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Effect of Dexamethasone on B7 Regulation and T Cell Activation in Neonates and Adults

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ABSTRACT

The safety of dexamethasone for neonates has been questioned, partly because of its multiple unspecific effects on the immune system. Specific effects of dexamethasone on co-stimulatory and immune suppressive functions of neonatal compared with adult macrophages (MΦ) are not known. We evaluated the effect of dexamethasone on the expression and regulation of MΦ B7 family receptors (B7-1, CD80; B7-2, CD86) and on their ability to co-stimulate T cells. Cord blood macrophages (CBMΦ) and MΦ from healthy adults (PBMΦ) were isolated, and cell surface markers were phenotyped by flow cytometry. In tissue culture, cells were exposed to dexamethasone, interferon-γ (IFN-γ), cAMP, or a T cell mitogen (αCD3) and examined for their capacity to activate or destroy T cells. CBMΦ were less able to up-regulate CD80 and CD86 than PBMΦ ($p < 0.05$). Dexamethasone inhibited the up-regulation of CD80, CD86, and HLA-DR on PBMΦ and even more so on CBMΦ ($p < 0.05$ *versus* PBMΦ for CD80 and CD86). In the presence of dexamethasone, stimulation with αCD3 MAb enhanced cytotoxic functions of PMBMΦ and CBMΦ with an increase in deleted T cells, a reduced fraction of enlarged T cells, and an inhibition of T cell CD28 up-regulation, which again were more pronounced with CBMΦ ($p < 0.05$ *versus* PBMΦ). In conclusion, neonatal MΦ are exquisitely sensitive to the inhibitory effects of dexamethasone on B7 expression. Although perhaps producing the desired therapeutic effect, dexamethasone may do so in newborns at the expense of a near complete paralysis of MΦ-dependent T cell function. **(*Pediatr Res* 57: 656–661, 2005)**

Abbreviations

APC, antigen presenting cell
BPD, bronchopulmonary dysplasia
αCD3 Mab, anti-CD3 monoclonal antibody
CBMΦ, cord blood monocyte-derived macrophages
CBMNC, cord blood mononuclear cells
IFN-γ, interferon-γ
MΦ, monocyte-derived macrophages
PBMΦ, peripheral blood monocyte-derived macrophages
PBMNC, peripheral blood mononuclear cells

Preterm newborns have been exposed to dexamethasone for the prevention or treatment of bronchopulmonary dysplasia (BPD) for many years (1,2). Dexamethasone is a synthetic glucocorticoid that reduces the recruitment of inflammatory cells (3,4) and thereby is thought to inhibit the development of BPD. Its effects on other developing organs such as the CNS were only recognized much later. Because follow up-studies provided evidence of abnormal neurodevelopment, early postnatal use of dexamethasone is not recommended any more (2). Nevertheless, glucocorticoids continue to be given to pregnant women to accelerate fetal lung development.

Besides the endocrinium, the primary target of dexamethasone is the immune system, which is incompletely developed in neonates. Evidence that the immunosuppressive effects of dexamethasone are primarily mediated *via* an inhibition of cytokine production has been produced. Mononuclear cells from adults and neonates respond differently to treatment with dexamethasone. With respect to proinflammatory cytokines, cord blood cells show an increased sensitivity toward the inhibitory action of dexamethasone compared with cells from adult donors, resulting in a more pronounced inhibition of IL-1β, IL-6, tumor necrosis factor-α, IL-12, IL-2, and IL-3 production (5,6).

Dexamethasone may directly inhibit T cell proliferation (7), induce apoptosis (8), promote long-lasting changes in the T cell receptor vβ repertoire (9), or decrease the CD4/CD8 ratio in infants with BPD (10). Dexamethasone also influences gene expression of cytokines and various functions of antigen pre-

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senting cells (APC), including B cells, dendritic cells, and monocytes/macrophages (11–13).

T cell activation in the neonate is impaired (7). Beside intrinsic T cell deficiencies, their reduced capacity to become activated largely results from an impaired function of and interaction with APC (14–16). The ability of APC, including monocyte-derived macrophages (MΦ), to induce co-stimulatory signals in T cells by engaging their CD28 receptors is critically important for the T cell response. The lack of co-stimulation leads to anergy or apoptosis of antigen-reactive T cells (17,18). The CD28 ligands are expressed on MΦ and belong to the B7 receptor family. Beyond controlling T cell activation and cell death, B7 ligation influences T cell differentiation and cytokine production (19).

In humans, the B7 family consists of at least two molecules, B7-1 (CD80) and B7-2 (CD86), which belong to the immunoglobulin superfamily (20). Both bind to ligands on T cells, CD28, and CTLA4 (CD152) (21,22). The B7/CD28 family consists of additional receptors, each of which may promote activating or terminating signals (reviewed in 22).

CD86 (B7-2) is constitutively expressed on MΦ (21). The up-regulation of CD80 and CD86 occurs after contact with nominal antigen, IFN-γ (23), lipopolysaccharide (24), or mitogen-activated T cells (25) with different kinetics (26). In adult donors, glucocorticoids were found to inhibit the activation-induced expression of B7 receptors in MΦ (27).

Cord blood macrophages (CBMΦ) *per se* exhibit a reduced co-stimulatory potential: CD80 and CD86 expression and up-regulation are significantly inhibited in CBMΦ compared with MΦ from adult donors (16). Consistent with this observation, MΦ-dependent T cell activation is reduced in neonates, and CBMΦ preferentially deliver negative signals to T cells (16).

In view of the immaturity of the neonatal immune system, we were interested in investigating the capacity of neonatal MΦ to respond to dexamethasone. To our knowledge, its impact on neonatal MΦ and on MΦ-dependent T cell reactions has not yet been studied. Because mechanisms that control B7 receptors bear large consequence for the T cell response, we tested the hypothesis that CBMΦ would exhibit an increased sensitivity toward dexamethasone-induced inhibition of B7 expression and on T cell proliferation compared with PBMΦ.

METHODS

Patients. The study protocol was approved by the Ethics Committee of the University of Tuebingen. All mothers gave written consent before they went into labor. Randomly selected, unrelated adult healthy volunteers donated blood and served as control subjects. All term neonates were delivered spontaneously and did not exhibit signs of infection, as defined by the clinical status, white blood cell count, and C-reactive protein. Mothers with amnion infections and prolonged labor were excluded. Umbilical cord blood was drawn from the fetal side of the placenta by puncture with a sterile needle, attached to a syringe without suction, and placed in heparin-coated tubes (100 IE/mL blood) immediately after ligation of the cord.

Cell cultures. Peripheral blood (PBMCs) and cord blood mononuclear cells (CBMNC) were isolated by Ficoll-Hypaque (Pharmacia LKB, Uppsala, Sweden) density gradient centrifugation. Washed cells were resuspended in RPMI 1640 (Sigma Chemical Co., St. Louis, MO) that contained 10% FCS (Sigma Chemical Co.) and incubated at 37°C in a humidified incubator with a 5% CO_2 atmosphere.

Preparation of mononuclear cell subsets. Unseparated mononuclear cells were placed at 3×10^5 cells/0.1 mL in flat-bottom 96-well microtiter plates (Falcon, Bedford, MA). For separating macrophages from lymphocytes, cells were plated at 3×10^6 cells per 1.5 mL in 60 × 15-mm culture vessels (NoK4-3802-4; Becton Dickinson, Mountain View, CA) 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 washed thoroughly twice and used as a source of macrophages. Usually, 85% of adherent cells expressed CD14.

For further eliminating contaminating nonadherent macrophages, the procedure above described was repeated with the fraction of nonadherent cells. Usually, <1% $CD14^+$ macrophages were found in the nonadherent fraction after this adherence cycle, as determined by FACS analysis.

Co-culture Experiments. Macrophage-enriched adherent cells (1×10^5/0.05 mL) and macrophage-depleted nonadherent cells were mixed (2×10^5/0.05 mL). Nonadherent cell fractions were not pooled from different donors. For equalizing allogeneic effects, CBMΦ and PBMΦ of one adult donor were co-cultured with nonadherent cells of a second adult donor.

Flow cytometry. A daily calibrated FACScan flow cytometer (Becton Dickinson) was used to perform phenotypic analysis. For preventing nonspecific binding, cells were incubated with 10% human serum on ice for 10 min before staining with FITC-, phycoerythrin-, or isotype-specific Ig-labeled MAb for 20 min over ice in the dark. MΦ were gated by forward, side scatter, and CD14 expression. For ensuring that larger cells were MΦ recently migrated and not lymphoblasts, a parallel analysis was performed with the MAb anti-CD3 (SK7). Dead lymphocytes were discriminated by propidium iodide (Molecular Probes, Eugene, OR; 5 μg/mL, 5 min). Propidium iodide–negative cells were counted and analyzed for expression of CD4 and CD8. T cell blasts were detected as $CD4^+$ or $CD8^{hi+}$ cells with enlarged size in the forward scatter as previously described (28).

Reagents. Human recombinant IFN-γ, a potent inducer of CD80 and CD86 (15), was purchased from R&D Systems (Minneapolis, MN). Cell-permeable dibutyryl-cAMP, up-regulating the expression of CD86 but not of CD80 on MΦ (27), was obtained from Sigma Chemical Co.

Fresh dilutions of dexamethasone (Sigma Chemical Co.) in PBS solutions were prepared for each experiment and added at concentrations from 10^{-9} to 10^{-4} mol shortly before addition of B7-inducing substances. Serum concentrations to 10^{-5} mol may be reached pharmacologically (5). Cultures that were incubated in the absence of dexamethasone served as controls. The T cell mitogen anti-CD3 MAb (OKT3, 1 μg/mL) was purchased from Ortho Diagnostics (Raritan, NJ).

Antibodies to CD14 (MΦP9), CD80 (L307.4), CD86 (IT2.2), HLA-DR (L243), CD3 (SK7), CD4 (SK3), CD8 (SK1), and CD28 (L293) and Ig-matched controls (IgG1, IgG2b) were purchased from Becton Dickinson (Heidelberg, Germany). Tissue culture experiments were repeated at least three times. Results are expressed in mean ± SD.

Data display and statistical analysis. Fluorescence intensities were determined, and the nonspecific background staining was subtracted. Statistical analysis was performed by using the decadic logarithm of the values of CD80, CD86, HLA-DR, and CD28. Using an ANOVA, we examined whether the variables were influenced by dexamethasone and age (both nominal effects). The variable "patient" (nominal) was nested under "age," and the nested variables were modeled as a random effect. Furthermore, an interaction between "age" and "dexamethasone" was considered.

Using an analysis of covariance (ANCOVA), we examined whether the decadic logarithm of numbers of resting or blast-forming T cells were influenced by time, the origin of the blood, and dexamethasone. The variable "patient" was nested and modeled as a random effect. Furthermore, 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

Effect of dexamethasone on CD80, CD86, and HLA-DR expression. CBMΦ and PBMΦ were incubated for 48 h with and without various concentrations of dexamethasone. Spontaneous and IFN-γ–induced CD80 and CD86 expressions were determined. For examining whether the IFN-γ–induced effect was specific for CD80 and CD86, HLA-DR densities were also detected. Representative histograms for CD80 expression on PBMΦ (left) and CBMΦ (right) are depicted in Fig. 1.

CBMΦ spontaneously expressed CD80, CD86, and HLA-DR in lower densities than PBMΦ ($p < 0.05$; Fig. 2). Dexamethasone further decreased CD80 and CD86 expression in both groups ($p < 0.05$ *versus* unstimulated control) but did

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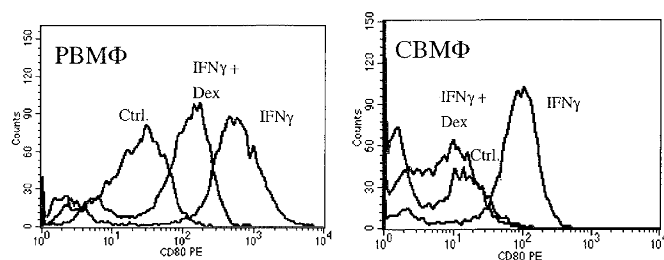

Figure 1. Dexamethasone inhibits IFN-γ–mediated CD80 up-regulation. PBMΦ (left) and CBMΦ (right) were cultured for 48 h in the absence or the presence of dexamethasone (10^{-6} mol) and/or IFN-γ (500 U/mL). Histograms of one experiment were overlain; background staining was $<10^1$ MFI (data not shown).

not influence the spontaneous HLA-DR expression. IFN-γ caused an up-regulation of all three receptors and was more pronounced on PBMΦ ($p < 0.05$ *versus* CBMΦ).

Dexamethasone inhibited the IFN-γ–mediated up-regulation of CD80, CD86, and HLA-DR. On PBMΦ, this inhibitory effect on CD80 and in both MΦ populations the inhibition of HLA-DR expression were dose dependent. Dexamethasone-induced CD80- and CD86-related effects were stronger on CBMΦ ($p < 0.01$ *versus* PBMΦ); the inhibition of HLA-DR up-regulation did not differ significantly ($p = 0.11$). Survival, as detected by propidium iodide staining, was not affected by the drug (data not shown).

To investigate whether dexamethasone-mediated effects on CD80 and CD86 were restricted to IFN-γ, we used different B7 inducers (Fig. 3). Stimulation with cAMP did not affect CD80 expression (Fig. 3*A*) but resulted in increased CD86 receptor densities in both groups (Fig. 3*B*), with CBMΦ being less receptive ($p < 0.05$ *versus* PBMΦ). The addition of dexamethasone to this group inhibited CD86 up-regulation, again more pronounced on CBMΦ ($p < 0.05$ *versus* PBMΦ). The pattern seen with αCD3 was similar to IFN-γ stimulation with both: an impairment of CBMΦ to up-regulate CD80 and CD86 and a stronger inhibitory effect of dexamethasone ($p < 0.05$ *versus* PBMΦ).

Effect of dexamethasone on MΦ-dependent αCD3-mediated T cell reactions. In the presence or absence of dexamethasone, mononuclear cells from adults (PBMNC) or cord blood (CBMNC) were incubated with αCD3. The frequencies of enlarged T cells (Fig. 4*A*) as a parameter of T cell activation, and the absolute numbers of viable T cells, a parameter of T cell survival (Fig. 4*B*), were assayed daily.

The fraction of enlarged T cells was increased in PBMNC ($p < 0.05$ after 48 and 72 h *versus* CBMNC; Fig. 4*A*). In contrast, the fraction of T lymphocytes that were deleted before cells had a chance to divide was higher in CBMNC ($p < 0.05$ *versus* PBMNC after 72 h).

Dexamethasone enhanced the fraction of initially deleted T cells in cord blood ($p < 0.05$ *versus* αCD3 and *versus* PBMNC) and inhibited T cell blast transformation in both groups ($p < 0.05$) but to a higher extent in CBMNC ($p < 0.05$ *versus* PBMNC after 72 h), consistent with the finding that the number of remaining T cells in this group was constantly decreasing.

For eliminating innate differences between neonatal and adult T cells, in particular their potentially different sensitivity toward dexamethasone, purified PBMΦ of one healthy adult donor, or CBMΦ, were co-cultured with MΦ-depleted nonadherent mononuclear cells of a second, unrelated healthy adult donor as a source of enriched T cells (Fig. 5).

CBMΦ diminished the fraction of proliferating T cells ($p < 0.05$ *versus* PBMΦ after 48 and 72 h; Fig. 5*A*) and enhanced the αCD3-mediated deletion of adult T cells ($p < 0.05$ *versus* PBMΦ after 72 h; Fig. 5*B*). Treatment with dexamethasone essentially showed findings identical to those depicted in Fig. 4. In the presence of CBMΦ, we found a significant impact on the decrease of remaining T cells and an almost abolished blast transformation ($p < 0.05$ *versus* PBMΦ after 48 and 72 h). Neither MΦ-depleted enriched T cells nor MΦ-enriched adherent cells showed significant proliferation or deletion in the presence of αCD3 (1 μg/mL; data not shown). In the described interval, we found no differences with regard to proliferation between PBMΦ that were co-cultured with autologous or allogeneic T cells (data not shown). Co-incubation of lower numbers of MΦ (5×10^{-4} MΦ) with T cells had less effect in both cord and peripheral blood (data not shown), indicating the importance of the local tissue environment and MΦ:T cell ratio *in vivo*.

In the αCD3-mediated reaction, engagement of B7 receptors with corresponding receptors on T cells leads to CD28 up-regulation on T cells, which undergo blast transformation (29,30). Using CBMΦ as a source of co-stimulatory receptors, αCD3-mediated CD28 up-regulation on T cells from healthy adult donors was impaired ($p < 0.05$ *versus* PBMΦ). Dexamethasone inhibited this αCD3-mediated CD28 up-regulation in both groups but significantly more so in the presence of CBMΦ ($p < 0.05$ *versus* PBMΦ; Table 1), further indicating the T cell inhibiting impact of the drug to be mediated *via* co-stimulatory molecules on APC.

DISCUSSION

Our data identify the CD80 and CD86 receptors as targets of dexamethasone-induced immune suppression and show that neonatal MΦ exhibit an increased sensitivity toward this drug-induced receptor inhibition. Functionally, this negatively influences the MΦ-dependent T cell activation (Figs. 4 and 5, Table 1). In addition and in contrast to PBMΦ, CD86 expression on neonatal MΦ is partially inhibited by the drug (Fig. 2*B*). Effects of dexamethasone on MΦ are not restricted to CD80 and CD86, because HLA-DR expression is inhibited as well (Fig. 2*B*). Compared with MΦ from adults, neonatal MΦ already show a reduced potential to up-regulate CD80, CD86, and HLA-DR (16) (Figs. 2 and 3). Thereby, MΦ-dependent T cell proliferation is inhibited in cord blood, and activation-induced cell death is promoted (Figs. 4 and 5).

MΦ possess the capacity to regulate the T cell response positively and negatively. We distinguished two principal cytokine-induced MΦ subsets. One, referred to as cytotoxic MΦ (Mc) (31), lacks B7 expression (32) and is induced by IL-10 (33). The second subset, referred to as helper MΦ (Mh) (31), is induced by IFN-γ and expresses CD80, CD86, or both. Disturbances in the Mh/Mc balance have been reported in various diseases (34–38). MΦ that express B7 family mole-

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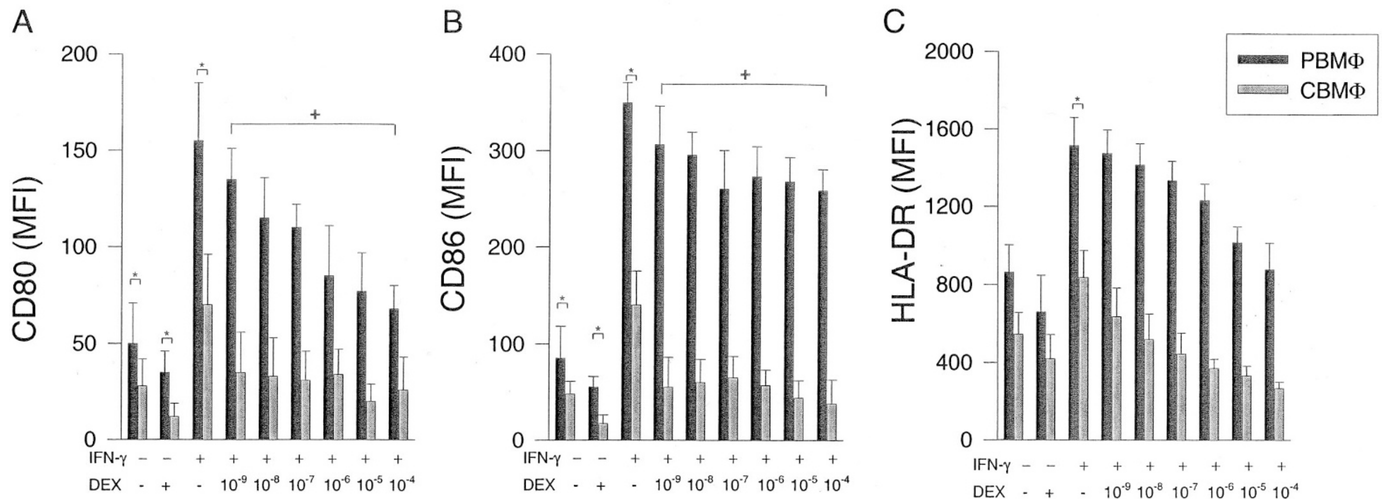

Figure 2. Inhibition of spontaneous and IFN-γ–induced expression of CD80 and CD86 by dexamethasone. PBMΦ (■) and CBMΦ (▦) were cultured for 48 h in the presence or absence of dexamethasone (concentrations as indicated in mol; $^{+}10^{-6}$ mol) and/or IFN-γ (500 U/mL). Dexamethasone was added 1 h before IFN-γ. Cells were harvested and phenotyped for CD80 (*A*), CD86 (*B*), or HLA-DR (*C*). Mean fluorescence intensities are depicted; five experiments are shown (mean ± SD); *$p < 0.05$ PBMΦ *vs* corresponding CBMΦ; $^{+}p < 0.05$ dexamethasone-mediated inhibition across different dexamethasone concentrations PBMΦ *vs* CBMΦ.

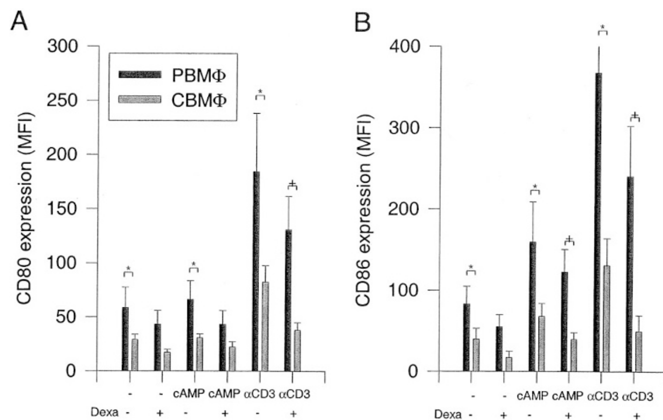

Figure 3. Dexamethasone-induced inhibition of cAMP- and αCD3-induced B7 expression. Mononuclear cells (2×10^5/0.1 mL) from healthy adults (■) and cord blood (▦) were cultured for 48 h alone or in the presence of cAMP (10^{-3} mol) or αCD3 (1 μg/mL). Three groups received dexamethasone (10^{-6} mol). Cells were harvested and phenotyped in triplicate for the mean fluorescence expression of CD80 (*A*) or CD86 (*B*). Five experiments are shown (mean ± SD). *$p < 0.05$ PBMΦ *vs* corresponding CBMΦ; $^{+}p < 0.05$ dexamethasone-mediated inhibition PBMΦ *vs* CBMΦ.

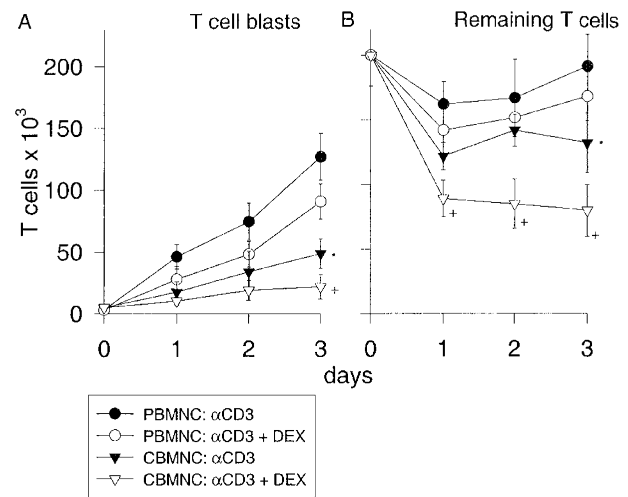

Figure 4. Dexamethasone inhibits αCD3-mediated T cell blast transformation and promotes cell death. Unseparated PBMNC of healthy adults (circles) and cord blood (triangles), each containing comparable amounts of MΦ, were stimulated with αCD3 (1 μg/mL). Dexamethasone (10^{-6} mol; open symbols) was added before αCD3. Cells were counted and phenotyped for CD4 and CD8 expression daily. T cell blasts (*A*) and remaining T cells (*B*) were detected. Three experiments are shown (mean ± SD); *$p < 0.05$ *vs* αCD3-treated PBMNC; $^{+}p < 0.05$ dexamethasone-mediated effect CBMNC *vs* PBMNC.

cules induce neither T cell anergy nor T cell destruction by apoptosis, because both reactions are blocked in the presence of co-stimulation (28–30,39–41). MΦ that lack CD80 and CD86 expression are incapable of preventing the induction of anergy or apoptosis in conjugated T cells and act as negative immune regulators (42–44). In addition, MΦ have the capacity to actively destroy T cells that they target for conjugate formation (44). MΦ may express CD95 ligand in high concentration, engaging the T cell CD95 receptor in the apoptotic destruction of the T cell (45).

The Mh/Mc balance reveals itself in the polyclonal αCD3-mediated stimulation of T cells in our experimental setting: T cell reactivity depends on the number of MΦ and their capacity to up-regulate co-stimulatory molecules (28,30). When exposed to αCD3 MAb, T cells mount a biphasic immune response, characterized by an initial decline in their number and a subsequent clonal expansion (Figs. 4 and 5). Only the fraction of T cells that manages to block apoptosis by engagement of CD28 co-stimulatory molecules gets properly activated by αCD3 MAb (39) (Table 1). Other T cells remain anergic or become deleted (39).

The presence of neonatal MΦ, which were impaired to up-regulate CD80 and CD86 after challenge with αCD3 MAb (16) (Fig. 3), led to a strong decline in remaining T cells and a reduction in proliferating cells (Figs. 4 and 5). Enhancing B7

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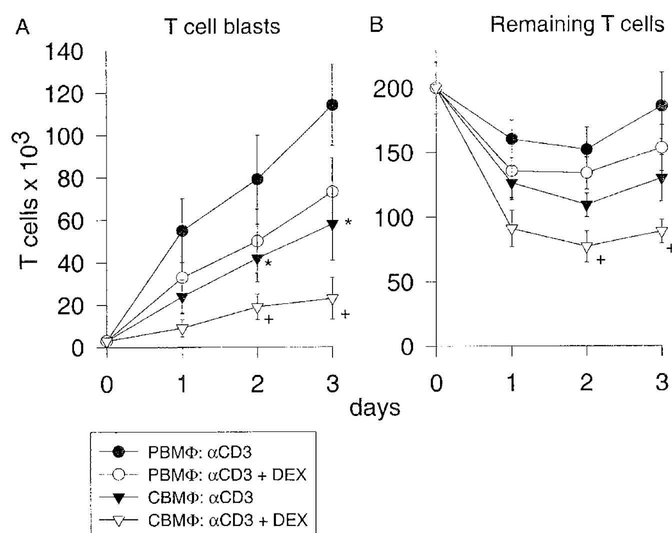

Figure 5. CBMΦ are more sensitive toward dexamethasone-mediated inhibition of αCD3-mediated T cell activation than PBMΦ. MΦ-depleted PBMNC (2×10^5) from one healthy adult donor were co-cultured with PBMΦ (1×10^5) from a second adult donor (circles) or 1×10^5 CBMΦ (triangles) and αCD3 (1 μg/mL) was added. Two groups received dexamethasone (10^{-6} mol) 2 h before addition of αCD3 (open symbols). Samples were taken daily, counted, and phenotyped for CD4 and CD8 expression. T cell blasts (*A*) and remaining T cells (*B*) were depicted. No differences were seen in the nonstimulated groups (data not shown). Four experiments are shown (mean ± SD); $^{*}p < 0.05$ *vs* αCD3-treated group with PBMΦ; $^{+}p < 0.05$ dexamethasone-mediated effect CBMΦ *vs* PBMΦ.

Table 1. *CD28 expression on T cells from adult healthy donors after stimulation with αCD3*

	Control (mean ± SD)	αCD3 (mean ± SD)	αCD3 + Dexa (mean ± SD)
PBMΦ	75 ± 22	175 ± 31*	134 ± 17*
CBMΦ	83 ± 18	117 ± 10*	80 ± 11†

MΦ-depleted PBMNC (2×10^5) from one healthy adult donor were co-cultured with PBMΦ (1×10^5) from a second adult donor or CBMΦ. Dexamethasone (10^{-6} mol) was added before addition of αCD3 (1 μg/mL). Samples were phenotyped for CD28 expression after 48 h. Five experiments are shown (mean ± SD). * $p < 0.05$ *vs* corresponding unstimulated control; † $p < 0.05$ dexamethasone-mediated inhibition *vs* PBMΦ. No significant differences were seen in the nonstimulated dexamethasone groups (data not shown).

expression either *via* exchange of a MΦ type (Fig. 5) or by addition of IFN-γ, was associated with increased T cell blast formation and a decline in the fraction of deleted T cells (25,30,39). These results underscore the observation that the neonatal T cell response can be turned on to almost adult-like levels by immunocompetent APC (14,16).

Deficiencies in IFN-γ production and effect by neonatal monocytes, which partially are attributed to their immaturity (46), are well documented (47). Marodi (48) identified a deficient cytokine receptor signaling pathway *via* signal transducer and activator of transcription-1 phosphorylation in response to IFN-γ, which may help to explain functional consequences. Reduced basal and IFN-γ–induced HLA-DR expression and up-regulation on CBMΦ underscore earlier reports (47,49–51). Dexamethasone additionally inhibits IFN-γ transcription (52). In accordance with our results, dexamethasone inhibits IFN-γ–induced HLA-DR expression on human monocytic cell lines (50); however, in contrast to CD80 and CD86, our results suggest no significant differences in the dexamethasone-mediated inhibition of HLA-DR up-regulation between PBMΦ and CBMΦ (Fig. 2*C*). Whether the drug affects the HLA-DR–mediated antigen-presenting capacity of CBMΦ in particular remains the subject of further investigation.

Dexamethasone inhibited the activation-induced up-regulation of CD86 on CBMΦ and to a lesser extent on PBMΦ (Fig. 3). In contrast to Girndt (27), who found no drug-related influence on the CD86 regulation in MΦ from adults, we used unpurified mononuclear cells for stimulation with IFN-γ and cAMP, suggesting additional indirect effects of dexamethasone, *e.g. via* T cells.

Although we tried to minimize potentially different effects of dexamethasone on neonatal *versus* adult T cells by co-culture, our experimental setup neither excludes drug-related effects on αCD3-stimulated T cells, which may influence MΦ secondarily, nor rules out allogeneic factors that may influence the αCD3 reaction in long-term cultures. Memory T cells, which are characterized by the membrane determinant CD45RO, are virtually absent in cord blood (7) and might account for the increased dexamethasone sensitivity of CBMNC. However, addition of identical amounts of CD45RO cells by co-incubating T cells of adult donors with CBMΦ or PBMΦ (Fig. 5) indicates a drug-mediated effect on MΦ.

The effects of dexamethasone on CD80 receptor inhibition in adults were found to be transmitted *via* the cytoplasmic glucocorticoid receptor, because it could be abrogated by the addition of the glucocorticoid receptor antagonist RU38486 (27). The CD80 up-regulation was similarly inhibited by equipotent doses of hydrocortisone and prednisolone (27). Compared with adults, neonatal adrenals are functionally immature, as reflected by decreased neonatal plasma cortisol concentrations (53) and a decreased density of glucocorticoid receptors in the hippocampus (54). The increased sensitivity of CBMΦ to dexamethasone therefore may reflect an immunologic compensation for the low levels of glucocorticoids in newborn plasma, so the neonatal immune system may be functionally balanced *in vivo*.

Here we confirm earlier observations (16) that the newborn macrophage system emphasizes negative rather than positive immune regulation, which may be prudent because in establishing an immune repertoire, the newborn must guard him- or herself most diligently against autoimmune reactions. Our data show that although dexamethasone tilts the Mh/Mc balance of both adult and neonatal MΦ toward Mc dominance, neonatal MΦ are significantly more sensitive to this effect of dexamethasone. The drug nearly abrogates the expression of co-stimulatory molecules and strongly enforces Mc activities. Therefore, although the neonatal lung may well respond to dexamethasone, the price that the neonate pays in excessively suppressed immune function may prove prohibitive.

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