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Major concern has emerged about the possible long term adverse effects of glucocorticoid treatment, which is frequently used for the prevention of chronic lung disease in preterm infants. Here we show that neonatal glucocorticoid treatment of rats increases the severity ($p < 0.01$) and incidence ($p < 0.01$) of the inflammatory autoimmune disease experimental encephalomyelitis in adult life. In search of possible mechanisms responsible for the increased susceptibility to experimental autoimmune encephalomyelitis, we investigated the reactivity of the hypothalamo-pituitary-adrenal axis and of immune cells in adult rats after neonatal glucocorticoid treatment. We observed that neonatal glucocorticoid treatment reduces the corticosterone response after an LPS challenge in adult rats ($p < 0.001$). Interestingly, LPS-stimulated macrophages of glucocorticoid-treated rats produce less TNF-$\alpha$ and IL-1$\beta$ in adult life than control rats ($p < 0.05$). In addition, splenocytes obtained from adult rats express increased mRNA levels of the proinflammatory cytokines IFN-$\gamma$ ($p < 0.01$) and TNF-$\beta$ ($p < 0.05$) after neonatal glucocorticoid treatment. Apparently, neonatal glucocorticoid treatment has permanent programming effects on endocrine as well as immune functioning in adult life. In view of the frequent clinical application of glucocorticoids to preterm infants, our data demonstrate that neonatal glucocorticoid treatment may be a risk factor for the development of autoimmune disease in man. The Journal of Immunology, 2000, 165: 5932–5937.
In the current study we investigated the effects of neonatal GC treatment on the susceptibility to and severity of EAE. In addition, we examined possible mechanisms underlying the permanent adverse effects of neonatal GC treatment on immune functioning.

Materials and Methods

Animals

Ten-day-pregnant Wistar rats (250–280 g; Harlan-CPB, Zeist, The Netherlands) were housed individually. They were kept under conventional conditions (dark phase, 1900–0700 h) with free access to commercial rat food and water. Pups were born on days 22–23 of gestation. On the day of birth (designated day 0), all pups were removed from the nests, and eight healthy pups (four females and four males) were randomly placed back with each dam. Pups were weaned at 21 days of age and housed two or three per cage.

Experimental design

Newborn rats were injected with dexamethasone 21-phosphate (DEX) on neonatal day 1 (0.5 μg/g body weight i.p.), day 2 (0.3 μg/g), and day 3 (0.1 μg/g). A second group of rats was injected with equal volumes of sterile pyrogen-free saline (SAL). A third group received no treatment (UNTR) and was left undisturbed. Only female offspring were used for the experiments. The various studies were performed using different groups of rats.

EAE induction

Starting 4 days before the administration of myelin basic protein (MBP), rats were handled each day. At 8 wk of age rats were injected s.c. in one hind footpad with 100 μl of an emulsion containing 1500 μg of MBP (isolated from guinea pig brain) in 1 ml of PBS mixed with 1 ml of IFA (Difco, Detroit, MI), to which 10 mg of Mycobacterium tuberculosis H37Ra was added (Difco). This emulsion is referred to as MBP/CFA in the following sections. Injections were performed under halothane anesthesia. From day 7 postimmunization (p.i.) onward, rats were examined daily (weight and clinical disease) until day 20 p.i. Neurologic aberrations were graded from 0 to 5: 0, no EAE; 0.5, loss of tip tail tone; 1, loss of tail tone; 2, partial tail paralysis; 3, complete tail paralysis; 4, hind limb paralysis; and 5, hind limb paralysis. Disease severity was scored by observers in a blinded fashion.

HPA axis activation

Before the start of the experiment, 9-wk-old female rats were moved to a separate room and handled each day for 4 days. On the day of the experiment (0900 h), rats were injected with LPS (2.5 μg/kg, 2.5 μg/ml i.p.). A control group was injected with equal volumes of saline. At 120 min after injection all rats were decapitated.

CORT assay

Trunk blood was collected in EDTA-containing tubes, centrifuged (3000 rpm, 10 min, 4°C), and stored (−20°C) until assay. Plasma CORT levels are expressed as nanograms per milligram of plasma.

Macrophage stimulation

Both 9- to 11-wk-old rats (Expt. 1) and 20- to 25-wk-old rats (Expt. 2) were used. After decapitation, 20 ml of ice-cold RPMI 1640 was injected into the peritoneum, and peritoneal macrophages (Mo) were harvested after gentle massage of the peritoneum (5 min). Cells were seeded in 24-well plates (5 × 10^4/well) in culture medium containing FBS. After 1 h, non-adherent cells were removed by dispensing the culture medium. Mo were cultured in the presence of varying concentrations of LPS. Culture supernatants were collected after 24 h and stored at −20°C until assay.

TNF-α, IL-1β, IL-12, and NO production

The TNF-α content in supernatant was determined by ELISA (U-Cytech, Utrecht, The Netherlands). The IL-1β content in supernatant was also determined by ELISA (Steve Poole, National Institute of Biological Standards and Control, Pottersbar, U.K.) in collaboration with Dr. A. van Dam (Department of Pharmacology, Vrije Universiteit, Amsterdam, The Netherlands). The IL-12p40/p70 content in the supernatant was measured by ELISA (BioSource, Camarillo, CA). OD was read at 450 nm.

In a separate set of experiments nitrite/nitrate was measured in supernatant of cells cultured in Iscove’s medium (24 h, 37°C, 5% CO2) supplemented with Nutridoma SP (Roche, Indianapolis, IN). Nitrate was converted to nitrite by the action of nitrate reductase from Aspergillus niger (Sigma, St. Louis, MO) (14). Briefly, supernatants were incubated with 40 μM NADPH (to initiate the reaction) and 14 μM of enzyme in a final volume of 50 μl of 20 mM Tris, pH 7.6. Reaction was terminated by addition of 50 μl of H2O. The metabolic product nitrite in the supernatant was quantified using Griess reagent (Promega, Madison, WI). OD was read at 540 nm.

Results

Neonatal DEX treatment reduces body weight

Neonatal DEX treatment reduced the body weights of the rats at 8 wk of age compared with those in SAL and UNTR groups (UNTR, 166.4 ± 2.51 g (n = 22); SAL, 167.3 ± 2.91 g (n = 23); DEX, 154.3 ± 3.70 g (n = 26); p < 0.05, by ANOVA).

Neonatal DEX treatment increases the severity and incidence of EAE

At 8 wk of age, rats were immunized with MBP/CFA. Seven days later, the first clinical signs of EAE appeared. In Fig. 1 the mean severity of disease is shown from day 7 until day 20 p.i. No differences were noted in the day of onset among the three experimental groups. Comparison of the mean cumulative score shows that DEX treatment results in increased severity of EAE compared
Neonatal DEX treatment reduces the endotoxin-induced increase in plasma CORT levels in adult rats

Enhanced susceptibility to autoimmune disease is correlated with a reduction in the CORT response (7). To examine the CORT response to an immune challenge, adult rats (9 wk of age) were injected i.p. with 2.5 μg/kg LPS and decapitated after 120 min, i.e., at the peak of the CORT response (15). In Fig. 2 it is shown that in control rats injection of LPS induced an 8-fold increase in the plasma CORT concentration compared with vehicle injection. In the group of rats that had been subjected to neonatal DEX treatment, a decreased LPS-induced CORT response was observed compared with that in the SAL group (p < 0.001, by ANOVA). No effects of neonatal SAL treatment on LPS-induced increases in plasma CORT levels were seen compared with the UNTR group (by ANOVA). We have no indication that the CORT response in DEX-treated animals followed a time course different from that in controls (data not shown).

Peritoneal macrophages are less capable of producing TNF-α and IL-1β after neonatal DEX treatment

Mφ mediate the LPS-induced activation of the HPA axis (15). To investigate whether reduced production of TNF-α and IL-1β by Mφ of neonatally DEX-treated rats plays a role in the reduced CORT response in these animals, Mφ from adult rats were isolated and stimulated with LPS. In Fig. 3 it is shown that LPS increases TNF-α and IL-1β secretion dose-dependently, with a maximum at 3–10 ng/ml LPS. Neonatal DEX treatment reduces the capacity of Mφ to produce TNF-α after LPS stimulation compared with the controls (Fig. 3A; treatment effect, p < 0.05, by ANOVA). Apart from a decrease in TNF-α production in rats treated neonatally with DEX, LPS-induced IL-1β production by Mφ was also decreased compared with that in controls (Fig. 3B; treatment effect, p < 0.05, by ANOVA). No significant effects of neonatal DEX treatment on LPS-induced NO production by Mφ were seen (data not shown). Interestingly, in 20- to 25-wk-old rats both TNF-α (DEX, 65.2 ± 5.1 U/ml; SAL, 112.6 ± 11.6 U/ml; p = 0.01, by ANOVA) and IL-1β (DEX, 156.8 ± 42.1 pg/ml; SAL, 374.3 ± 79 pg/ml; p = 0.03, by ANOVA) production by LPS-stimulated (10 ng/ml) Mφ were decreased in DEX-treated rats compared with control rats.

Since IL-12 plays a central role in the induction of EAE (16), in a separate series of experiments we determined the amount of IL-12 (p40/p70) in supernatants after stimulating Mφ with LPS (Fig. 4). A dose-dependent increase in the production of IL-12...
Neonatal DEX treatment increases cytokine mRNA expression after in vitro stimulation of spleen cells

An increase in EAE susceptibility in DEX-treated rats could be caused by an increased ability to produce proinflammatory cytokines (17). To investigate this possibility, spleen cells obtained from naive 8-wk-old DEX-treated rats and control rats were cultured in the presence of Con A. mRNA expression was detected after 48 h of stimulation. Fig. 5 shows the mRNA expression of a representative experiment as a percentage of a household gene (L32) in both DEX-treated and SAL-treated rat spleen cells. Stimulation of rat spleen cells resulted in a relatively high mRNA expression of TNF-β, IL-2, IFN-γ, and TNF-α. Furthermore, significant production of IL-6 and IL-10 was found. Interestingly, neonatal DEX treatment increased the expression of the cytokines IFN-γ (p < 0.01, by t test), and TNF-β (p < 0.05, by t test). Although the mean mRNA expression of IL-2 and of TNF-α were also increased in the neonatally DEX-treated rats, this was not statistically significant (0.05 < p < 0.1, by t test). No effects were seen on the expression of IL-6 and of IL-10 (Fig. 5). Furthermore, there was no effect of the injection procedure on cytokine mRNA expression (data not shown).

Discussion

This is the first report demonstrating that neonatal DEX treatment leads to enhanced susceptibility of EAE in adult life. Furthermore, our data show reduced endotoxin-induced plasma CORT levels after neonatal DEX treatment. Moreover, endotoxin-induced TNF-α and IL-1β production by Mφ is affected over the long term by neonatal DEX treatment. Finally, the mRNA expression of Th1-type cytokines in the spleen was profoundly increased in adult rats after neonatal DEX treatment. Apparently, exposure to GC in early neonatal life has permanent programming effects on both immunocompetence and neuroendocrine functioning in later life. Our data, which show decreased reactivity of the HPA axis after neonatal GC exposure, are supported by other studies (18–20). Furthermore, we show that the HPA response in neonatally DEX-treated rats is decreased after an immune challenge. The latter suggests that during the induction and course of EAE, the HPA response is reduced in treated animals. The endotoxin-induced activation of the HPA axis is mediated via LPS-induced IL-1β, TNF-α, and IL-6 production by Mφ (21–23). Our data, which showed decreased IL-1β and TNF-α production by Mφ after in vitro stimulation with LPS, suggest that the diminished Mφ response is responsible for the reduced HPA reactivity in adult life after neonatal DEX treatment. The latter conclusion does not exclude the possibility that neonatal DEX exposure also affects one or more components of the HPA axis itself. The preliminary observation that neonatally DEX-treated rats have a decreased HPA response after exposure to a novel environment supports this idea (data not shown).

The reduced cytokine response of Mφ from DEX-treated rats to an endotoxin challenge suggests that these Mφ are less capable of mounting an adequate response to invading pathogens. This could have important consequences for the susceptibility to pathogens of infants who have been treated with GCs in early life. Interestingly, in one study increased reports of infectious diseases in infants were recorded after antenatal GC treatment (3). Our data strongly suggest that the increased incidence may be due to a relatively lower activity of Mφ in these infants.

IL-12 has been shown to be a key cytokine in the regulation of EAE (16, 24). Moreover, GCs can directly inhibit the production of IL-12 by monocytes and dendritic cells (25–27). However, we did not find differences in LPS-induced IL-12 production by Mφ obtained from adult rats after neonatal SAL or DEX treatment. This suggests that increased susceptibility to EAE in DEX-treated rats cannot be explained by increased production of IL-12 in these rats.

It is important to realize that not every neonatal manipulation exerts the same effect on immune responsiveness in later life. We found increased susceptibility to EAE after neonatal GC treatment. However, decreased susceptibility to autoimmunity in later life has been reported when rat pups were subjected to maternal deprivation from days 1–28 of life (28). Maternal deprivation is regarded as a stressor for the pups. It may be possible that exposure in neonatal life to GC, on the one hand, or to stress, on the other hand, leads to opposite effects with regard to disease susceptibility in adult life. Interestingly, in humans certain perinatal environmental stress factors (e.g., neonatal anesthesia and surgery) have been suggested to increase the susceptibility to develop allergy (29). It may well be that some manipulations (such as early life stress factors) increase susceptibility to allergy and asthma, whereas others (such as administration of GC in early life) increase susceptibility to autoimmunity disease. Thus, the nature of the stimulus may be a decisive factor for the outcome.

Could the increased susceptibility to EAE in the DEX-treated animals be caused by the relatively low reactivity of the HPA axis in these rats? It is known that endogenous plasma CORT levels

![Figure 5](http://www.jimmunol.org/)

**Figure 5.** Neonatal DEX treatment increases the mRNA expression of proinflammatory cytokines after in vitro stimulation of spleen cells. Rats were decapitated at 9 wk of age. Spleens were dissected, and single-cell suspensions were cultured in the presence of Con A (1 μg/ml) for 48 h. mRNA was isolated, and the expression of cytokines was determined by RPA. Data are the mean and SEM from one experiment that is representative of two independent experiments. SAL, Neonatal saline treatment (n = 6); DEX, neonatal DEX treatment (n = 6). Statistical analysis was performed on two experiments. *p < 0.05; **p < 0.01; #p = 0.07 (by Student’s t test).
rise during the clinical phase of EAE (30). These enhanced plasma CORT levels are thought to prevent the immune response from overshooting. For example, Lewis rats with relatively reduced HPA reactivity compared with Fischer rats are more susceptible to autoimmune disease than Fischer rats (8, 9). Administration of GC inhibits autoimmune disease in Lewis rats, whereas administration of the GR antagonist RU486 increases the severity of disease in these animals (8, 31). In addition, enhanced plasma CORT levels may favor the production of Th2 cytokines over Th1 cytokines (25, 32, 33), thus reducing the severity of EAE (34). Because of their lower HPA reactivity, DEX-treated animals may therefore be less capable of suppressing the inflammation. Moreover, their Th1/Th2 balance may be shifted more toward Th1, resulting in a more severe EAE in the DEX-treated animals.

The profound increased mRNA expression of the Th1 cytokines IFN-γ and TNF-β is in line with the hypothesis that neonatal DEX treatment shifts the Th1/Th2 balance toward Th1. Moreover, neonatal DEX treatment may have altered not only the HPA axis but also neuroendocrine circuitry at a higher brain level. Kurosawa et al. reported that neonatal GC treatment increased the norepinephrine, dopamine, and serotonin contents of several brain regions, including the hypothalamus (35). This is interesting because high serotonin levels promote cell-mediated immune responses (36). Dopamine may also play a pivotal role in this phenomenon. In an earlier publication we described that the sensitivity for a dopaminergic agonist is associated with Th1/Th2 balance and induction of autoimmunity, i.e., EAE (9). We demonstrated that a low sensitivity to the dopaminergic agonist apomorphine is associated with a shift toward Th1 cytokines, resulting in relatively more IFN-γ mRNA expression. Moreover, Rots et al. reported that a low sensitivity to dopamine is functionally associated with a relatively low activity of the HPA axis (37). Therefore, we propose that neonatal DEX treatment has altered the Th1/Th2 balance on the basis of a permanent shift in neuroendocrine circuitry involving dopamine and serotonin and consequently changes in HPA axis function.

Taken together, early neonatal GC treatment increases the susceptibility to EAE. This suggests that early neonatal GC treatment may be a risk factor for MS or other proinflammatory autoimmune diseases. The mechanisms involved will be a low reactivity of the HPA axis in adult rats after neonatal DEX treatment and increased Th1 cytokine production. Furthermore, neonatally GC-treated rats have a long term decreased production of cytokines by macrophages. This may lead to increased susceptibility to bacterial infections in later life. Clearly, studies in man are urgently needed that focus on the consequences of neonatal GC therapy in infants for susceptibility to (auto)immune diseases in later life.

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References


