hormonal changes. Dinarello *et al.* (3) have shown that substances present in crude preparations of human chorionic gonadotropin suppressed neonatal leukocyte pyrogen release. These hormonal changes, anesthesia-related phenomenon, and gestational age, known to affect the ontogeny of almost all immune responses in humans (9), are currently under investigation in our laboratories.

This study and other recent work (3, 4) raise questions in extrapolating "defects" discovered in cord blood to the resistance to an event that occurs in the first or second wk of life, the usual time of onset of neonatal *Herpes simplex* infection (10, 12). Prospective studies of neonates' NKC and ADCC function postpartum are clearly necessary to evaluate these "defects".

Table 1. *Natural killer cytotoxicity of adult and cord blood mononuclear cells*[1]

Uninfected target cells						Infected target cells					
Adult (9)	Vaginal (9)	P	Adult (11)	C-section without labor (11)	P	Adult (9)	Vaginal (9)	P	Adult (11)	C-section without labor (11)	P
25.4 ±7.2	8.8 ±2.7	$P^3 < 0.05$ P^5 = N.S.	32.0 ±10.1	10.5 ±2.7	P^4 = N.S.	28.4 ±4.2	16.6 ±3.4	$P^3 < 0.05$ $P^5 < 0.05$	30.7 ±4.0	7.6 ±2.8	$P^4 < 0.001$

[1] Values are expressed as mean ± S.E.
[2] ( ) = Number of samples in each group.
[3] Comparison of adult and vaginal.
[4] Comparison of adult and C-section without labor.
[5] Comparison of vaginal and C-section without labor.

Table 2. *Antibody-dependent cellular cytotoxicity of adult and cord blood mononuclear cells*[1]

Adult (10)[2]	Vaginal (10)	P	Adult (7)	C-section without labor (b)	P
57.4 ± 6.9	50 ± 5.9	P^3 = N.S. $P^5 < 0.05$	58.5 ± 7.4	30.4 ± 7.2	P^4 = N.S.

[1] Values are expressed as mean ± S.E.
[2] ( ) = Number of samples in each group.
[3] Comparison of adult and vaginal.
[4] Comparison of adult and C-section without labor.
[5] Comparison of vaginal and C-section without labor.

REFERENCES AND NOTES

1. Anderson, D. C., Pickering, L. K., and Feigin, R. D.: Leukocyte function in normal and infected neonates. J. Pediatr., *85:* 420 (1974).
2. Ching, C. and Lopez, C.: Natural killing of herpes simplex virus type I-infected target cells: normal human responses and influence of antiviral antibody. Infect. Immun., *26:* 49 (1979).
3. Dinarello, C. A., Shparber, M., Kent, E. F. Jr., and Wolff, S. M.: Production of leukocyte pyrogen from phagocytes of neonates. J. Infect. Dis., *144:* 337 (1981).
4. Frazier, J. P., Cleary, T. G., Pickering, L. P., Kohl, S., and Ross, P. J.: Comparison of human neonatal polymormonuclear leukocyte activity following vaginal and C-section deliveries. Pediatr. Res. (abstract) *15:* 595 (1981).
5. Kohl, S., Frazier, J. P., Greenberg, S. B., Pickering, L. K., and Loo, L. S.: Interferon induction of natural killer cytotoxicity in human neonates. J. Pediatr., *98:* 379 (1981).
6. Kohl, S., Frazier, J. P., Pickering, L. K., and Loo, L. S.: Normal function of neonatal polymorphonuclear leukocytes in antibody-dependent cellular-cytotoxicity to herpes simplex virus infected cells. J. Pediatr., *98:* 783 (1981).
7. Kohl, S., Shaban, S. S., Starr, S. E., Wood, P. A., and Nahmias, A. J.: Human neonatal and maternal monocyte-macrophage and lymphocyte-mediated antibody dependent cytotoxicity to cells infected with herpes simplex. J. Pediatr., *93:* 206 (1978).
8. McConnachie, P. R., Rachelefsky, G., Stiehm, E. R., and Terasaki, P. I.: Antibody-dependent lymphocyte killer function and age. Pediatrics, *52:* 795 (1973).
9. Miller, M. E.: Host defenses in the human neonate. pp. 1–112 (Grune and Stratton, New York, NY, 1978).
10. Nahmias, A. J. and Visintine, A. M.: Herpes Simplex. In: Jack S. Remington and Jerome O. Klein Ed. Infectious diseases of the fetus and newborn infant, pp. 156–190 (W. B. Saunders Company, Philadelphia, PA, 1976).
11. Shore, S. L., Milgrom, H., Wood, P. A., and Nahmias, A. J.: Antibody-dependent cellular cytotoxicity to target cells infected with herpes simplex viruses: functional adequacy in the neonate. Pediatrics, *59:* 22 (1977).
12. Whitley, R. J., Nahmias, A. J., Visintine, A. M., Fleming, C. L., and Alford, C. A.: The natural history of herpes simplex virus infection of mother and newborn. Pediatrics, *66:* 489 (1980).

13. Requests for reprints should be addressed to: Dr. Steve Kohl, Associate Professor of Pediatrics, Program in Infectious Diseases and Clinical Microbiology, P.O. Box 20708, Houston, TX 77025.
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