Neonatal Dexamethasone Treatment Increases Susceptibility to Experimental Autoimmune Disease in Adult Rats

Joost M. Bakker, Annemieke Kavelaars, Patrick J. G. H. Kamphuis, Pieter M. Cobelens, Harmke H. van Vugt, Frank van Bel and Cobi J. Heijnen

*J Immunol* 2000; 165:5932-5937; doi: 10.4049/jimmunol.165.10.5932
http://www.jimmunol.org/content/165/10/5932

References

This article cites 36 articles, 16 of which you can access for free at: http://www.jimmunol.org/content/165/10/5932.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Neonatal Dexamethasone Treatment Increases Susceptibility to Experimental Autoimmune Disease in Adult Rats

Joost M. Bakker,²*† Annemieke Kavelaars,* Patrick J. G. H. Kamphuis, ‡ Pieter M. Cobelens,* Harmke H. van Vugt,* Frank van Bel,† and Cobi J. Heijnen*  

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-α and IL-1β 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-γ (p < 0.01) and TNF-β (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 (auto)immune disease in man. The Journal of Immunology, 2000, 165: 5932–5937.

Early neonatal glucocorticoid (GC) therapy has been used successfully for the prevention of chronic lung disease after neonatal respiratory distress syndrome in preterm infants (1, 2). GCs are administered to these preterm infants in high doses for several weeks (1). Therefore, growing concern has arisen for the safety of neonatal GC therapies on long term development of the child. Antenatal GC therapy has been found to increase the number of hospital admissions because of infectious diseases in the first years of life (3). It may well be that interference of GC with the developing immune system is responsible for this effect. It is important to realize that infants are treated postnatally with GC in even higher doses than prenatally. Studies in man are not conclusive, but long term effects of postnatal GC exposure on neurodevelopmental outcome (1) and on motor functions and somatic growth (4) have been reported. However, very little is known about the long term effects of neonatal GC treatment on immune functioning.

To investigate possible effects of neonatal GC treatment, we used a new animal model for early GC treatment. It has been suggested that the development of the human fetus during the last trimester of pregnancy is comparable to the development of the rat in the first weeks of neonatal life (5). In the hospital neonatal GC therapy is mainly applied in the 26th to 33rd weeks, which would normally be in the last trimester of pregnancy. Therefore, the rat can be used as an animal model to study the effects of early neonatal GC therapy in the premature infant. Furthermore, the dose and time scheme of GC treatment were used according to the method of Cummings et al. (1) and current clinical protocols for corticosteroid treatment. Finally, outbred rats were chosen to increase the clinical relevance of this model.

Experimental autoimmune encephalomyelitis (EAE) is an experimental autoimmune inflammatory disease that serves as an animal model for multiple sclerosis (6). The hypothalamo-pituitary-adrenal (HPA) axis is believed to play a major role in determining susceptibility to EAE (7). High plasma corticosterone (CORT) levels during disease are thought to suppress the inflammation (7). Indeed, Sternberg et al. (8) found that Lewis rats, which are highly susceptible to EAE, have reduced plasma CORT levels. Also in other rat strains an inverse correlation was found between disease susceptibility and plasma CORT response (8, 9).

GCs play a major role in the development of the immune system (10), implying that the immune system may be a potential target for the detrimental effects of GC in early life. GC inhibit cytokine expression by macrophages and T cells at both transcriptional and post-transcriptional level (reviewed in Ref. 11). Lymphocytes of transgenic mice with overall reduced GC receptor (GR) expression have increased proliferative responses (12), suggesting that these lymphocytes have an altered cytokine production. On the other hand, the expression of an antisense GR transgene in immature thymocytes, which specifically interferes with thymocyte maturation, decreases susceptibility to autoimmune disease (13). However, very few data, if any, exist that show the effects of neonatal GC exposure on cytokine production and susceptibility to autoimmune disease in later life.
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 (UNT). 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 tonus; 1, loss of tail tonus; 2, partial tail paralysis; 3, complete tail paralysis; 4, hind limb paralysis; and 5, hind limb paralysis. Disease severity was scored by observers and recorded daily. The day of onset was calculated from the day of the first clinical sign.

Results

On the first day of life, neonatal rats were treated with DEX or saline (SAL). Control rats were treated with vehicle only. Neonatal DEX treatment increases the severity and incidence of EAE.

FIGURE 1. The expression of clinical signs of EAE in adult rats after neonatal DEX treatment. Rats were treated with DEX or vehicle on days 1, 2, and 3 of life. At 8 wk of age, EAE was induced. Data are the mean ± SEM of clinical disease, which was scored from day 7 p.i. until day 20 p.i. UNTR, No neonatal treatment (n = 22); SAL, neonatal saline treatment (n = 23); DEX, neonatal dexamethasone treatment (n = 26).
with the controls (UNTR, 5.48 ± 0.92; SAL, 7.80 ± 1.58; DEX, 11.69 ± 1.23; DEX vs SAL, p < 0.01, by ANOVA). The mean cumulative score for EAE in the SAL group was not different from the score in the UNTR group (by ANOVA). Neonatal DEX treatment also led to a higher incidence of disease, defined as the percentage of animals with a disease score of 2 or more, compared with the controls (DEX group, 73.1% (n = 26); SAL group, 34.7% (n = 23); p < 0.01, by χ² test). No effects of neonatal SAL treatment on the incidence of disease were seen compared with non-treatment (n = 22).

**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 naïve 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 show 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 autoimmune 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/)

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).
NEONATAL GLUCOCORTICOID TREATMENT INCREASES EAE


