FcγRI and FcγRIII on polymorphonuclear leucocytes in cord blood

N Takahashi, H Nishida, T Kuratsuji

Abstract

FcγRI and FcγRIII expression on polymorphonuclear leucocytes in cord blood from seven normal infants was investigated by flow cytometry. FcγR expression on fresh polymorphonuclear leucocytes in whole blood samples and in blood samples incubated with or without interferon gamma (IFN-γ) for 48 hours was also studied. The percentage of FcγRIII positive polymorphonuclear leucocytes in cord blood (73-3%) was significantly lower than that in adult controls (95-9%). The mean fluorescence intensity of FcγRIII was significantly increased on cord polymorphonuclear leucocytes by the incubation with IFN-γ. Fresh cord polymorphonuclear leucocytes expressed only a small amount of FcγRI as adult polymorphonuclear leucocytes. The percentage of FcγRII positive polymorphonuclear leucocytes induced with IFN-γ was significantly higher in cord blood (62-3%) than in adult controls (30-3%). It is possible that decreased expression of FcγRII is a factor in the susceptibility of newborns to infection. High expression of FcγRI stimulated with IFN-γ in neonates could have a compensatory role against decreased immunological function.

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Human polymorphonuclear leucocytes have several host defence functions, including phagocytosis and release of both reactive oxygen intermediates and specific granule proteins. Each of these functions can be triggered by FcγR.3,7,8 which is the receptor for the Fc portion of IgG. Three classes of human FcγR (FcγRI, FcγRII, and FcγRIII) have been identified.4,10 FcγRIII (cluster designation CD16) binds monomeric IgG with low affinity6 but recognises IgG complexes7 and IgG coated particles.8 A second type of FcγR with low affinity for monomeric IgG is designated FcγRII (CD32).7 FcγRI (CD64) binds the Fc region of monomeric human IgG1 and IgG3 with high affinity.4,10 Human polymorphonuclear leucocytes normally express two distinct types, FcγRII and FcγRIII.3,7,8 Freshly isolated polymorphonuclear leucocytes do not express detectable quantities of FcγR.2 However, polymorphonuclear leucocytes treated with interferon gamma (IFN-γ) for 16 or 24 hours expressed a greater amount of FcγRII on their surface.1,2 Although all three types of FcγR are capable of mediating phagocytosis and antibody dependent cell mediated cytotoxicity (ADCC),3,7,8 FcγRIII provides the major binding capacity of neutrophils for immune complexes and IgG opsonised particles7 and contributes to ADCC. FcγRI induced by IFN-γ on polymorphonuclear leucocytes is able to mediate the activation of ADCC2 at 10 to 100-fold lower antibody concentrations than control polymorphonuclear leucocytes.2

FcγRI expression on polymorphonuclear leucocytes is known to be increased in patients with immunological defects such as chronic granulomatous disease11 and leucocyte adhesion deficiency.12 It is possible that FcγRII expression on polymorphonuclear leucocytes is increased in newborn infants, as newborn infants are susceptible to infections because of functionally impaired polymorphonuclear leucocytes. To our knowledge, however, no report refers to FcγRI expression on neonatal polymorphonuclear leucocytes, and there are no reported studies on the effects of IFN-γ on the expression of FcγRIII on neonatal polymorphonuclear leucocytes. In this report we found a significant difference between cord blood and adult peripheral blood in the expression of FcγRI and FcγRIII on polymorphonuclear leucocytes, especially changes of expression due to IFN-γ.

Subjects and methods

SUBJECTS

The subjects studied were seven normal infants with a gestational age ranging from 37–40 weeks and birth weight from 2554–3365 g. Five subjects were born vaginally and two were delivered by caesarean section. Informed consent was obtained from all the parents of the subjects. Seven healthy adult donors were used as control subjects.

BLOOD SPECIMENS

Cord blood was collected with heparin from the umbilical vein of the seven infants. For the control subjects 3 ml of peripheral blood was obtained.

MONOCLONAL ANTIBODIES

The monoclonal antibodies used were 32-2-FITC (Medarex, West Lebanon, NH) for FcγRI, FITC labelled anti-Leu-11a (Beckton-Dickinson, San Jose, CA) for FcγRIII, PE labelled anti-Leu-M3 (Beckton-Dickinson) for CD14, a monocyte antigen, PE labelled 4B4-RDI (Coulter Immunology, Hialeah, FL) for CD29, an eosinophil antigen, and antime
IgG₂ antibody (Beckton-Dickinson) for a negative control.

CELL SUSPENSIONS AND DIRECT IMMUNOFLOUORESCENCE STAINING
Blood samples were initially divided into aliquots of 100 μl. In order to investigate FcγR expression on fresh polymorphonuclear leucocytes in whole blood, the blood samples were washed in phosphate buffered saline (PBS) without calcium and magnesium (PBS−) with 0.02% EDTA. After centrifugation at 1500 rpm for five minutes, the pellets were resuspended in PBS containing 0.1% sodium azide. The cells were incubated for 30 minutes on ice with 32.2-FITC, anti-Leu-11A, anti-Leu-M3, and 4B4-RD1, respectively. In order to investigate the effect of IFN-γ on FcγR expression of polymorphonuclear leucocytes, 100 μl blood samples were added to 900 μl Roswell Park Memorial Institute 1640 medium containing 10% fetal calf serum and incubated with or without 100 U/ml IFN-γ for 48 hours at 37°C in an incubator in an atmosphere of 5% carbon dioxide. After incubation, the samples were washed in PBS−. After centrifugation at 1500 rpm for five minutes, the pellets were resuspended and were incubated for 30 minutes on ice with the above mentioned four kinds of monoclonal antibodies. After incubation with monoclonal antibodies, the samples were centrifuged at 1500 rpm for five minutes. The pellets were lysed with hypotonic solution. After one wash in PBS/0.1% sodium azide, the cells were resuspended in PBS−.

FLOW CYTOMETRY
Measurements were performed with a flow cytometer (EPICS-C; Coulter Electronics, Hialeah, FL). Forward and 90 degree light scatter and fluorescence emission were measured simultaneously using an argon ion laser tuned at 488 nm as a light source. Cell debris, red blood cells, lymphocytes, and most monocytes were electronically eliminated on the basis of their light scatter signal. Histograms of the fluorescence of approximately 10 000 viable polymorphonuclear leucocytes were recorded. Signals were converted to a logarithmic scale using logarithmic minimum amplifiers. The mean fluorescence intensity and the percentage of positive cells were analysed. In order to calculate the percentage of positive cells, the threshold value was set at 10 channels on the fluorescence intensity, as non-specific binding of FITC-IgG₂ to polymorphonuclear leucocytes did not exceed 10 channels on the fluorescence intensity.

STATISTICAL METHODS
Statistical analysis was carried out with the Student's t test for unpaired observations in comparison between cord blood and adult peripheral blood, and for paired observations comparing data after the incubation with and without IFN-γ; p values less than 0.05 were considered significant.

Results

FCγRIII EXPRESSION ON FRESH POLYMORPHONUCLEAR LEUCOCYTES
The mean (SD) percentage of FCγRIII positive polymorphonuclear leucocytes in the subjects was 73.3 (17.5)% (n=6, one case was excluded because of sample coagulation) and was significantly lower (p<0.01) than that (95.9 (1.8)%; n=7) seen in adult controls (fig 1A). However, the mean fluorescence intensity of FCγRIII positive polymorphonuclear leucocytes in cord blood was similar to that in adult controls (fig 1B).

FCγRIII EXPRESSION ON POLYMORPHONUCLEAR LEUCOCYTES AFTER 48 HOURS INCUBATION WITH OR WITHOUT IFN-γ
IFN-γ had no effect on the percentage of FCγRIII positive polymorphonuclear leucocytes in both cord blood and adult controls (fig 2A). However, the mean fluorescence intensity of FCγRIII was significantly increased by the incubation with IFN-γ only in cord blood (fig 2B). In contrast, IFN-γ had no effect on the mean fluorescence intensity of FCγRIII in adult controls. The difference of the mean fluorescence intensity of FCγRIII between polymorphonuclear leucocytes in cord blood and the adult controls seen after 48 hours incubation without IFN-γ disappeared after incubation with IFN-γ.

A typical distribution for polymorphonuclear leucocytes incubated for 48 hours with

![Figure 1](http://fn.bmj.com/)

(A) Percentage of FCγRIII positive polymorphonuclear leucocytes; individual values and mean (SD).

(B) Mean fluorescence intensity of FCγRIII positive polymorphonuclear leucocytes; individual values and mean (SD).
FcyRI and FcyRIII on polymorphonuclear leucocytes in cord blood

or without IFN-γ is shown in fig 3. In cord blood, not only was the mean fluorescence intensity of FcyRIII on polymorphonuclear leucocytes after incubation without IFN-γ significantly lower than in adult controls, but the fluorescence distribution curve was broader and skewed to the left. This means the cord blood has more heterogeneity of numbers of receptor per cell than in adult controls. This fluorescence distribution curve in cord blood was steepened by IFN-γ. This change of the fluorescence distribution curve due to IFN-γ in cord blood caused an increase in mean fluorescence intensity, as shown in fig 2B. These results show that IFN-γ increases the amount of FcyRIII per polymorphonuclear leucocytes in the case of cord blood and that IFN-γ decreases the heterogeneity of the amount of FcyRIII per polymorphonuclear leucocyte in the case of cord blood. No significant change in mean fluorescence intensity was observed in adult controls.

FCyRI EXPRESSION ON FRESH POLYMORPHONUCLEAR LEUCOCYTES

In both cord blood and the adult controls, polymorphonuclear leucocytes expressed FcyRII minimally. The mean percentage of FcyRII positive polymorphonuclear leucocytes in cord blood and in the adult controls was 1.3% and 0.5%, respectively.

FCyRI EXPRESSION ON POLYMORPHONUCLEAR LEUCOCYTES AFTER 48 HOURS INCUBATION WITH OR WITHOUT IFN-γ

As shown in fig 4A, a mean (SD) of 18.2 (19.1)% (n=7) of polymorphonuclear leucocytes in cord blood, and 5.0 (4.7)% (n=7) on polymorphonuclear leucocytes in the adult controls, expressed FcyRI (no significant difference). However, the percentage of FcyRI positive polymorphonuclear leucocytes was significantly increased by IFN-γ in both cord blood and adult controls. The percentage of FcyRII positive polymorphonuclear leucocytes induced with IFN-γ was significantly higher in cord blood than in adult controls (p<0.02).

IFN-γ had no effect on the mean fluorescence intensity of FcyRII positive polymorphonuclear leucocytes in either cord blood or adult controls (fig 4B). A typical distribution of FcyRI positive polymorphonuclear leucocytes incubated for 48 hours with or without IFN-γ is shown in fig 5.
leucocytes in cord blood incubated with IFN-γ showed significantly increased expression of FcyRI compared with adult controls. The fluorescence distribution curve of FcyRI was broader in cord polymorphonuclear leucocytes stimulated with IFN-γ than in adult controls, and had two peaks in four cases of cord blood.

Discussion
We investigated FcyRI and FcyRIII expression on polymorphonuclear leucocytes in cord blood. The percentage of FcyRIII positive polymorphonuclear leucocytes was significantly lower in cord blood than in adult controls. The mean fluorescence intensity of FcyRIII on polymorphonuclear leucocytes in cord blood was significantly increased by incubation with IFN-γ. Untreated fresh cord polymorphonuclear leucocytes expressed only a small amount of FcyRI. The percentage of increase in FcyRII positive polymorphonuclear leucocytes after incubation with IFN-γ was significantly higher in cord blood than in adult controls.

The human neonate is highly susceptible to severe bacterial and fungal infections. A number of abnormalities have been described in the host defence system of newborn infants; one of the most important appears to be in polymorphonuclear leucocyte function. In healthy term neonates the major neutrophil abnormality is impaired chemotaxis. Bactericidal killing by the newborn’s granulocytes has also been reported to be decreased.

It is possible that neonatal FcyRIII expression is impaired and leads to a decrease of bactericidal activity. However, FcyRIII expression on neonatal polymorphonuclear leucocytes is controversial. There was no significant difference between adult and cord polymorphonuclear leucocytes in the percentage of cells that expressed FcyRIII when examined in whole blood or after stimulation with FMLP. On the other hand, Masuda et al reported that the percentage of antibody coated E rosette forming neutrophils was significantly lower in cord blood than the percentage of adult polymorphonuclear leucocytes. The mean peak fluorescence was significantly different between polymorphonuclear leucocytes isolated by centrifugation from adult and neonatal blood. In terms of the percentage of positive cells, the expression of FcyRIII on fresh cord polymorphonuclear leucocytes in whole blood was reduced in our present study.

Heterogeneity of FcyRIII expression after 48 hour culture in cord blood was noted. Heterogeneity in neonates has already been reported concerning chemotaxis, adherence, and FcyR expression. Our observations agreed with those previous reports. It has never been found that heterogeneity of polymorphonuclear leucocytes in neonates is decreased by cytokines. The effects of IFN-γ on the expression of FcyRIII in adults are reported to be various. Petroni et al identified that IFN-γ had no effect on expression of FcyRII or FcyRIII. However, the opposite down regulatory effect was also observed in samples in which the initial levels of FcyRIII were high. It is possible that neutrophils from some individuals had been exposed to stimulation in vivo. IFN-γ may act as down regulators of FcyRIII on cells from stimulated donors and up regulators for unstimulated donors.

In patients with some immunological defects such as leucocyte adhesion deficiency and chronic granulomatous disease, FcyRI expression on polymorphonuclear leucocytes is increased. Therefore high FcyRI expression in these patients is thought to play a compensatory part. Although newborn infants have susceptibility to infection and are thought to be in a state of immunodeficiency, no report has dealt with FcyRI expression on neonatal polymorphonuclear leucocytes. From our results in this study, cord polymorphonuclear leucocytes appear to express only a small amount of FcyRI in the resting state. IFN-γ is a potent activator of polymorphonuclear leucocyte functions. This activation may depend in part on the induction of FcyRI. High FcyRI expression in patients with immunological defects that is not related to treatment might be due to endogenous IFN-γ production. Disease states that induce high endogenous production of IFN-γ would be expected to result in circulating polymorphonuclear leucocytes with a high expression of FcyRI.
cord polymorphonuclear leucocytes do not have any exposure to IFN-γ, they could have only small amounts of FcγRI.

We found that cord polymorphonuclear leucocytes expressed a relatively high percentage of FcγRI only after 48 hours incubation and a significantly higher percentage due to IFN-γ than adult controls. Terminally differentiated circulating myeloid cells (polymorphonuclear leucocytes) are short lived cells, incapable of proliferation, in which no or minimum RNA and protein synthesis occurs. It is possible that neonatal polymorphonuclear leucocytes are not differentiated by the final state and has a flexibility capable of FcγR expression. It is also possible that neonatal polymorphonuclear leucocytes have different subpopulations from adult polymorphonuclear leucocytes. The fluorescence distribution curve of FcγRI was broader in cord blood and in some cases had two peaks. The two peaks observed in this study might be due to contamination by other cells such as monocytes or eosinophils. Therefore we investigated the percentage of CD14 positive cells, indicative of monocytes, or CD29 positive cells, which indicates eosinophils, in the same cytograms on flow cytometry. We observed only a small percentage of such cells: less than 2% in each case. We excluded the possibility that our observations were due to increased numbers of eosinophils or monocytes. It is possible that there are two or more subpopulations in cord polymorphonuclear leucocytes, as shown in chemotaxis. Finally, we found the state of polymorphonuclear leucocytes in cord blood was very different from adult controls in terms of FcγR. It is possible that decreased expression of FcγRIII is a factor in the susceptibility of newborns to infection. The administration of IFN-γ might increase the expression of FcγRI and FcγRIII on cord polymorphonuclear leucocytes in vivo and might be clinically effective against bacterial infection. We speculate that high expression of FcγRI on cord polymorphonuclear leucocytes stimulated with IFN-γ could play a compensatory part against decreased immunological function.

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