

Age-related changes in polymorphonuclear neutrophil characteristics in infants born to human immunodeficiency virus type 1 seropositive mothers

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In infants, the major components of the innate immune system appear weakened, and it has been shown that both polymorphonuclear neutrophil (PMN) production and function are immature. This study was conducted to assess the expression of a number of receptors important to normal PMN function and the integrity of PMN degranulation in cord blood and in uninfected children of varying ages born to human immunodeficiency virus type 1 (HIV-1) seropositive mothers. Although the expression of L-selectin (CD62L) on PMN did not differ between the infants aged 12, 15 and 18 months, the expression of the interleukin-8 (IL-8) receptors CXCR1 and CXCR2, and the complement 5a (C5a) receptor CD88 displayed a similar pattern, with the highest levels expressed on PMN from infants in the 12 month old age group, and declining with age. It was also observed that PMN from a substantial proportion of the younger infants were unresponsive to a variety of stimuli including IL-8, C5a, stromal cell-derived factor (SDF)-1 α , SDF-1 β , and phorbol 12-myristate 13-acetate (PMA), with the proportions of children showing positive (adult-like) PMN degranulation responses increasing with age. Exposure to HIV-1 did not appear to be the cause of impaired degranulation responses, since a similar proportion of cord blood PMN from uninfected infants born to HIV-1 infected and HIV-1 uninfected mothers were unresponsive. The altered expression of these important receptors and inefficient agonist-induced degranulation in early life may contribute to the increased susceptibility of infants to secondary microbial infections.

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Polymorphonuclear neutrophils (PMN) are key effector cells in non-specific host defence (1), and as a result of infections or tissue injury are rapidly recruited from the bloodstream to sites of inflammation. The migration of PMN occurs by a coordinated cascade, with an initial interaction of circulating leucocytes with endothelial cells being mediated by L-selectin (CD62L), which produces a characteristic 'rolling' of the leucocytes on the vascular walls, a prerequisite to firm attachment and subsequent extravasation (2). The immunomodulatory and inflammatory properties of chemotactic factors such as interleukin-8

(IL-8) and complement 5a (C5a) are mediated by specific binding to the IL-8 receptors, CXCR1 and CXCR2 and the C5a receptor (CD88), respectively. Opsonized microorganisms are phagocytosed by PMN and killed by oxygen-dependent or oxygen-independent mechanisms. The former occurs when the PMN undergo respiratory burst which results in the production of a series of toxic oxygen intermediates. The non-oxidative armature of PMN, on the other hand, involves the action of potent antimicrobial polypeptides contained within cytoplasmic granules, which are released by degranulation.

Newborns and young infants are at increased risk of prolonged infections with infectious agents that require the induction of strong cellular immune responses for clearance. In infants, the major components of the innate immune system appear weakened, where both PMN production and function are immature. During infection, neutrophils are rapidly recruited into the affected tissues from the circulating neutrophil pool, and cells from the bone marrow storage pool are released to compensate. Evidence that a quantitative deficiency exists in the neonatal neutrophil storage pool in the bone marrow is supported by work with full-term babies (3) and animal models (4). This quantitative deficiency in cellular host defence compromises infants, as neutropenia may ensue during infection as a consequence of possessing a small neutrophil marrow storage pool. Functional defects in adhesion, chemotaxis, and bactericidal activity of neonatal PMN have also been reported (5, 6). Further limitations in the innate immune system of infants are observed in monocytes/macrophages, natural killer (NK) cells, and the complement system that remain below adult levels (7), with all of these factors predisposing infants to rapidly overwhelming infections. In addition to maturation-induced differences, immune abnormalities could also be expected in uninfected infants born to human immunodeficiency virus type 1 (HIV-1) seropositive mothers, resulting from exposure of the foetus to HIV-1 proteins or even whole viral particles.

Although the phagocytic cells of the innate immune system, especially neutrophils, have been reasonably well studied in newborn infants, this has usually been with blood from the umbilical cord as the extent of studies in any individual infant is severely limited by the volume of blood obtainable because of the baby's size. The main limitation of these reports has been identified as the use of cord blood rather than peripheral blood cells, where the distinction between developmental effects and the consequences of perinatal stress is unclear. A number of unanswered questions therefore remain concerning the time required for the maturation of infant PMN to acquire adult-like function, and the potential physiological role of the limitation of innate immunity in the early life context. This study was therefore conducted to assess the expression of a number of receptors important to normal PMN function, and the integrity of PMN degranulation responses in uninfected infants of varying ages born to HIV-1 seropositive mothers, the majority exposed to short-course antiretroviral

treatment, to determine if age-related differences existed in receptor expression or in the capacity of PMN to degranulate. In addition to examining maturation-associated changes, the effect of exposure to HIV-1 on PMN receptor expression and the integrity of degranulation responses in cord blood was assessed, since the majority of infants born to HIV-1 seropositive mothers would be uninfected at birth.

Materials and methods

Study samples

The infants enrolled for this study were all born to HIV-1 seropositive mothers at the Chris Hani Baragwanath Hospital in Soweto, which served as one of South African sites of the United Nations AIDS-sponsored clinical trial of short-course zidovudine-lamivudine (ZDV-3TC) to prevent mother-to-child transmission of HIV-1 (the Perinatal Transmission study, 8). Inclusion criteria for the trial were availability for enrolment at <38 wk gestation, haemoglobin > 80 g/l, and no prior antiretroviral drug exposure. None of the mothers (range: 16–30 yr of age) had any other microbial infections, and all mothers had CD4 T-cell counts > 200 cells/ μ l at the time of delivery. Women enrolled on the clinical trial were randomized to one of four groups. In the first arm, women received 300 mg ZDV and 150 mg 3TC twice daily from 36 wk. From the onset of labour, they received 300–600 mg ZDV, then 300 mg every 3 h and 150 mg 3TC every 12 h until delivery, and for 1 wk postpartum. Neonates were given 4 mg ZDV and 2 mg 3TC per kg twice daily for 1 wk. Women in the second arm of the trial received only the intrapartum and postpartum components of the regimen, while those in the third arm received only the intrapartum component. The fourth arm of the study, a placebo group, was suspended soon after sample collection for the present study had begun.

Venous blood samples were collected from infants of clinical trial participants at follow-up time points designated as part of the trial to determine HIV-1 infection outcome. A total of 198 blood samples were collected from infants aged between 4.5 and 18 months (4.5, 6, 9, 12, 15 and 18-month-old infants were used in this study). As a result of limited blood volumes, cross-sectional analysis was performed with as many tests as possible being conducted for each specimen, with 155 of the samples used for the evaluation of PMN degranulation and 43 used for PMN receptor expression analysis. Of the

155 infants recruited in this study to assess PMN degranulation responses, 153 were confirmed as remaining HIV-1 uninfected up to 18 months of follow-up. The two children who became infected with HIV-1 were excluded from the final analysis of the proportions of positive C5a- and IL-8-induced degranulation responses for each age group. All of the children used for PMN receptor expression analysis were confirmed as remaining HIV-1 uninfected up to 18 months of age. Cord blood samples were also collected from 13 infants born to HIV-1 uninfected mothers and from 12 infants born to mothers on a post-exposure prophylaxis (PEP) trial that was also conducted at Chris Hani Baragwanath Hospital. Women who had either received no prenatal care or who received prenatal care at community clinics where HIV-1 testing and access to antiretroviral drugs was unavailable were eligible for the PEP trial, where nevirapine was not used during labour, but given to the baby postnatally. All samples were collected into ethylenediamine-tetraacetic acid (EDTA) vacutainers (Becton Dickinson, San Jose, CA, USA) and processed within 6 h of collection. This study was approved by the University of the Witwatersrand Committee for Research on Human Subjects (Medical), and patients were recruited after informed consent had been obtained and the confidentiality of all records ensured.

Reagents

Recombinant human IL-8 was obtained from Boehringer Mannheim (Mannheim, Germany). Recombinant human C5a, phorbol 12-myristate 13-acetate (PMA), cytochalasin B, *p*-nitrophenyl- β -D-glucuronide and Histopaque-Ficoll were from Sigma Chemical Co. (St Louis, MO, USA). Stromal cell-derived factor (SDF)-1 α and SDF-1 β were obtained from R&D Systems (Minneapolis, MN, USA). Mouse monoclonal antibodies (mAbs) to CXCR1 (9H1) and CXCR2 (10H2) were supplied by Genentech, Inc. (San Francisco, CA, USA). Fluorescein isothiocyanate (FITC)-conjugated CD88 and CD62L, and the mouse isotype antibodies IgG1 and IgG2a, used as controls for CXCR1 and CXCR2 respectively, were obtained from Serotec (Oxford, UK). Secondary antibody was FITC-conjugated goat anti-mouse (GAM) obtained from Dako (Glostrup, Denmark). Fluorescence-activated cell sorter (FACS) lysing solution (10X concentrate) was from Becton Dickinson. The cell fixative was 1.5% (v/v) formaldehyde (Merck, Darmstadt, Germany) with 2% (w/v) bovine serum albumin (BSA; Sigma Chemical Co.).

Fluorescent-labelling of PMN in whole blood

Labelling of cells with CXCR1 and CXCR2 antibodies was performed using an indirect staining method as previously described (9). Briefly, 5 μ l of CXCR1 or CXCR2 antibody was added to 50 μ l whole blood (2.5 μ g/ml final concentration). Control antibodies for CXCR1 and CXCR2 staining were 5 μ l of mouse IgG1 or IgG2a, respectively, added to 50 μ l whole blood. The samples were then incubated with the antibodies for 20 min at room temperature, and washed twice with 3 ml of wash solution. After washing, 5 μ l of the secondary antibody, FITC-conjugated GAM, was then added to each of the samples, which were again incubated for 20 min at room temperature. Samples were then washed, the residual erythrocytes lysed with 2 ml of 1X FACS lysing solution, washed again and the cells resuspended in 200 μ l fixative which was 1.5% (v/v) formaldehyde containing 2% (w/v) BSA. Samples were stored at 4°C until analysis.

Labelling of cells for CD88 and CD62L expression was performed by adding 5 μ l of FITC-conjugated CD88 or CD62L antibody to 50 μ l whole blood. As a control, 5 μ l IgG1-FITC was added to 50 μ l whole blood. Samples were incubated with the antibodies for 20 min at room temperature, and the red blood cells lysed with 2 ml 1X FACS lysing solution. The cells were then washed and resuspended in 200 μ l of fixative. Samples were stored at 4°C until analysis.

Flow cytometry

All samples stained were acquired and analysed on a Becton Dickinson FACSsort flow cytometer with a 488 nm argon laser. Granulocyte populations were gated using forward (FSC) and side light scatter (SSC) characteristics. Data was analysed using CELLQUEST version 3.1 software (Becton Dickinson) and expressed as the percentage of cells expressing CXCR1, CXCR2, CD88, or CD62L and their respective fluorescence intensities.

Separation of neutrophils

Anticoagulated whole blood was diluted 1:1 with phosphate-buffered saline (PBS) and centrifuged for 30 min at room temperature on a Histopaque-Ficoll gradient. Following removal of the mononuclear cell layer, the remaining PMN/erythrocyte layer was over-layered onto a

secondary Ficoll gradient and centrifuged as before. The PMN layer was then removed from the second gradient, the residual erythrocytes lysed with a solution of 0.15 M NH_4Cl , 10 mM KHCO_3 , and 1 mM sodium EDTA, pH 7.2, and the PMN washed twice with PBS. Viability of the purified PMN suspensions were >98% as determined by trypan blue exclusion.

β -glucuronidase bioassay

Polymorphonuclear neutrophil degranulation was measured in response to IL-8, C5a, SDF-1 α , SDF-1 β , and PMA by the release of β -glucuronidase as described by Schröder et al. (10). The concentration of PMN was adjusted to $10^7/\text{ml}$ and cytochalasin B added to a final concentra-

tion of 5 $\mu\text{g}/\text{ml}$. Aliquots of 100 μl of the cell suspension were placed in a 96-well round-bottom plate and incubated for 15 min at 37°C. Human IL-8 test samples at a low (15.63 ng/ml) and a high (500 ng/ml) input concentration, and human C5a test samples at a low (15.63 ng/ml) and a high (1000 ng/ml) input concentration, each in a total volume of 100 μl , were added to separate wells and the plate was incubated for a further 30 min at 37°C. Human SDF-1 α and SDF-1 β were tested at a low (31.25 ng/ml) and a high (1000 ng/ml) input concentration, while PMA was tested at 1 and 25 ng/ml as the low and high input concentrations respectively. The cells were then pelleted at 300 g for 10 min at 4°C and 100 μl of the supernatant transferred to the wells of a 96-well flat-bottom plate containing

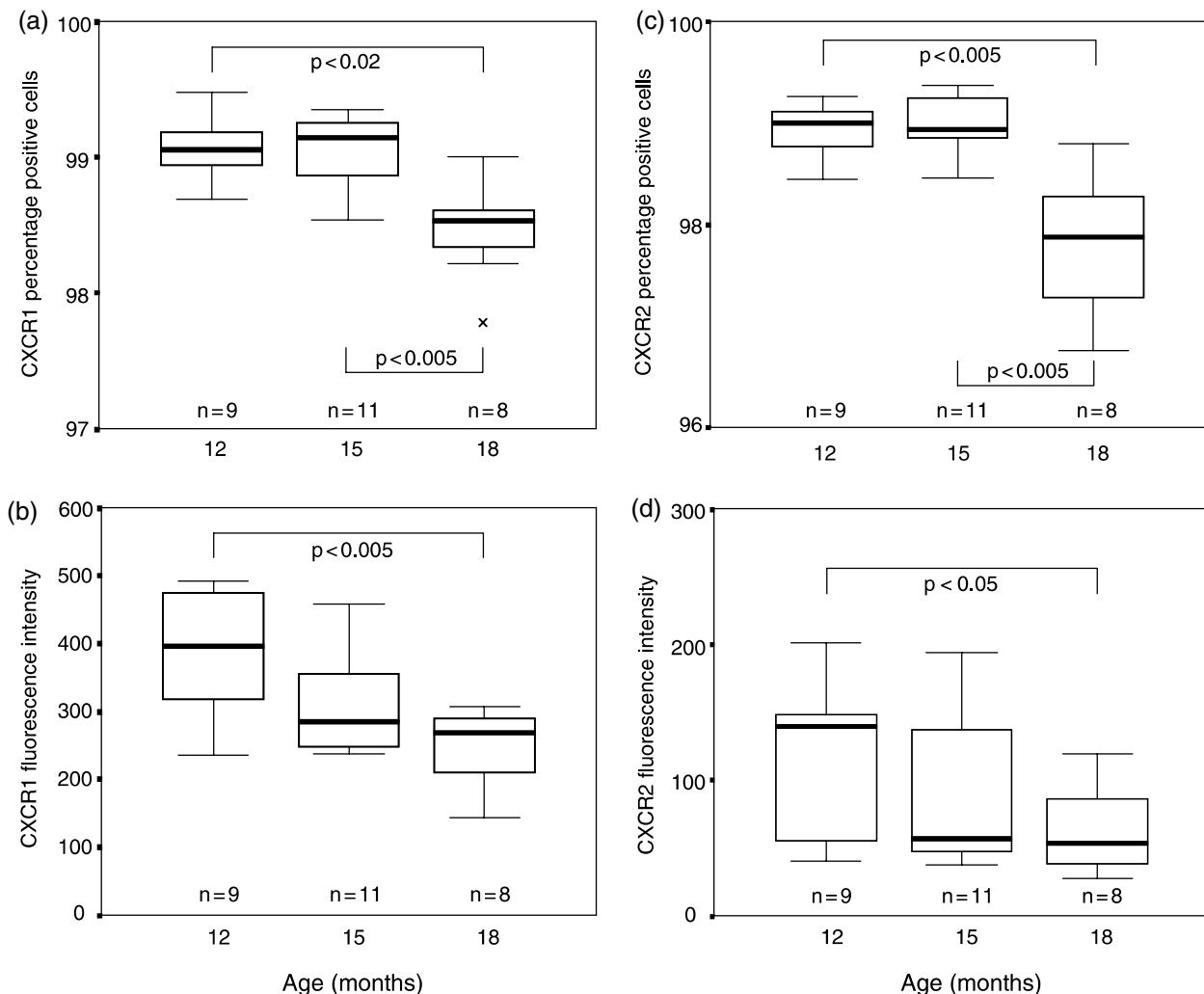


Fig. 1. CXCR1 and CXCR2 staining of polymorphonuclear neutrophil (PMN) from infants aged 12, 15 and 18 months. PMN were gated and the proportion of cells expressing CXCR1 (a) and CXCR2 (c) were determined. Corresponding relative fluorescence intensities of CXCR1 (b) and CXCR2 (s) are also shown. Data are presented as medians (horizontal bar), 25th and 75th percentiles (boxes), and 10th and 90th percentiles (bars). Significant differences between groups are indicated.

100 μ l 0.01 M *p*-nitrophenyl- β -D-glucuronide in 0.1 M sodium acetate pH 4.0. The plate was incubated overnight at 20°C, the reaction stopped with 100 μ l 0.4 M glycine buffer (pH 10) and the absorbance read at 405 nm. For the determination of total β -glucuronidase content in PMN, 5×10^5 cells were lysed in 100 μ l of 1% Triton-X/PBS (v/v). The release of β -glucuronidase at different IL-8 and C5a concentrations was calculated as the optical density (OD) at 405 nm obtained at a particular IL-8 or C5a concentration divided by the total OD 405 nm of the PMN lysate, expressed as a percentage. Degranulation responses were defined as either positive, negative (reciprocal), or unresponsive as previously described (11, 12). For analysis in this study, all infants in a particular age group showing an unresponsive or reciprocal PMN

degranulation response were grouped together to allow comparison against the number of positive degranulation responses in that group.

Results

Expression of CXCR1, CXCR2, CD88 and CD62L on whole blood PMN in relation to age of the infants

The expression of CXCR1 and CXCR2 on PMN was determined for 28 infants ranging between 12 and 18 months of age, while CD88 and CD62L expression was measured for 32 infants aged between 12 and 18 months. The proportion of PMN expressing CXCR1 was significantly lower in infants aged 18 months than those in the 12 ($p < 0.02$) and 15 month ($p < 0.005$) age groups (Fig. 1a). CXCR1 fluorescence intensity

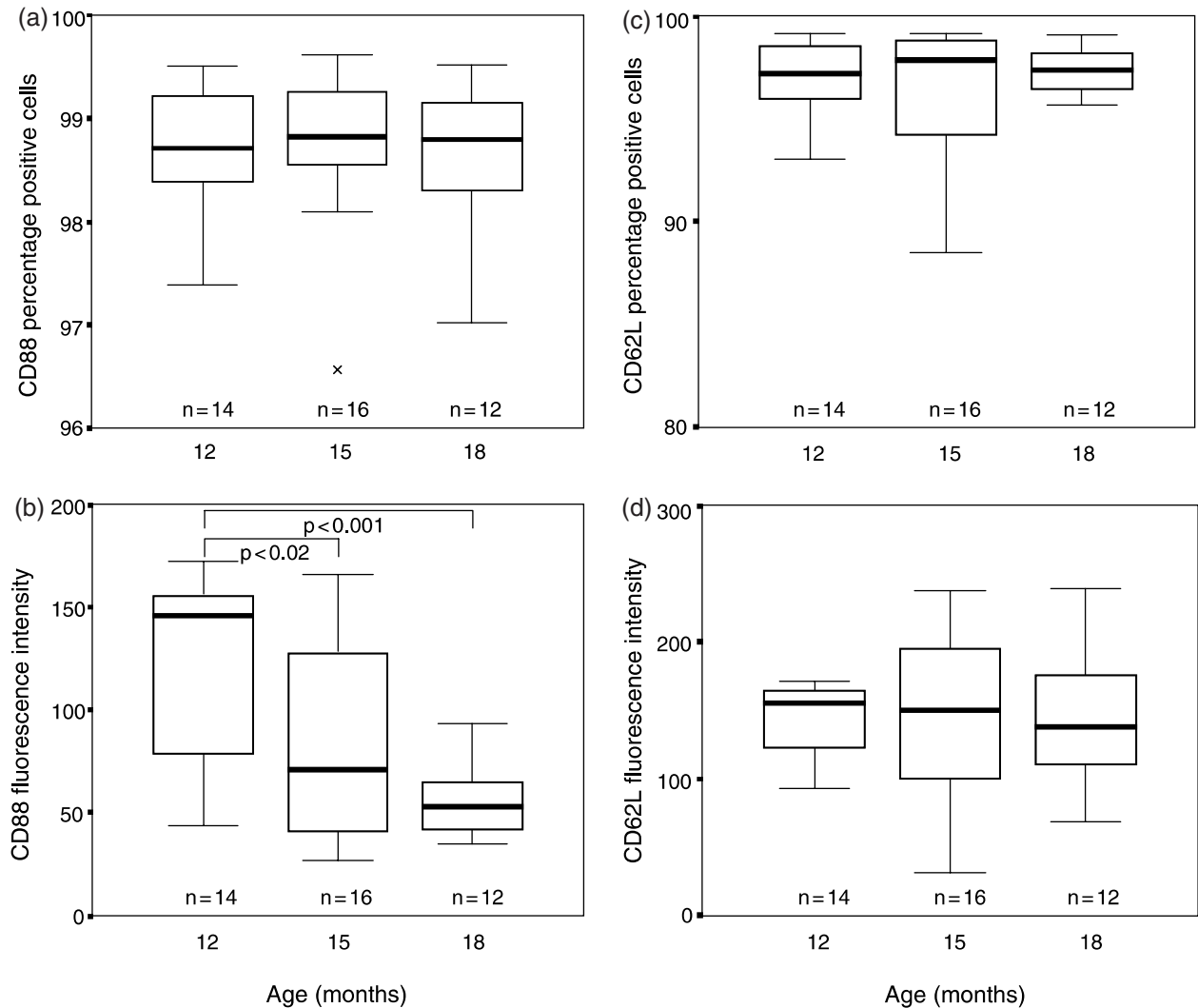


Fig. 2. CD88 and CD62L staining of polymorphonuclear neutrophil (PMN) from infants aged 12, 15 and 18 months. PMN were gated and the proportion of cells expressing CD88 (a) and CD62L (c) were determined. Corresponding relative fluorescence intensities of CD88 (b) and CD62L (d) are also shown. Data are presented as medians (horizontal bar), 25th and 75th percentiles (boxes), and 10th and 90th percentiles (bars). Significant differences between groups are indicated.

also declined with increasing age of the children, and was significantly lower in infants aged 18 months compared with the 12 month old age group ($p < 0.005$) (Fig. 1b). Similar age-related variations to CXCR1 were also seen for the other IL-8 receptor, CXCR2, where the percentage of PMN expressing CXCR2 was significantly reduced in infants aged 18 months compared with those in the 12 ($p < 0.005$) and 15 month ($p < 0.005$) age groups (Fig. 1c). The fluorescence intensity of CXCR2 on PMN was significantly lower in infants in the 18 month

age group than those aged 12 months ($p < 0.05$) (Fig. 1d), and while CXCR2 fluorescence intensity was reduced in the 15 month age group compared the 12-month-old infants, this did not attain significance ($p > 0.05$). Although no differences were observed in the proportion of PMN expressing CD88 between the three age groups (Fig. 2a), CD88 fluorescence intensity was significantly lower in the infants aged 15 ($p < 0.02$) and 18 months ($p < 0.001$) compared with those in the 12 month age group (Fig. 2b). No age-related variations between the

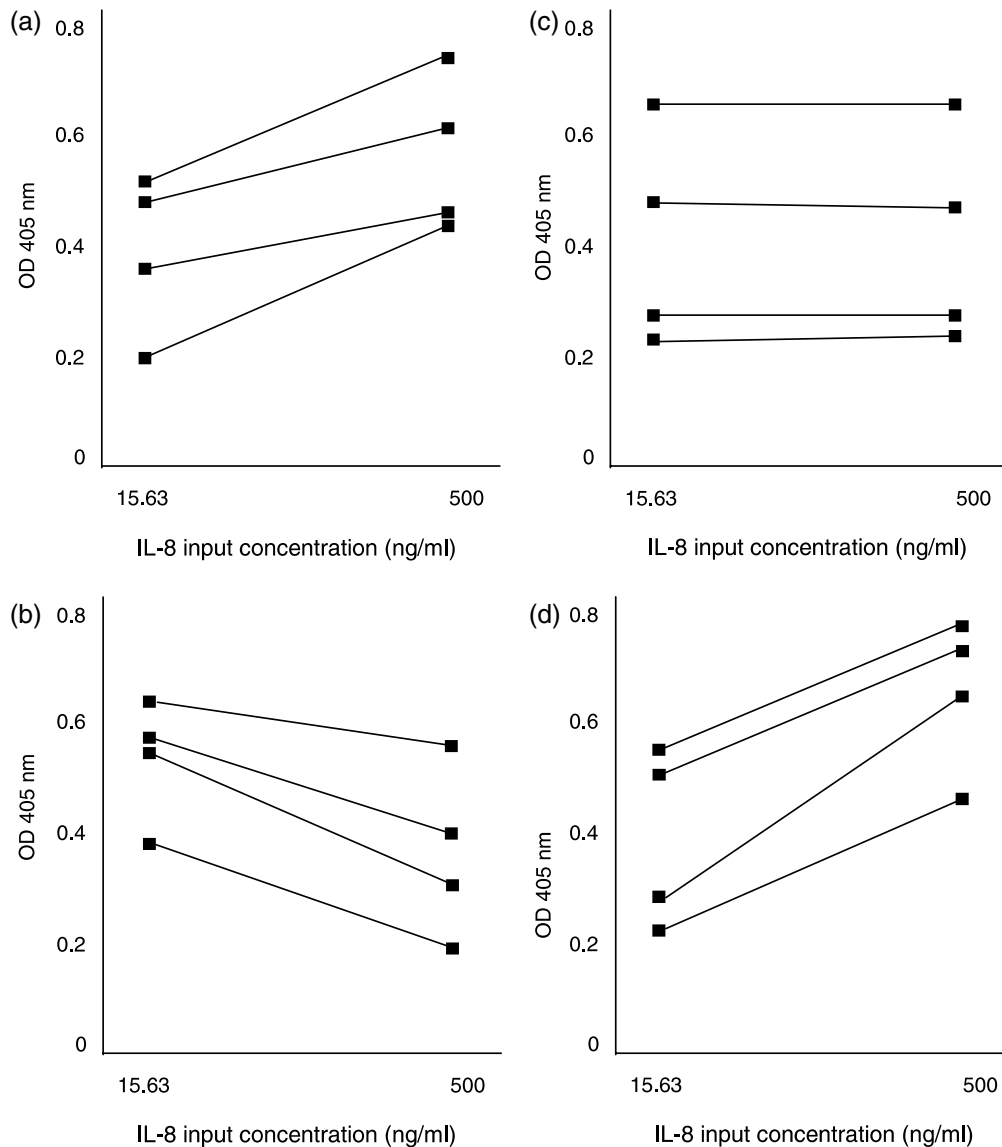


Fig. 3. Representative data showing interleukin-8 (IL-8) stimulated degranulation assays to illustrate types of responses defined in this study as either positive (greater release of β -glucuronidase at the higher input concentration of IL-8 than at the lower) (a), negative/reciprocal (greater release of β -glucuronidase at the lower input concentration of IL-8 than at the higher) (b) or unresponsive (no difference in release of β -glucuronidase between the two concentrations of IL-8 used) (c) assays. For comparison normal adult IL-8-induced PMN degranulation responses are also shown (d).

three study groups were found with respect to either the percentage of PMN expressing CD62L (Fig. 2c) or their respective fluorescence intensities (Fig. 2d).

Degranulation of PMN in infants of different ages in response to IL-8, C5a, SDF-1 α , SDF-1 β and PMA

In order to evaluate the integrity of PMN degranulation responses over time in the developing infant, β -glucuronidase release in response to a variety of stimuli was measured in infants of varying ages. Degranulation in response to IL-8 and C5a was measured in infants ranging from 4.5 to 18 months of age. PMN from 43 infants were tested for IL-8-induced degranulation while 52 infants were tested for C5a-induced degranulation. Fig. 3 shows the different types of PMN degranulation responses defined in this study, demonstrating representative positive (a), negative (b) and unresponsive (c) results obtained in IL-8-stimulated infant assays. For comparison healthy adult IL-8-induced PMN degranulation responses are also shown (Fig. 3d). Fig. 4 shows the proportion of children in each age group who showed a positive (healthy adult-like) PMN degranulation response when stimulated with either IL-8 or C5a. The proportion of infants with a positive degranulation response increased with increasing age for both IL-8 and C5a stimulation.

Degranulation was also measured in response to SDF-1 α , SDF-1 β , and PMA for 22, 16, and 22 infants, respectively. As these assays were only carried out towards the end of the PETRA trial, only older children were available, and in this case responses were determined for infants ranging from 12 to 18 months of age. Similar trends were, however, seen compared with IL-8- and C5a-

induced degranulation, where a greater proportion of positive responses were seen in the older children, and by 18 months of age, 100% of children showed positive SDF-1 α , SDF-1 β and PMA-induced degranulation responses (Fig. 5). Of the 155 infants recruited in this study to assess PMN degranulation responses, 153 were confirmed as remaining HIV-1 uninfected up to 18 months of follow-up. A sample from one of the children who became infected with HIV-1 [HIV-1 polymerase chain reaction (PCR)-negative at 6 wk, HIV-1 PCR-positive at 3 months] was collected at 9 months of age, and this child showed unresponsive C5a-induced PMN degranulation at this time point. The other infected child (timing of infection unknown) was tested for IL-8-induced PMN degranulation at 12 months of age, and in this case a positive degranulation response was observed. These two children were excluded from the final analysis of the proportions of positive C5a- and IL-8-induced degranulation responses for each age group. Similar proportions of impaired responses were observed in infants born to mothers randomized to all four arms of the clinical trial (data not shown), and although the numbers for each group were small, there was no indication that prior exposure to short-course antiretroviral drugs had any effect on the integrity of PMN degranulation responses.

Ability of cord blood PMN from HIV-1 exposed uninfected infants and HIV-1 unexposed infants to degranulate in response to IL-8, C5a, SDF-1 α , SDF-1 β and PMA

Although all the infants analysed in the first section of this study remained HIV-1 uninfected despite being born to HIV-1 seropositive mothers, exposure to the virus may have contributed to the

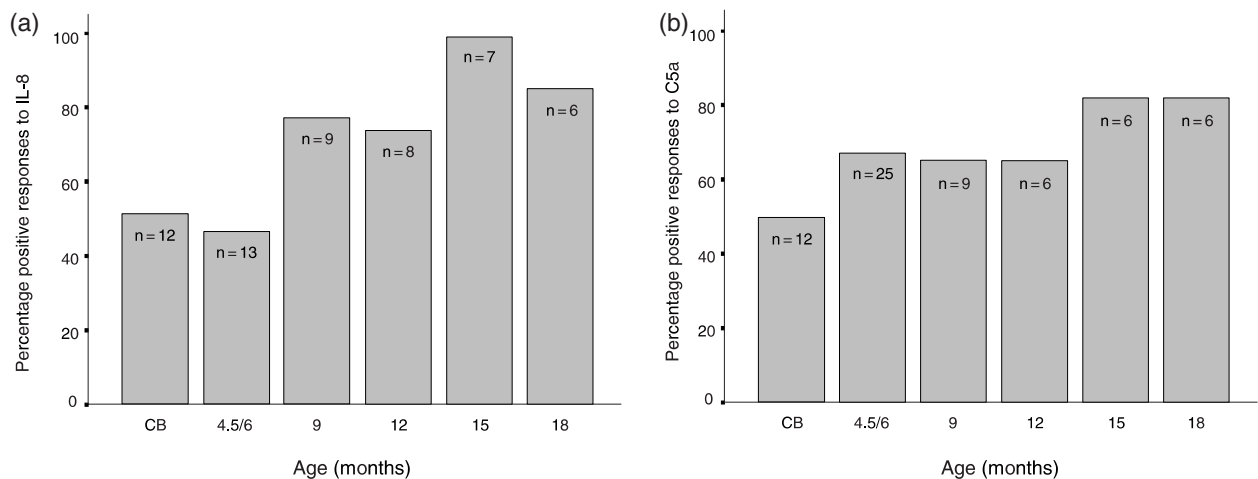


Fig. 4. Percentage positive degranulation responses of cord blood polymorphonuclear neutrophil (PMN) and PMN from infants between 4.5 and 18 months of age in response to interleukin-8 (IL-8) (a) and complement 5a (C5a) (b).

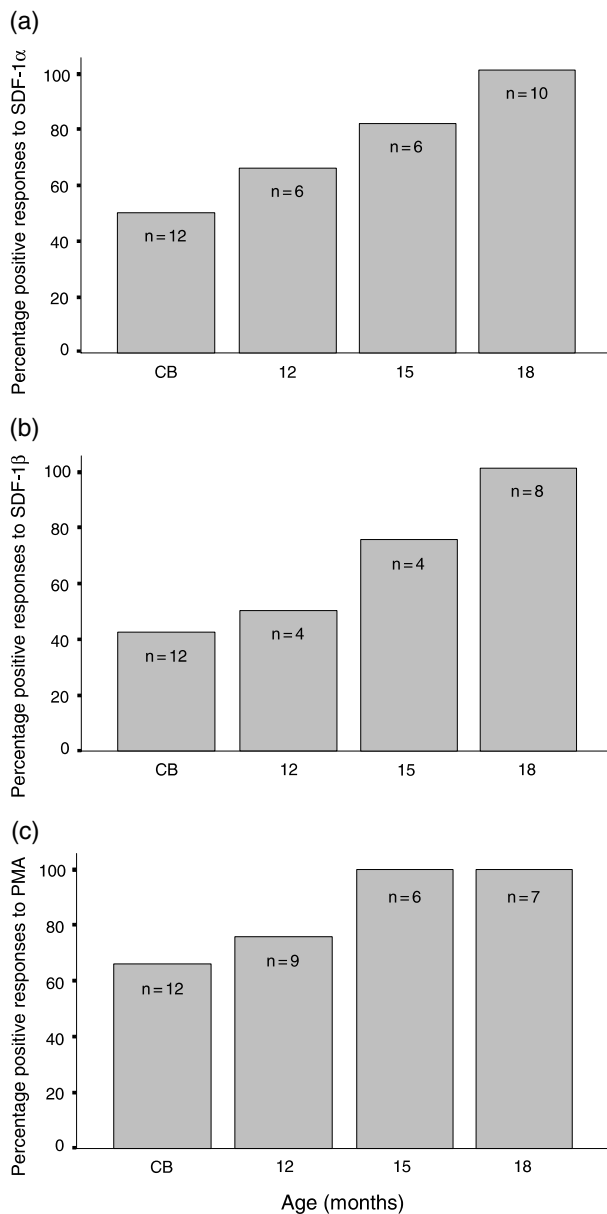


Fig. 5. Percentage positive degranulation responses of cord blood polymorphonuclear neutrophil (PMN) and PMN from infants between 12 and 18 months of age in response to stromal cell-derived factor (SDF)-1 α (a), SDF-1 β (b) and phorbol 12-myristate 13-acetate (PMA) (c).

impaired PMN degranulation responses that were observed. Therefore, in addition to measuring PMN degranulation responses in infants of varying ages, the ability of cord blood PMN from infants born to both HIV-1 infected and HIV-1 uninfected mothers to degranulate was also assessed. Degranulation in response to IL-8, C5a and PMA was measured in cord blood PMN from 13 infants born to HIV-1 uninfected mothers, while degranulation in response to IL-8, C5a, SDF-1 α , SDF-1 β and PMA was determined for cord blood PMN from infants of 12 HIV-1

Table 1. Frequencies of IL-8, C5a, SDF-1 α , SDF-1 β and PMA induced cord blood PMN degranulation responses

	Positive responses, n (%)	Reciprocal/ unresponsive, n (%)
HIV-1 uninfected mothers (n = 13)		
IL-8	8 (61.5)	5 (38.5)
C5a	7 (53.8)	6 (46.2)
PMA	9 (69.2)	4 (30.8)
HIV-1 infected mothers (n = 12)		
IL-8	6 (50.0)	6 (50.0)
C5a	6 (50.0)	6 (50.0)
SDF-1 α	6 (50.0)	6 (50.0)
SDF-1 β	5 (41.7)	7 (58.3)
PMA	8 (66.7)	4 (33.3)

IL-8, interleukin-8; C5a, complement 5a; SDF, stromal cell-derived factor; PMA, phorbol 12-myristate 13-acetate; PMN, polymorphonuclear neutrophil; HIV-1, human immunodeficiency virus type 1.

infected mothers. The proportions of positive and reciprocal/unresponsive degranulation responses for cord blood PMN from infants born to the HIV-1 uninfected controls and the HIV-1 seropositive mothers are shown in Table 1.

Cord blood PMN from a substantial proportion of infants born to the HIV-1 uninfected mothers were reciprocal/unresponsive to IL-8 [five of 13 (38.5%)], C5a [six of 13 (46.2%)] and PMA [three of 12 (25%)]. Cord blood PMN from infants born to the HIV-1 infected mothers also showed large proportions of reciprocal/unresponsive degranulation reactions when stimulated with either IL-8 [six of 12 (50%)], C5a [six of 12 (50%)], SDF-1 α [six of 12 (50%)], SDF-1 β [seven of 12 (58.3%)], or PMA [four of 12 (33.3%)]. The percentage of positive cord blood PMN responses for the different stimuli observed in infants of HIV-1 infected mothers are also shown in Figs 4 and 5 to allow comparison with the proportions of positive responses observed for the different aged infants. No significant differences were, however, observed between cord bloods PMN responses from infants born to the HIV-1 uninfected and HIV-1 infected mothers when comparing the proportions of positive or reciprocal/unresponsive PMN reactions for the different stimuli used ($p > 0.05$).

Discussion

In this study, the expression of a number of receptors vital to normal PMN function were quantified, and PMN degranulation was measured to assess the integrity of PMN responses in infants of varying ages. Although the expression of CD62L on PMN did not differ between the

infants aged 12, 15 and 18 months, the expression of the IL-8 receptors CXCR1 and CXCR2, and the C5a receptor CD88 displayed a similar pattern, with the highest levels expressed on PMN from infants in the 12 month old age group, and declining with age. For the IL-8 receptors, both the proportion of PMN expressing these receptors as well as their respective fluorescence intensities declined with age, while for CD88, only fluorescence intensity showed age-related differences.

The recruitment of leucocytes from the bloodstream to sites of inflammation is controlled by a sequential cascade of events which involves several adhesion molecules, with the initial interaction between circulating leucocytes and endothelial cells being mediated by CD62L (2). According to most reports, PMN derived from neonates adhere less well than cells obtained from adults (13, 14), which is thought to be caused by a failure to regulate CD62L expression. Previous studies have demonstrated a significantly reduced surface expression of CD62L on PMN from newborns compared with adult PMN (15, 16), although age-related differences in CD62L expression in developing infants have not been reported. No differences in CD62L expression were observed between the different age groups in this study indicating that CD62L expression on PMN may rise rapidly within the first few months of life after the neonatal period to adult-like levels. Further longitudinal studies are required to measure baseline levels of CD62L on cord blood PMN, and to follow infants from birth to determine changes in CD62L expression during early life.

As for CD62L, no reports concerning age-related modulation of the expression of the IL-8 receptors, CXCR1 and CXCR2 in developing infants have been reported. In this study, expression of both IL-8 receptors and CD88 declined with age. Since samples for this section of the study were collected from the PETRA study participants at the latter end of the trial, we were only able to collect 12, 15 and 18-month-old infants that therefore represented a fairly narrow range of ages. The collection of cord blood specimens and a larger number of samples from infants younger than 12 months of age would provide more information about the expression of these receptors at a time when major immune system development and maturation is occurring.

Neutrophil intracellular granules serve as a source of hydrolytic enzymes and antimicrobial proteins, which during phagocytosis are released into the extracellular space. Although decreased in number, the mobilization of all granule types

by a variety of stimuli is comparable in PMN from adults and infants (17). In particular, exocytosis of β -glucuronidase, the marker of PMN degranulation assessed in this study, has been found to be equivalent in PMN from adults and infants (17, 18). No previous reports have, however, evaluated the integrity of PMN degranulation responses over time in the developing infant, and therefore in this study, PMN degranulation in children of varying ages was measured to assess the maturity and development of these responses. It was observed that PMN from a substantial proportion of the younger infants were reciprocal/unresponsive, with the proportions of children showing positive PMN degranulation responses increasing with age. We have previously evaluated the integrity of IL-8-induced PMN degranulation responses in HIV-1 infected and uninfected adults (11), and all uninfected adults assessed were shown to have positive degranulation responses. In addition, we have previously measured PMN degranulation, in response to IL-8 and C5a, in a group of children vertically infected with HIV-1 and a group of uninfected control children (12). All the uninfected control children in this study were also found to have normal positive degranulation responses to IL-8, with 14 of 15 showing a positive response to C5a with PMN from one control child, aged 30 months, being unresponsive.

These results support that PMN from infants are immature in function, with an improvement in the integrity of degranulation responses as the child ages. Considering the infants in this study were all born to HIV-1-infected mothers, it is possible that impaired degranulation responses may have been a consequence of exposure to HIV-1. Although not reported for PMN function, developmental and functional abnormalities have been shown in CD4 and CD8 T-lymphocyte populations from HIV-1 exposed uninfected infants (19–21). HIV-1 exposure effects on PMN function could also be anticipated considering the direct effects of HIV-1 on PMN that have been reported. These include the HIV-1 transactivator protein (Tat) that can stimulate PMN chemotaxis, calcium mobilization and respiratory burst (22), and a peptide derived from the N-terminus region of the HIV-1 envelope protein gp41 that induces directional migration and calcium mobilization in PMN (23). It is unlikely however that exposure to HIV-1 could explain the defects in PMN degranulation observed in this study, considering that a large proportion of PMN from cord blood of infants born to HIV-1 uninfected mothers were also

unresponsive. There was also no indication that prior exposure to antiretroviral drugs was associated with impaired degranulation, since defective responses were observed in infants born to mothers randomized to all four arms of the clinical trial. Since antiretroviral drugs were only administered before and during delivery to the mothers and in some cases for 1 wk postpartum to the neonates, and infant samples were only collected at the earliest at 4.5 months of age, these results do not, however, preclude the possibility that the drugs may alter certain neutrophil functions if tested at the time of administration.

While the proportions of children showing positive degranulation responses increased with age for all the stimuli used, 100% of SDF-1 α , SDF-1 β and PMA-induced responses were positive by 18 months of age, while reciprocal/unresponsive IL-8- and C5a-induced degranulation responses were still observed at 18 months, indicating that PMN degranulation in response to these two stimuli may take longer to mature than for SDF-1 α , SDF-1 β and PMA. Although PMA-induced PMN degranulation is not receptor-dependent, SDF-1 α , and SDF-1 β trigger PMN degranulation via the HIV-1 co-receptor CXCR4. The expression of CXCR4 on PMN has been found to be lowest in 4.5-month-old infants, increasing to adult-like levels by 15 months of age, which may explain improvements in PMN degranulation as children grow older. Increased receptor expression cannot, however, explain improvements in IL-8- and C5a-induced degranulation with age as the expression of these receptors actually declined with increasing age of the children. In the context of HIV-1 infection of adults, there is a generalized reduction in the expression of CXCR1, CXCR2, and CD88 on PMN (9, 24), although it appears that infection of young children with HIV-1 may play a lesser role in the overall modulation of CXCR2, and especially that of CXCR1 (12). In HIV-1 infected adults, where we have previously observed that individuals that had the poorest ability to degranulate were also those who had the lowest density of CXCR1 on their PMN (11), this could not explain impaired IL-8-induced degranulation responses in the HIV-1-infected children in our previous study (12), as CXCR1 expression was largely unaltered in the HIV-1 disease groups. In addition, impaired C5a-induced PMN degranulation in HIV-1 infected adults has been found not to be associated with the reduced expression of CD88 (24). Collectively these results indicate that although altered receptor expression may influence PMN function, there are probably a

number of factors involved which result in impaired responses (25).

In conclusion, these results show that substantial age-related modulation of the expression of a number of receptors important in PMN function is evident in early life, and that in addition to functional defects in adhesion, chemotaxis, and bactericidal activity of neonatal PMN that have previously been reported (5, 6), deficiencies exist in degranulation responses in young infants, although it appears that these responses mature over time. As PMN play such a vital role in the clearance of invasive organisms, the altered expression of these important receptors and inefficient agonist-induced degranulation in early life may contribute to the increased susceptibility of infants to secondary microbial infections.

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