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Diminished Response to Interleukin-10 and Reduced Antibody-Dependent Cellular Cytotoxicity of Cord Blood Monocyte-Derived Macrophages

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ABSTRACT: Monocyte-derived macrophage (MΦ) subsets are generated by antagonistic induction pathways. A helper MΦ-type (Mh-MΦ) is induced by interferon gamma (IFN-γ), whereas a cytotoxic MΦ-type (Mc-MΦ), induced by interleukin-10 (IL-10), is a potent mediator of antibody-dependent cellular cytotoxicity (ADCC). Compared with MΦ from healthy adults [peripheral blood monocyte-derived macrophages (PBMΦ)], cord blood MΦ (CBMΦ) were found less capable of generating Mh-MΦ. Here we tested the hypothesis that their generation of Mc-MΦ via IL-10 is also impaired. MΦ surface markers were phenotyped. IL-10 protein and mRNA production were detected after stimulation [αCD3 monoclonal antibody (mAb)]. CBMΦ or PBMΦ were co-cultured with MΦ-depleted mononuclear cells of adults and CD4-targeting antibodies as models for ADCC were added. In cord blood, we found diminished αCD3-induced IL-10 protein and mRNA production ($p < 0.05$ *versus* adults). Basal CD16 and HLA-DR expressions on CBMΦ of preterm and full-term neonates were lower ($p < 0.05$ *versus* PBMΦ). IL-10 had reduced effects on CD16 up- and HLA-DR down-modulation on CBMΦ ($p < 0.05$ *versus* PBMΦ). CD4-directed receptor modulation and deletion were reduced in the presence of CBMΦ ($p < 0.05$ *versus* PBMΦ). IL-10 failed to enhance their ADCC capacity, which was in contrast to PBMΦ ($p < 0.05$). These data suggest that CBMΦ have an impaired cytotoxic capacity via lower sensitivity toward IL-10. **(*Pediatr Res* 60: 152–157, 2006)**

Qualitative characteristics of immune responses are regulated by immune cell subsets through their production of distinct cytokines. TH1 cytokines, *e.g.* IFN-γ, play an important role in the inflammatory response and promote cell-mediated responses against bacteria, whereas TH2 cytokines, *e.g.* IL-10, induce antibody synthesis and promote immunoregulatory signals. Evidence exists that the neonatal period is characterized by a TH2-dominated immune response with a hyporesponsive inflammatory component (1,2). In contrast, clinical observation reveals a more severe parainfectious systemic inflammatory response in neonates than in children and adults, often with detrimental sequelae (3). Consistent with these findings, neonates show an enhanced capacity to produce proinflammatory cytokines during infections (4) and inflammatory diseases, *e.g.* bronchopulmonary dysplasia (5) or periventricular leukomalacia (6).

Cytokines orchestrate T cell immune responses either directly or indirectly via antigen presenting cells, *e.g.* blood monocytes or MΦ. Via antagonistic cytokine induction pathways, at least two MΦ subsets, displaying distinct phenotypes and functions, have been distinguished (7): IFN-γ generates a costimulatory helper MΦ-type (Mh) which expresses and up-regulates B7 family receptors (CD80, B7-1 and CD86, B7-2) (8). Its engagement of T cell CD28 family molecules influences T cell survival, activation, and TH1/TH2 cytokine production [for review, see Salomon and Bluestone (9)]. In contrast, IL-10 not only mediates inhibitory and immunoregulatory signals, but induces a cytotoxic MΦ-type (Mc) (8), which fails to up-regulate HLA-DR and B7 molecules. Mc-MΦ instead express Fc-gamma III receptors (CD16) in high density (7). Mc-MΦ are potent mediators that destroy antibody-coated, *e.g.* virus-infected, cells by ADCC via induction of apoptosis (10).

We found CBMΦ less capable of generating Mh-MΦ due to diminished IFN-γ effects on B7 molecules, thereby inhibiting MΦ-dependent T cell activation (11,12). Here, we tested the hypothesis that their generation of Mc-MΦ via IL-10 in cord blood is also impaired.

MATERIALS AND METHODS

Patients. The study protocol was approved by the Ethics Committee of the University of Tuebingen. All mothers had given written consent before they went into labor. Randomly selected, adult healthy donors served as controls.

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Abbreviations: ADCC, antibody-dependent cellular toxicity; **αCD4,** mAb anti-CD4 monoclonal antibody; **CBMΦ,** cord blood monocyte-derived macrophages; **CBMNCs,** cord blood mononuclear cells; **gp120,** HIV glycoprotein 120; **IFN-γ,** interferon gamma; **MΦ,** monocyte-derived macrophages; **PBMΦ,** peripheral blood monocyte-derived macrophages; **PBMNCs,** peripheral bloodmononuclear cells

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All term neonates were delivered spontaneously and did not exhibit signs of infection, as defined by the clinical status, and, if necessary, C-reactive protein. Ten preterm neonates (medium gestational age 29.5 wk; range, 25.5–32.6 wk; medium birth weight, 1350 g; range, 650–1690 g) were delivered for maternal reasons [cervix insufficiency ($n = 5$), preeclampsia ($n = 4$), or placental ablation ($n = 1$)]. Maternal antibiotics and antenatal steroids (up to 4 wk before labor) were given to seven of 10 preterm neonates, four infants were from multiparous pregnancies. Although six preterm infants were treated with antibiotics, none developed serological or clinical signs of early-onset bacterial infection. Mothers with amnion infections or prolonged labor were excluded. Umbilical cord blood was drawn by puncture with a sterile needle and placed in heparin-coated tubes (100 IE/mL blood) immediately following cord ligation.

Cell cultures. Peripheral blood and cord blood mononuclear cells (PBMNCs and CBMNCs) were isolated by density gradient centrifugation (Biochrom AG, Berlin, Germany). Washed cells were resuspended in VLE RPMI 1640 (Biochrom), containing 10% FCS (Biochrom). Cells were counted in an ultraplane Neubauer hemocytometer, placed at 2×10^6 cells/mL in flat-bottom, 24-well cell culture plates (Costar, Bodenheim, Germany) and incubated at 37°C.

Preparation of mononuclear cell subsets. To separate MΦ from lymphocytes, cells were plated at 3×10^6 cells per 1.5 mL in 60 × 15-mm culture vessels (NoK4-3802-4, BD Biosciences, Heidelberg, Germany) in the incubator and allowed to adhere for 60 min. Nonadherent cells were gently removed by repeatedly pipetting 500 μL of RPMI buffer into the cultures. Remaining adherent cells were twice thoroughly washed and used as a source of MΦ. Usually, 95% of adherent cells were viable and 85% expressed CD14. Remaining $CD14^-$ cells were negative for the natural killer cell receptor CD56. To further eliminate contaminating nonadherent MΦ, the procedure described above was repeated with the nonadherent cell fraction. Usually, <1% $CD14^+$ MΦ were found in the nonadherent fraction after this adherence cycle, as determined by fluorescence-activated cell sorter analysis.

Co-culture experiments. MΦ-enriched adherent cells (1×10^6/0.5 mL) and MΦ-depleted nonadherent cells (2×10^6/0.5 mL) were mixed. Nonadherent cell fractions were not pooled from different donors. To equalize allogeneic effects, CBMΦ and PBMΦ of one adult donor were cocultured with nonadherent, nonirradiated cells of a second adult donor. In none of the coculture experiments did we see any significant T cell deletion in the absence of deleting reagents, as observed earlier (10). Anti-CD3-mediated T cell proliferation required the presence of MΦ or the addition of a co-mitogenic anti-CD28 mAb (L293 low azide, 1 μg/mL, BD Biosciences,) and was assessed by Vybrant CFDA SE Cell Tracer Kit (Molecular Probes, Eugene, OR) according to the manufacturer's protocol.

IL-10 quantification by electroimmunoassay. IL-10 was quantified in supernatants using human IL-10 OptEIA (BD-PharMingen, Freiburg, Germany) according to the manufacturer's instructions. The OD was measured photometrically at 450 nm in an enzyme-linked immunosorbent assay reader Spectra (Tecan, Frankfurt, Germany). Samples were analyzed in duplicate and blotted against a standard curve (Mathcad Professional 2000). The assay detected values as low as 7.8 pg/mL.

Flow cytometry. A FACScan flow cytometer (BD Biosciences) was used. To prevent nonspecific binding, cells were incubated with 10% human serum on ice for 10 min before staining with fluorescein-isothiocyanate-, phycoerythrin-, or isotype-specific immunoglobulin-labeled mAb for 20 min in the dark. MΦ were gated by forward scatter, side scatter, and CD14 expression. Dead lymphocytes were discriminated by propidium iodide (PI) (Molecular Probes, 5 μg/mL, 5 min). PI-negative cells were counted, double stained for CD4/CD3 and CD8/CD3, and analyzed for expression of CD4 and CD8. The percentage of deleted T cells was determined as previously described (13) applying the formula: percentage of deleted T cells = 1 − remaining T cells = 1 − (T cells cultured with deleting reagent at day 2: T cells cultured without deleting reagent at day 2) × 100.

mRNA quantification. For RNA extraction, the MagNA Pure LC RNA Isolation Kit I (Roche, Mannheim) was used as described previously (14). Synthesis of cDNA was performed using the First Strand cDNA Synthesis Kit (Roche). Amplification and quantification of cDNA were performed using a standard LightCycler protocol and external standardization, based on fluorescence resonance energy transfer (FRET) with specific oligonucleotides (14). External standards consisting of purified polymerase chain reaction products were quantified by spectral photometry, followed by serial dilution to obtain defined amounts of input copy numbers. All results were normalized against the expression of the housekeeping gene ALAS (5-aminolevulinate synthase gene)

Reagents. To target CD4 T cells that we used a chimeric humanized antibody (anti-CD4 mAb, αCD4; ch 412, 1 μg/mL; gift from Gerd Riethmuller, Munich, Germany) and immune complexes, consisting of recombinant HIV-1 gp120SF2 (1 μg/mL, kindly provided by K. Steimer, Chiron Biocine, Emeryville, CA) and serum from an HIV seropositive donor (New York Medical College, Valhalla, NY; final dilution 1:1000) as source of anti-gp120 (αgp120) antibody as previously described (15). The T cell mitogen anti-CD3 (αCD3 mAb; OKT3, 5 μg/mL) was used as a soluble antibody and purchased from Ortho Diagnostics (Raritan, NJ). Antibodies to CD14 (MΦP9), CD80 (L307.4), HLA-DR (L243), CD3 (SK7), CD56 (NCAM16.2), CD8 (SK1), CD28 (L293), and immunoglobulin (Ig)-matched controls (IgG1, IgG2b) were purchased from BD Biosciences. Remaining CD4 T cells were detected by OKT4 (Ortho), which is a non-cross reactive antibody to ch412 (10).

Data display and statistical analysis. Fluorescence intensities were determined and the nonspecific background staining was subtracted. Results are expressed as mean ± SD. Statistical analysis was performed by using the decadic logarithm of the values of CD16, HLA-DR, and CD80. Using analysis of variance, it was examined whether the variables were influenced by IL-10 or age (both nominal effects). Using an analysis of covariance, it was examined whether the decadic logarithm of numbers of remaining T cells were influenced by time or the origin of the blood. The variable "patient" was nested and modeled as a random effect; an interaction between "patient" and "day" was considered. Values of $p < 0.05$ were considered statistically significant. Statistical analysis was performed using the Sigmaplot 2000 software for Windows (SPSS, Chicago, IL).

RESULTS

IL-10 production after stimulation with a T cell mitogen. To compare IL-10 production in mononuclear cells of cord blood and peripheral blood of adults (CBMNCs and PBMNCs), we used an *in vitro* stimulation with αCD3 mAb (Fig. 1). Supernatants were analyzed at 6, 12, 24, and 48 h. Unstimulated mononuclear cells produced low IL-10 concentrations after 48 h. We found a substantial increase in IL-10 protein production in PBMNCs, which was in contrast to CBMNCs ($p < 0.05$; Fig. 1*A*) and also evident at the mRNA transcriptional level after 24 h (Fig. 1*B*). In the presence of MΦ (Fig. 1 *C*), the addition of a co-mitogen (anti-CD28 mAb, αCD28 mAb) to αCD3 mAb markedly enhanced T cell proliferation in PBMNCs and CBMNs (not shown), but induced only little IL-10 production in CBMNCs after 48 h (Fig. 1 *C*). In the absence of MΦ (Fig. 1*D*), IL-10 production of PBMNCs was lower in the stimulated groups ($p < 0.05$ *versus* corresponding groups with MΦ).

IL-10-mediated effects on CD16 and HLA-DR expression on MΦ. To analyze IL-10-mediated effects on MΦ phenotypes, CD16 and HLA-DR expression on CBMΦ of preterm and full-term neonates, as well as on PBMΦ, were assessed after stimulation for 48 h (Fig. 2). Basal receptor expressions were reduced on CBMΦ ($p < 0.05$ *versus* PBMΦ), with lower densities on preterm neonates ($p < 0.05$ *versus* full-term neonates). IL-10 up-regulated CD16 dose dependently on PBMΦ ($p < 0.05$ *versus* unstimulated PBMΦ), but only slightly on CBMΦ ($p > 0.05$; Fig. 2*A*). IL-10-induced HLA-DR down-modulation on PBMΦ was dose dependent as well and more pronounced ($p < 0.05$ *versus* unstimulated PBMΦ), whereas this effect was only marginal on CBMΦ ($p > 0.05$ *versus* unstimulated CBMΦ; Fig. 2*B*). For both parameters, IL-10-mediated effects were not different between CBMΦ of term and preterm neonates ($p > 0.05$). MΦ survival did not differ between groups, but in the presence of IL-10, MΦ cell death increased from 15% to 26% (500 ng/mL IL-10) after 48 h (not shown).

MΦ-mediated CD4-directed T cell deletion. As shown previously, IL-10 enhances cytotoxic MΦ function by facilitating T cell deletion via ADCC (7,10). Before analyzing this

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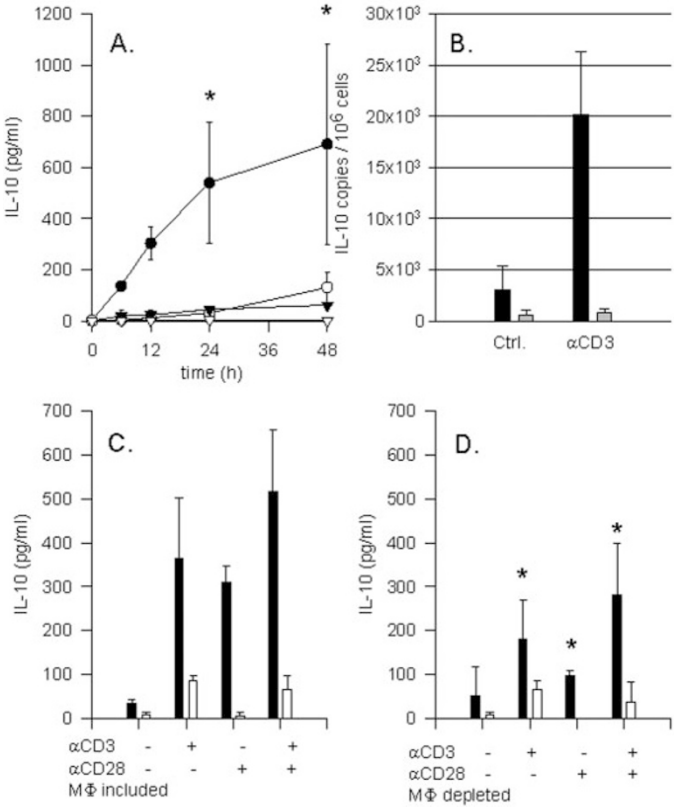

Figure 1. IL-10 protein and mRNA production: 4×10^6/mL PBMNCs (*solid columns*) or CBMNCs (*open columns*) were incubated without (*triangles*) or with (*circles*) αCD3 mAb (5 μg/mL) for 48 h. Supernatants were analyzed for IL-10 protein. For 6 and 12 h five experiments and for 24 and 48 h eight experiments are depicted (*A*); $*p < 0.05$. Cells were probed for IL-10 mRNA; three experiments are depicted (*B*). In the presence (*C*) or absence (*D*) of MΦ, PBMNCs (*black bars*) and CBMNCs (*white bars*) were additionally stimulated with αCD28 mAb (1 μg/mL) for 48 h ($n = 3$); $*p < 0.05$ *vs* presence of MΦ.

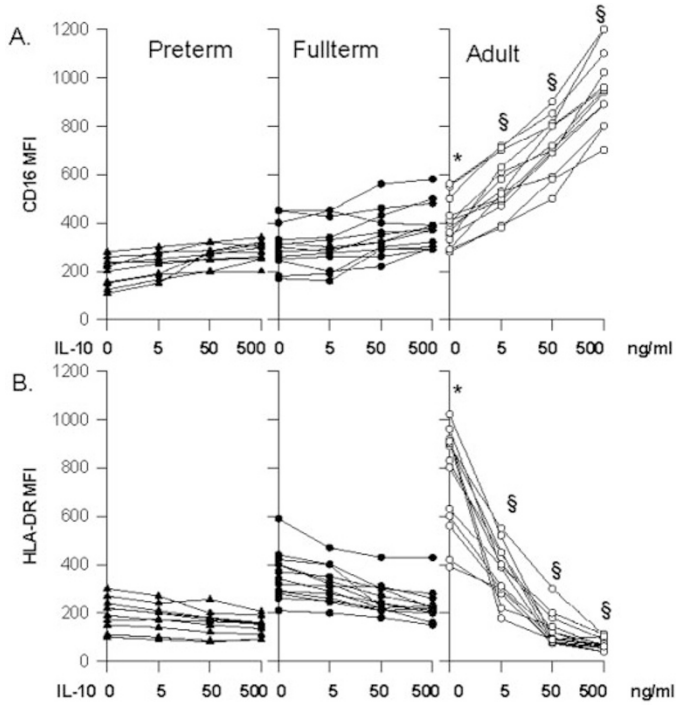

Figure 2. CD16 and HLA-DR expression profiles: 2×10^6 MΦ of preterm neonates ($n = 10$, *solid triangles*), full-term neonates ($n = 13$, *solid circles*), and adults ($n = 13$, *open circles*) were treated with IL-10 for 48 h (concentrations indicated). CD16 (*A*) and HLA-DR (*B*) densities are expressed (arbitrary fluorescence intensity units above baseline per donor). $*p < 0.05$ preterm *vs* full term *vs* adult; §$p < 0.05$ effect of IL-10 on adults *vs* full-term and preterm neonates.

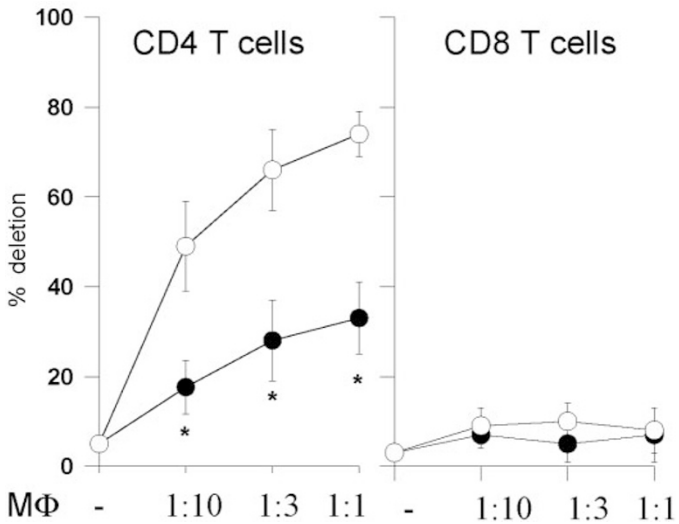

Figure 3. MΦ-mediated CD4-directed T cell deletion: 2×10^6 nonadherent cells of one adult donor were co-incubated with 2×10^5, 6×10^5, 2×10^6 either CBMΦ (full-term neonates, *solid circles*) or PBMΦ of a second adult donor (*open circles*) in the presence of αCD4 (1 μg/mL) for 48 h. The remaining cells were counted, assayed for CD4 and CD8 and are expressed as percentage of deleted cells ($n = 3$, $*p < 0.05$ PBMΦ *vs* CBMΦ).

function, we assessed the basal ADCC capacity of PBMΦ and CBMΦ, directed against CD4 T cells. MΦ-depleted PBMNC of one adult donor were incubated either with CBMΦ or PBMΦ of a second unrelated adult donor. We chose this system to avoid a potentially different sensitivity toward ADCC-mediated apoptosis between neonatal and adult T cells. MΦ were added in different concentrations in the absence or presence of a CD4 T cell–directed deleting antibody (αCD4 mAb) for 48 h (Fig. 3). CD4 T cell deletion was dependent on the presence of MΦ and their concentrations added. Although both CBMΦ and PBMΦ induced CD4 T cell deletion ($p < 0.05$ *versus* αCD4-treated MΦ-depleted PBMNCs), it was stronger in the presence of PBMΦ in all MΦ: lymphocyte ratios ($p < 0.05$ *versus* CBMΦ, Fig. 3*A*). The deletion was specific for CD4 T cells; CD8 T cells remained unaffected (Fig. 3*B*).

CD4 T cell apoptosis-preceding events were shown to be CD4 receptor down-modulation and co-modulation of other surface receptors *e.g.* CD3 and CD28 (8). These phenomena were found on the remaining CD4 T cells with both MΦ subsets (Fig. 4). In the presence of PBMΦ *versus* CBMΦ, CD4 down-modulation was 63 *versus* 25%, CD3 co-modulation was 35 *versus* 13%, and CD28 co-modulation 40% *versus* 66% (Table 1, all $p < 0.05$). Neither down-modulation nor deletion occurred in the absence of αCD4mAb (not shown).

Effect of IL-10 on enhancement of MΦ cytotoxicity. To compare IL-10 cytotoxicity, MΦ-depleted PBMNCs of one adult donor were cocultured either with CBMΦ or PBMΦ of

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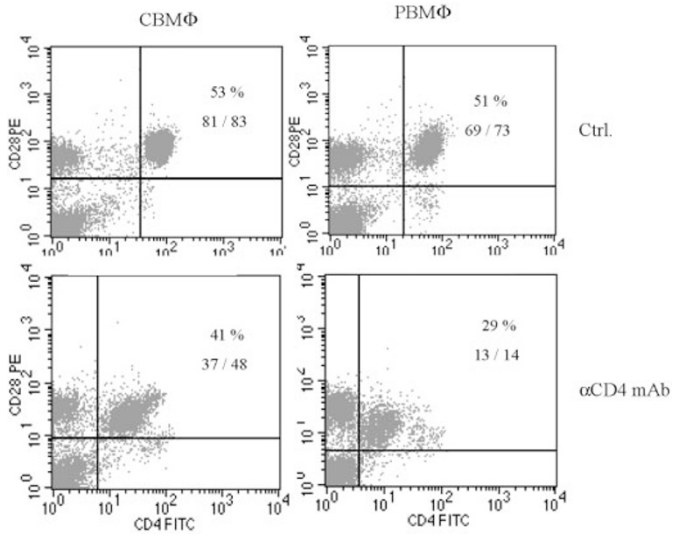

Figure 4. Receptor-modulation on CD4 T cells: 2×10^6 MΦ-depleted PBMNCs from one healthy adult donor were cocultured with 1×10^6 either CBMΦ (full-term neonate, *left*) or PBMΦ (*right*) from a second adult donor in the absence (*top*) or presence (*bottom*) of αCD4 mAb (1 μg/mL) for 48 h. Samples were double stained for CD4 and CD28 expression. Numbers represent the percentage of CD4 T cells and their CD4 (*left*) and CD28 (*right*) mean fluorescence intensities. No down-modulation occurred in the absence of MΦ or the CD4-negative fraction (not shown).

Table 1. *Receptor down-modulation on CD4 T cells*

Coculture	MΦ-depleted PBMNCs + PBMΦ		MΦ-depleted PBMNCs + CBMΦ	
Antibody	No mAb	αCD4 mAb	No mAb	αCD4 mAb
CD4 (MFI)	63 (20)	23 (8)	64 (15)	48 (10)*
CD3 (MFI)	131 (20)	80 (17)	137 (23)	119 (25)*
CD28 (MFI)	73 (14)	25 (12)	91 (14)	55 (10)*

MΦ-depleted PBMNCs (2×10^6) were cocultured with 1×10^6PBMΦ from a second adult donor or CBMΦ in the presence or absence of αCD4 mAb (1 μg/mL). CD4, CD3, and CD28 expression on CD4 T cells was assessed after 48 h. Three experiments are shown (mean ± SD). *$p < 0.05$ *vs* αCD4 mAb-treated group with PBMΦ. No differences were seen on $CD4^-$ cells (not shown).

a second unrelated adult donor for 48 h as described above. Targets again were CD4 T cells, forming MΦ conjugates either by αCD4 mAb or immune complexes, consisting of immune-complexed HIV-envelope protein gp120. IL-10 was added to both groups (Fig. 5). Again, CBMΦ and PBMΦ induced T cell deletion via antibody and immune complexes ($p < 0.05$ *versus* corresponding groups without deleting substances, not shown). PBMΦ were more effective though in eliminating CD4 T cells by both, αCD4 mAb and immune complexes ($p < 0.05$ *versus* CBMΦ; first *versus* third column). Addition of IL-10 increased MΦ cytotoxicity in cultures with PBMΦ ($p < 0.05$ *versus* deleting substance only), which was in contrast to CBMΦ ($p > 0.05$). IL-10 (50 ng/mL) did not affect CD4 or MΦ survival (not shown).

DISCUSSION

The scope of our experiments was to determine the production of IL-10 and its influence on phenotype and cytotoxic function of CBMΦ *versus* PBMΦ. We first found a diminished IL-10 protein and mRNA production in dCD3-

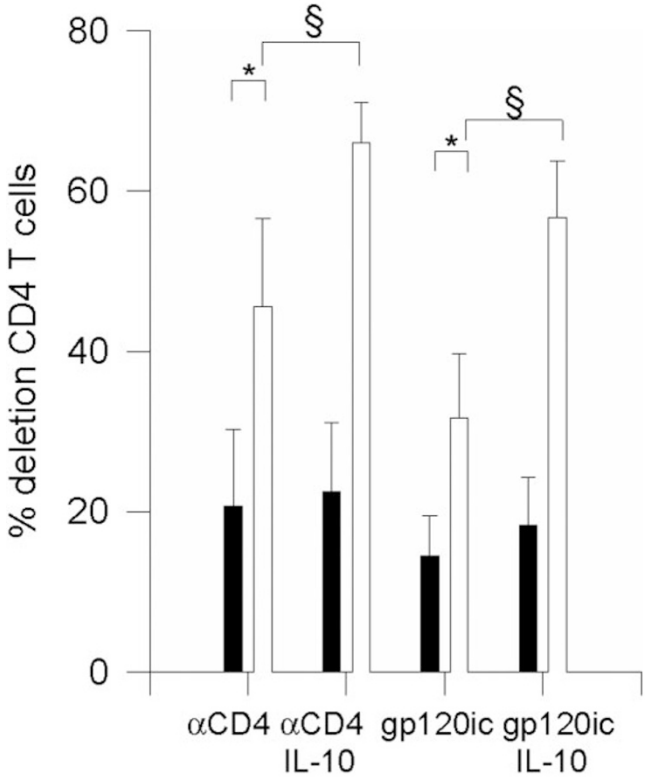

Figure 5. Effects of IL-10 on the cytotoxic capacity of MΦ: 2×10^6 MΦ-depleted PBMNCs from one adult donor were cocultured with 1×10^6 PBMΦ (*open columns*) from a second adult donor or CBMΦ (full-term neonate, *solid columns*) in the absence or presence of αCD4 (1 μg/mL), or gp120/αgp120 immune complexes and IL-10 (50 ng/mL) for 48 h. Remaining T cells were counted and phenotyped ($n = 4$). No changes in the CD8 T cell fraction were seen (not shown); *$p < 0.05$ PBMΦ *vs* CBMΦ, §$p < 0.05$ IL-10 + αCD4 or gp120/αgp120 (gp120ic) *vs* αCD4 or gp120/αgp120.

stimulated CBMNCs (Fig. 1). Second, the basal CD16 and HLA-DR expression on, and the cytotoxic capacity mediated by CBMΦ were reduced (Figs. 2–4). Third, IL-10-mediated effects on CBMΦ phenotype (Fig. 2) and their cytotoxic function (Figs. 4 and 5, Table 1), were impaired. Taken together, our data suggest CBMNCs to produce less IL-10 and CBMΦ to be less sensitive toward IL-10 and less capable of generating Mc-MΦ in our experimental setup. These results are consistent with recent work suggesting that the neonatal immune response is not only deficient in promoting activation (11) but also inhibition (4,5).

IL-10 is produced by various cells, including monocytes, MΦ, and T cells. Although neonates are capable of producing considerable amounts of IL-6 and IL-10 when induced via endotoxins, *e.g.* during sepsis (16,17), results of neonatal IL-10 production are conflicting and depend on the stimulation used (17–19). We found a profoundly diminished IL-10 mRNA and protein production in CBMNCs stimulated with anti-CD3 (Fig. 1). Anti–CD3-stimulated neonatal T cells already were shown to produce significantly less IL-10 than T cells from adults (18). A more detailed characterization of IL-10 production by naïve (CD45RA) *versus* memory (CD45RO) CD4 T cells revealed the latter subset to produce significantly more IL-10 (18). This subset is virtually absent in healthy neonates and may partly explain the reduced IL-10

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production in CBMNCs (Fig. 1*A–C*), most prominent in the absence of MΦ (Fig. 1*D*). Moreover, IL-10 production of naïve neonatal CD45RA T cells (18) and monocytes (19) were found to be reduced in similar experimental settings as well as after stimulation with endotoxin (4,19). Although the addition of a co-mitogen (anti-CD28 mAb) increased T cell proliferation in the presence of MΦ (not shown), we did not find a significant increase in IL-10 production compared with anti-CD3 only (Fig. 1*C*), which may be due to a relatively high mitogen concentration (5 μg/mL).

CD16 and HLA-DR expression on CBMΦ were lower in preterm than in full-term neonates (Fig. 2), confirming earlier reports (12,20,21). Conflicting phenotypic results showing equivalent or even increased CD16 expression on MΦ from preterm neonates (22) may be related to maternal steroids or other medication. Glucocorticoids were shown to selectively deplete $CD16^+$ monocytes in adults (23). Because antenatal steroids were administered to 70% of our preterm infants (Fig. 2), further analysis on their influence on MΦ survival is required. $CD16^+$ MΦ represent a differentiated MΦ subset that does not produce IL-10 (24,25) but strongly induces ADCC via CD95L and CD16 (26). $CD16^+$ MΦ increase in neonatal sepsis (27) and are involved in parainfectious tissue destruction in adults (28). Taking the vulnerability of neonatal tissues into account, the reduced MΦ CD16 expression and induction in preterm neonates may be of advantage *in vivo*.

To our knowledge, neonatal and adult MΦ-mediated ADCC, analyzed by flow cytometry on a single-cell basis (Table 1, Figs. 3–5), have not yet been compared. Previous experiments detecting ADCC by ^{51}Cr release revealed diminished cytotoxicity of CBMΦ toward chicken erythrocytes when conjugates were formed by heteroantibodies via Fc-gamma I receptors (CD64) (29). Our ADCC model targets CD4 T cells and involves Fc-gamma III (CD16) because ADCC is blocked by CD16 receptor blockade (15).

CD4 T cell receptor densities declined after conjugate formation with CD4-specific antibodies (Fig. 4, Table 1), confirming earlier reports (30). Co-modulation of surface receptors is a preceding event of T cell apoptosis (31) and involves the CD95/CD95L system (10). Because MΦ express and secrete CD95L (32) and IL-10 is capable of up-regulating CD95L (33), it is conceivable that neonatal MΦ do not primarily destroy lymphocytes that exhibit (auto)antibodies on their surface, but may form conjugates with nonlymphoid antibody-reactive tissues and induce apoptosis (34). Increased CD95L concentrations were found in healthy neonates (35) and maternal or neonatal inflammatory diseases (36). Therefore, a reduced CBMΦ cytotoxicity could be a means to counterbalance apoptotic hyperreactions.

It is conceivable that our results are influenced by cells in the nonadherent fraction, which may have secondary effects on MΦ, or by the purification process via adherence. The latter may preactivate more mature monocytes and, although negative for natural killer cells, also select nonmonocytic cells. However, when we negatively selected MΦ by magnetic bead depletion, the differences in ADCC capacity between CBMΦ and PBMΦ remained, suggesting the role of the CD14 negative cells in the MΦ-enriched environment to be of minor impact.

IL-10 was found to promote only minimal effects on neonatal T cells with respect to induction of B-cell helper functions compared with adults (18). Its inhibitory effect on proinflammatory cytokine production was shown to be lower in preterm infants than in adults, indicating a reduced IL-10 response (4). Our data suggest CBMΦ to be less sensitive toward IL-10 with respect to CD16 up- and HLA-DR down-regulation (Fig. 2), although this may be balanced by the genuinely lower receptor densities *in vivo*. In contrast to PBMΦ, we found no IL-10-mediated ADCC enhancement on CBMΦ (Fig. 5). Whether these findings are due to lower IL10 receptor densities, which are crucial in IL-10 signaling (37), compromised IL-10 binding properties to the receptor, and/or altered intracellular signal transduction in CBMΦ has to be elucidated.

With respect to IL-10-mediated effects, our data should be interpreted with caution. We used an *in vitro* system with relatively small sample sizes. In addition, for reasons of sample volumes required, we have not yet longitudinally investigated kinetics of cord and peripheral blood of newborns. Our model may therefore not properly reflect the neonatal situation several days postnatally. This was a heterogeneous group of preterm neonates, and although none of them developed early-onset infection, we cannot rule out effects of multiparous pregnancy or maternal antibiotics or antenatal steroids on their immunologic profile. To examine these parameters, as well as interindividual variations, further experiments elucidating pathophysiological mechanisms are required.

In conclusion, our results provide further evidence that the neonatal immune response is not dominated by either pro- or anti-inflammatory reaction patterns but that the plasticity of both sides is restricted compared with adults. As a consequence, a diminished stability and following hyperreactions to both sides are to be expected.

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