

## Cytotoxicity of lymphocytes in the newborn

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**SUMMARY** Antibody-dependent, phytohaemagglutinin-induced, and spontaneous cytotoxicity was studied in 44 term and 60 preterm newborn babies, all of whom were healthy and of normal weight for gestational age. Twenty-seven adults were used as controls. Antibody-dependent cytotoxicity was low in term babies particularly in the preterm ones during the first 4 days of life, but soon rose to adult levels. Phytohaemagglutinin-induced cytotoxicity was low both in term and preterm babies compared with adult levels, and remained low throughout the neonatal period although it began to rise. Spontaneous cytotoxicity was lower in preterm babies than in term ones during the first 2 weeks of life, and lower too than in adults. These findings indicate decreased cytotoxic ability of neonatal leucocytes especially during the first 4 days of postnatal life particularly in preterm babies, suggesting either lack of effector cells or that the cells are functionally immature.

It is well known that the newborn baby has a reduced resistance to infection; thus in recent years considerable attention has been paid to host defence mechanisms during the neonatal period. Low levels of immunoglobulins,<sup>1</sup> complement,<sup>2</sup> and lysozyme<sup>3</sup> have been found in neonatal blood, and functional immaturity of the phagocytic cells seems to contribute significantly to the impaired host defences.<sup>4</sup>

However, the function of lymphocytes—which are well known to play a central role in the immunological mechanisms during the neonatal period—has not been studied extensively, despite the fact that new techniques have been developed for studying the function of human lymphocytes *in vitro*.<sup>5</sup>

The aim of the present work was to study the cytotoxicity of neonatal lymphocytes—that is their ability to kill target cells *in vitro*. There have been a few studies of this particular lymphocyte function in the newborn,<sup>6-8</sup> but results so far have been inconclusive.

### Neonates and methods

Cell-mediated cytotoxicity was studied in 44 term and 60 preterm newborn babies. Each was healthy and of normal weight for gestational age. The birthweight of the premature babies ranged from 1700 to 2200 g (gestational age, 31-33 weeks). As no differences were found between babies weighing above and those weighing below 2000 g, both groups were combined.

**Term babies.** These were placed in one of two groups according to postnatal age.

#### Group 1

Babies aged between 1 and 4 days.

#### Group 2

Babies aged between 5 and 14 days.

**Preterm babies.** These were placed in one of three groups according to postnatal age.

#### Group 1

Babies aged between 1 and 4 days.

#### Group 2

Babies aged between 5 and 14 days.

#### Group 3

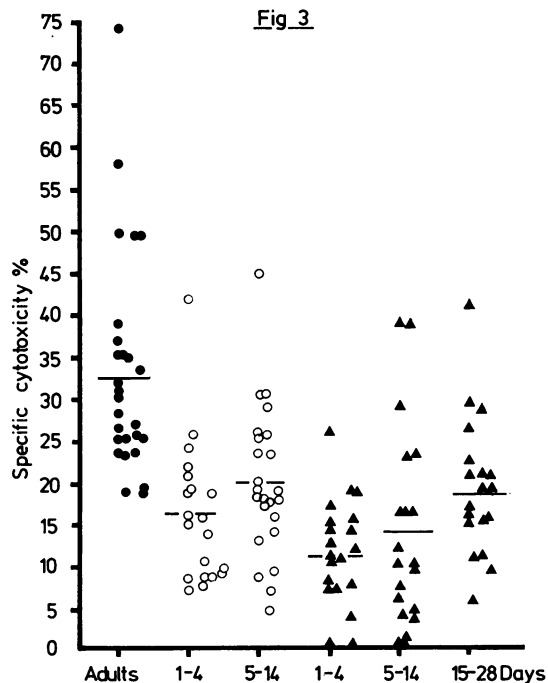
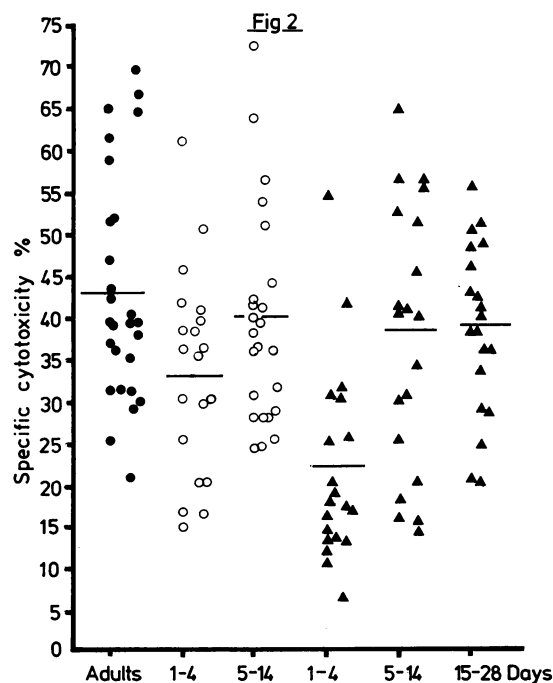
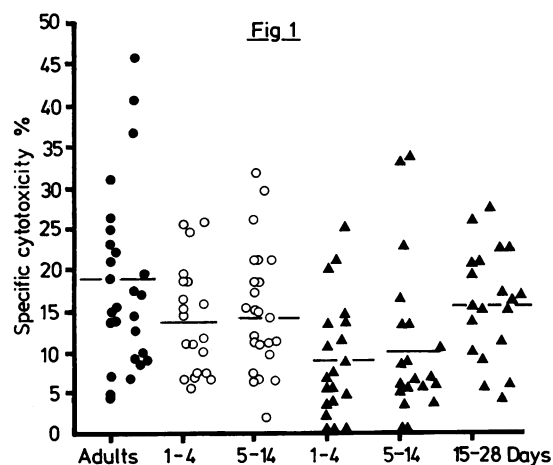
Babies aged between 15 and 28 days.

In each experiment a 'control' sample of venous blood was drawn from a healthy adult, so that by the end of the experiments there were 27 adult controls. In each case 0.5-1 ml of blood was obtained from a peripheral vein and anticoagulated with preservative-free heparin. All samples were obtained after informed consent had been given.

Cell-mediated cytotoxicity was assayed by a whole-blood microtechnique.<sup>5,9</sup> Dilution of heparinised blood 1:10 was made up with Eagle's minimum essential medium supplemented with antibiotics, non-essential amino-acids, glutamine,

and 10% fetal bovine serum. Target cells were Chang human liver cells, labelled by incubation at 37°C for 1 hour with 100  $\mu$ Ci  $^{51}$ Cr chromate (Radiochemical Centre, Amersham). The Chang cells were counted and made up to  $2 \times 10^4$  per ml of Eagle's minimum essential medium, plus 10% fetal bovine serum. Cultures were set up in triplicate with 500  $\mu$ l blood suspension and 500  $\mu$ l Chang cell suspension. In

some of the tubes only the medium had been added to Chang cells. Three separate sets of tubes were set up: one with no reagents other than blood and Chang cells, one with added heat-inactivated rabbit anti-Chang antiserum (concentration 3/100 000), and one with phytohaemagglutinin (PHA) (concentration 3:1000). These concentrations of antiserum and PHA have been found to induce maximal killing of



Figs 1-3 Specific cytotoxicity of blood (Fig. 1) against Chang cells only; (Fig. 2) against Chang cells in the presence of anti-Chang antiserum; (Fig. 3) against Chang cell cells in the presence of PHA (3:1000).

Chang cells by mononuclear cells. In the absence of such effector cells neither antibody nor PHA can induce any isotope release apart from one that occurs spontaneously from Chang cells in medium alone.<sup>7</sup> The tubes were incubated for 20 hours, after which the percentage release of <sup>51</sup>Cr into the cell-free supernatant and in the residue was assessed. From this percentage release 'specific cytotoxicity' was calculated by correcting for baseline release from Chang cells with medium alone, and the maximum <sup>51</sup>Cr release that would be reached if the number of effector cells were increased indefinitely.<sup>10</sup>

## Results

Values for cytotoxic activity in both adult and neonatal blood had an arithmetically normal distribution. They were therefore, expressed and plotted arithmetically.

There were no significant differences between adults and term babies in spontaneous cytotoxic activity of blood against Chang cells, although the values were lower during the first 4 days of life. In the preterm baby this cytotoxicity was appreciably lower than for adults or term babies during the first 2 weeks of life, after which it rose to normal adult values (Fig. 1 and Table 1).

Antibody-dependent cytotoxicity in term and preterm babies during the first days of life was significantly lower than that of adults, but rose to adult values between the 4th and 14th day of life (Fig. 2). In the preterm baby during the first 4 days of life it was significantly lower than in term babies of the same age (Table 2).

Table 1 Spontaneous cytotoxicity (blood + Chang cells)

Comparisons	P
Preterm	
1-4 days v. adults	<0.01
5-14 days v. adults	<0.02
1-4 days v. preterm 15-28 days	<0.01
5-14 days v. preterm 15-28 days	<0.05
1-4 days v. term 1-4 days	<0.05

Table 2 Specific cytotoxicity (blood + Chang cells + anti-Chang antibody)

Comparisons	P
Preterm	
1-4 days v. adults	<0.001
1-4 days v. preterm 5-14 days	<0.001
1-4 days v. preterm 15-28 days	<0.001
1-4 days v. term 1-4 days	<0.01
Term 1-4 days v. adults	<0.01

Table 3 Specific cytotoxicity (blood + Chang cells + PHA)

Comparisons	P
Term	
1-4 days } v. adults	<0.001
5-14 days }	
Preterm	
1-4 days } v. adults	<0.001
5-14 days }	
15-28 days }	<0.01
1-4 days v. preterm 15-28 days	<0.01
Term	
4-14 days v. preterm 5-14 days	<0.05

PHA-induced cytotoxicity in term babies was significantly lower than in adults. It tended to rise after the 4th day, but not appreciably (Fig. 3). In the preterm baby this cytotoxicity was even lower during the first few days of life, but it rose significantly after the 14th day (Table 3). Despite these rises, adult values were not reached in preterm or term babies during the neonatal period. Values for term babies were significantly higher than those for preterm ones only between the 4th and the 14th day (Table 3).

## Discussion

**Antibody-dependent cell-mediated cytotoxicity (ADCC).** This is shown by various types of effector cells depending on the type of target-cells used in the particular experimental system.<sup>11</sup>

The K-cell has been described as a non-phagocytic, non-glass-adherent mononuclear cell which is independent of the thymus.<sup>10,12</sup> It does not carry surface immunoglobulins,<sup>13</sup> but has membrane receptors for the Fc portion of the IgG molecule.<sup>10</sup> Although morphologically similar to fairly small lymphocytes, the precise lineage of the K-cell is unknown.<sup>14</sup> In our experiments the target-cells were coated with a low concentration of IgG antibody and were killed by means of an extracellular non-phagocytic mechanism of K-cells which bind to the targets by their specific Fc receptors for IgG.<sup>15</sup>

We found antibody-dependent cytotoxicity to be low in term neonates and to be even lower in preterm babies during the first 4 days of life. It then rose to adult values. Very little work has been done on this particular lymphocyte function in neonates. Campbell *et al.*<sup>7</sup> found fairly good K-cell activity in cord blood using Chang cells as targets. Shore *et al.*<sup>16</sup> found that the ADCC of cord mononuclear cells against *Herpes simplex* virus-infected Chang cells was moderately reduced, but Kohl *et al.*<sup>8</sup> using the same system, found it was normal compared with that exhibited by adult mononuclears.

Finally McConnachie *et al.*<sup>6</sup> reported that the ADCC was extremely low in term infants 24–26 hours old. In their assays however, they used human lymphocytes sensitised with HL-A antibodies as target cells.

It should be pointed out that in all these investigations separated mononuclear cells were used, whereas we used whole blood in our experiments, resembling the *in vivo* state more closely.

In view of the good ADCC activity found in cord blood<sup>7</sup> and the low cytotoxic activity observed by us during the first 4 postnatal days it can be assumed that effector cells leave the blood stream temporarily in order to recirculate. Interestingly, during that time there is a decrease in total lymphocyte numbers in peripheral blood.<sup>17</sup> Hallberg and Hallberg<sup>18, 19</sup> reported that the proportion of Fc-receptor bearing lymphocytes is very low during the first days of life and tends to rise with increasing neonatal age. Since ADCC is dependent on cells with these particular receptors, this finding supports our observations. However, apart from reduced numbers, functional immaturity of the effector cells in the neonate cannot be excluded.

The phenomenon of PHA-induced cell-mediated cytotoxicity was first described by Holm *et al.*<sup>20</sup> Analysis of the system used by us with Chang cells as targets, has shown that two types of effector cells are concerned. Both are lymphocytic, but one of them carries Fc receptors and the other does not.<sup>21</sup> Neither cell has surface immunoglobulin nor are such cells surface-adherent.<sup>28</sup>

The exact mechanism of killing in PHA-induced cytotoxicity is unknown. T-cell mediated cytotoxicity is thought to result from a single collision between effector and target cell.<sup>22–24</sup>

Very little work has been done to test this lymphocyte function in neonatal blood. Carr *et al.*<sup>25</sup> detected considerable PHA-induced cytotoxicity of cord blood lymphocytes against chicken red blood cells, but they gave no comparative data for adult effector cells. However, PHA-induced cytotoxicity was found to be very low in cord blood if Chang cells were used as targets.<sup>7</sup>

In our experiments PHA-induced cytotoxicity was found to be very low both in term and preterm babies throughout the neonatal period and although it rose during this time it did not reach adult values. The low cytotoxicity in our experiments could indicate either a lack of phagocytic cells or that such cells were functionally immature. Alternatively, the activity of the effector cells could be suppressed temporarily by some inhibitory factor in neonatal blood—that is alpha-fetoprotein.<sup>26</sup>

Spontaneous cytotoxicity was found to be

somewhat lower in term babies and appreciably lower in preterm babies than in adults. This sort of cytotoxicity is a function which appears to be mediated by cells which have many of the properties of K-cells, but which are probably not identical.<sup>27</sup> The cytolytic mechanism in spontaneous cytotoxicity and ADCC appear to be distinct since there is no evidence for an antibody in this spontaneous cytotoxicity system.<sup>27</sup>

The finding that the blood of preterm babies has less spontaneous cytotoxic capacity than the blood of term babies could be explained by the fact that there are fewer lymphocytes circulating in the preterm baby's blood<sup>17</sup> or that they are immature.

In conclusion, our findings indicate decreased cytotoxic ability of neonatal leucocytes, especially during the first 4 days of life and particularly in term babies.

## References

- Dossett J H. Microbial defenses of the child and man. *Pediatr Clin North Am* 1972; **19**: 355–72.
- Adinolfi M. Levels of two components of complement (C'4 and C'3) in human fetal and newborn sera. *Dev Med Child Neurol* 1970; **12**: 306–8.
- Xanthou M, Agathopoulos A, Sakellariou A, Economou-Mavrou C, Tsingoglou S, Matsaniotis N. Serum levels of lysozyme in term and preterm newborns. *Arch Dis Child* 1975; **50**: 304–7.
- Miller M E. *Host defenses in the human neonate*. New York: Grune & Stratton, 1978: 59–68.
- Thompson R A. *Techniques in clinical immunology*. Oxford: Blackwell, 1977: 191–5.
- McConnachie P R, Rachelefsky G, Stiehm E R, Terassaki P I. Antibody-dependent lymphocyte killer function and age. *Pediatrics* 1973; **52**: 795–800.
- Campbell A C, Waller C, Wood J, Aynsley-Green A, Yu V. Lymphocyte subpopulations in the blood of newborn infants. *Clin Exp Immunol* 1974; **18**: 469–82.
- Kohl S, Shaban S S, Starr S E, Wood P A, Nahmias A J. Human neonatal and maternal monocyte-macrophage and lymphocyte-mediated antibody-dependent cytotoxicity to cells infected with herpes simplex. *J Pediatr* 1978; **93**: 206–10.
- Gale D, MacLennan I C M. A method of measuring antibody- and phytohaemagglutinin-induced lymphocyte-dependent cytotoxicity using whole blood. *Clin Exp Immunol* 1976; **23**: 252–7.
- MacLennan I C M. Antibody in the induction and inhibition of lymphocyte cytotoxicity. *Transplant Rev* 1972; **13**: 67–90.
- MacDonald H R, Bonnard G D, Sordat B, Zawodnik S A. Antibody-dependent cell-mediated cytotoxicity. Heterogeneity of effector cells in human peripheral blood. *Scand J Immunol* 1975; **4**: 487–97.
- Perlmann P, Perlmann H, Wigzell H. Lymphocyte mediated cytotoxicity *in vitro*. Induction and inhibition by humoral antibody and nature of effector cells. *Transplant Rev* 1972; **13**: 91–114.
- Greenberg A H, Hudson L, Shen L, Roitt I M. Antibody-dependent cell-mediated cytotoxicity due to a 'null' lymphoid cell. *Nature New Biol* 1973; **242**: 111–3.

- <sup>14</sup> Roitt I. *Essential immunology*, third edition. Oxford: Blackwell, 1977: 160-2.
- <sup>15</sup> Lovchik J C, Hong R. Antibody dependent cell-mediated cytotoxicity (ADCC): analyses and projections. *Prog Allergy* 1977; **22**: 1-44.
- <sup>16</sup> Shore S L, Milgrom H, Wood P A, Nahmias A J. Antibody-dependent cellular cytotoxicity to target cells infected with herpes simplex viruses: functional adequacy in the neonate. *Pediatrics* 1977; **59**: 22-8.
- <sup>17</sup> Xanthou M. Leucocyte blood picture in healthy full-term and premature babies during neonatal period. *Arch Dis Child* 1970; **45**: 242-9.
- <sup>18</sup> Hallberg T, Hallberg A. Lymphocyte markers in newborn infants. *Acta Pathol Microbiol Scand [C]* 1976; **84**: 477-84.
- <sup>19</sup> Hallberg A, Hallberg T. Evolution of three lymphocyte markers in newborn preterm infants. *Int Arch Allergy Appl Immunol* 1977; **55**: 102-11.
- <sup>20</sup> Holm G, Perlmann P, Werner B. Phytohaemagglutinin-induced cytotoxic action of normal lymphoid cells on cells in tissue culture. *Nature* 1964; **203**: 841-3.
- <sup>21</sup> Wisløff F, Frøland S S, Michaelsen T E. Characterization of subpopulations of human lymphoid cells participating in phytohemagglutinin and concanavalin A-induced cytotoxicity. *Int Arch Allergy Appl Immunol* 1974; **47**: 488-97.
- <sup>22</sup> Henney C S. T cell mediated cytotoxicity: consideration of the role of a soluble mediator. *J Reticuloendothel Soc* 1975; **17**: 231-5.
- <sup>23</sup> Biberfeld P, Johansson A. Contact areas of cytotoxic lymphocytes and target cells. An electron microscopic study. *Exp Cell Res* 1975; **94**: 79-87.
- <sup>24</sup> Martz E. Multiple target cell killing by the cytolytic T lymphocyte and the mechanism of cytotoxicity. *Transplantation* 1976; **21**: 5-11.
- <sup>25</sup> Carr M C, Lieber E, Fudenberg H H. *In vitro* cytolysis by human fetal lymphocytes. *Cell Immunol* 1970; **1**: 455-60.
- <sup>26</sup> Dattwyler R J, Tomasi T B. Inhibition of sensitization of T-cells by alpha-fetoprotein. *Int J Cancer* 1975; **15**: 942-5.
- <sup>27</sup> Kay H D, Bonnard G D, West W H, Herberman R B. A functional comparison of human Fc-receptor-bearing lymphocytes active in natural cytotoxicity and antibody-dependent cellular cytotoxicity. *J Immunol* 1977; **118**: 2058-66.
- <sup>28</sup> Waller C A, Campbell A C, MacLennan I C M. Two populations of lymphocytes involved in phytohaemagglutinin-induced cytotoxicity of a dividing target cell. *Scand J Immunol* 1976; **5**: 931-9.

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### British Paediatric Association

#### Annual meetings

1982	20-24 April	Aviemore Centre, Scotland
1983	12-16 April	York University
1984	10-14 April	York University
1985	16-20 April	York University