Neonatal immune challenge exacerbates experimental colitis in adult rats: potential role for TNF-α

Sarah J. Spencer,* Niall P. Hyland,* Keith A. Sharkey, and Quentin J. Pittman
Hotchkiss Brain Institute and Institute of Infection, Immunity, and Inflammation, Department of Physiology and Biophysics, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada

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Spencer SJ, Hyland NP, Sharkey KA, Pittman QJ. Neonatal immune challenge exacerbates experimental colitis in adult rats: potential role for TNF-α. Am J Physiol Regul Integr Comp Physiol 292: R308–R315, 2007. First published September 14, 2006; doi:10.1152/ajpregu.00398.2006.—Early life events and childhood infections have been associated with the development and onset of inflammatory bowel disease in adulthood. However, the consequences of neonatal infection in the development and severity of colitis are not established. We investigated the effects of a neonatal (postnatal day 14) or juvenile (postnatal day 28) immune challenge with LPS on 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced damage and weight loss, as well as on food intake and body temperature in adult rats. Neonatally (n)LPS-treated rats developed more severe colitis than control animals, reflected in a greater loss of weight and a significantly increased macroscopic tissue damage score. These findings were associated with a hypothermic response after TNBS treatment in nLPS rats, but not in neonatally saline-treated rats receiving TNBS. These differences were not seen after TNBS in rats that had received LPS on postnatal day 28. Plasma corticosterone was measured as an index of adult hypothalamic-pituitary-adrenal (HPA) axis activation as was TNF-α, a proinflammatory cytokine associated with inflammatory bowel disease. Four days after TNBS treatment, plasma corticosterone was unaltered in all groups; however, TNF-α was significantly increased in adult TNBS-treated rats that had LPS as neonates compared with all other groups. In conclusion, neonatal, but not later, exposure to LPS produces long-term exacerbations in the development of colitis in adults. To test this hypothesis, we examined, in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

IBD is known to be associated with abnormal activation of the mucosal immune system, particularly with an imbalance between pro- and anti-inflammatory cytokines (13, 36). There is recent evidence to suggest that an immune challenge experienced during early development can affect this cytokine balance in the long term, at least in response to a further immune stress. For example, the circulating proinflammatory cytokine response to an immune challenge is attenuated in adult rats that have been treated as neonates with a similar challenge (20). An immune challenge experienced during early development affects many other aspects of adult physiology, such as the febrile (10) and cyclooxygenase-2 responses to a similar immune challenge (10), as well as the animals’ ability to cope with brain trauma such as global ischemia (40) and peripheral insults that induce pain (11) as adults. The adult hypothalamic-pituitary-adrenal (HPA) axis is also affected by immune challenge during the neonatal period (20, 21, 23, 37, 38), and long-term alterations to components of the HPA axis, such as corticotropic-releasing hormone, have been shown to play a particular role in neonatal psychological stress-induced intestinal mucosal dysfunction (39).

We therefore hypothesized that exposure to an immune challenge in early life will affect the severity of experimental colitis in adulthood. To test this hypothesis, we examined, in male Sprague-Dawley rats, the effects of neonatal exposure to a single injection of the bacterial endotoxin LPS on the severity of colitis induced by intracolonic infusion of TNBS in adulthood. In addition, we measured the proinflammatory cytokine TNF-α, which is known to be affected in IBD (13, 36), as well as corticosterone as an indicator of changes to the HPA axis (39).

MATERIALS AND METHODS

Animals. Pregnant Sprague-Dawley rats (Charles River) were maintained at 22°C on a 12:12-h light-dark cycle (7:00 AM–7:00 PM) with pelleted rat chow and water available ad libitum. Ten days after

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birth, i.e., postnatal day (P)10, litters were culled to 12 pups. All litters were weaned at P21, and male rats were kept and housed three or four animals per cage until they reached ~8 wk of age. All procedures were conducted in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the University of Calgary Animal Care Committee.

**Preconditioning immune challenge.** At P14 the pups were removed from their mother for ~5 min and subjected to intraperitoneal injections of either LPS (Escherichia coli, serotype 026:B6, 100 μg/kg; L-3755, Sigma, St. Louis, MO) in 1 ml/kg pyrogen-free saline or an equivalent volume of pyrogen-free saline. This time point was chosen to enable comparison with other known alterations in adult physiology that occur after a P14 immune challenge (10, 11, 20, 21, 40). To determine whether any effects on adult colitis were specifically related to a challenge during early development, a separate group of animals was given intraperitoneal LPS (100 μg/kg) at P28. Ears were clipped for identification at time of injection, and the pups were returned to their home cages and left undisturbed, except for weaning and the usual cleaning and feeding procedures, until experimentation. Approximately equal numbers of animals from each litter received LPS or saline, and after weaning each cage contained both LPS- and saline-pretreated pups. Efforts were made to ensure that animals from each experimental group were representative of each litter. Experiments were conducted on 86 P14-treated animals and 23 P28-treated animals selected from 21 litters over a period of 12 mo.

**Adult manipulations: implantation of temperature monitors.** When the animals reached ~8 wk of age, they were briefly anesthetized with halothane (induced at 4% and maintained at 2%). Sterile, silicone-coated temperature data loggers (SubCue Dataloggers; Calgary, AB, Canada) were then implanted into the abdominal cavity. Briefly, a small incision was made in the skin and muscle with sterile techniques, and a data logger was inserted. The muscle layer was then sutured with resorbable sutures, the skin was clipped with wound clips, and a topical analgesic was applied. Each surgery took ~5 min. Animals were thereafter housed separately.

**Adult manipulations: induction of colitis.** Three to four days after implantation of the temperature monitors, the rats were briefly anesthetized with halothane (induced at 4% and maintained at 2%) and subjected to administration of either TNBS [0.5 ml of 50 mg/ml in 50% (vol/vol) ethanol; Caledon Labs, Edmonton, AB, Canada] or an equivalent volume of sterile saline (0.9%) into the lumen of the colon through a polyethylene catheter inserted rectally 7 cm proximal to the anus. All injections were administered between 10:00 AM and 1:00 PM. A subset of the animals was weighed immediately after TNBS or saline administration, as well as on each subsequent day until the experiment was terminated.

**Adult measurements: consumption and excretion.** To measure food and water consumed as well as feces and urine excreted, a subset of the animals (35 of the 86 P14 treated) was housed in metabolic cages and measurements were taken daily from the day before TNBS or saline administration to the day before (feces, urine) or the day of (chow, water) termination of the experiment. Weights were measured to one decimal place with a top-loading balance. Pieces of food pellet that had fallen into the tray of the cage were included in the measurements, but food “dust” was not (this was minimal). Negligible leak occurred from the water bottles, and they were filled each day to the same level to ensure that any leak would be similar from each bottle.

**Tissue collection.** Three days after administration of TNBS or saline, rats were brought to a temperature-controlled room and allowed to acclimatize overnight. This 3-day time point represents the period during which TNBS-induced macroscopic damage and myeloperoxidase activity are at their greatest (days 1–4; Ref. 9). On the following day between 10:00 AM and 12:00 PM, 50 of the 86 P14-treated rats were deeply anesthetized with pentobarbital sodium (80–100 mg/kg ip) and perfused via the left cardiac ventricle with 4°C phosphate-buffered saline. In each case, the descending aorta was clamped to avoid perfusion of the gut. The remaining rats were decapitated quickly, and trunk blood was collected in heparinized tubes. After immediate centrifugation, plasma was removed and then snap frozen and stored at −80°C for subsequent assay for TNF-α and corticosterone. An unfrozen sample was immediately assessed for osmolality with a vapor pressure osmometer. From all rats, the colon was removed and assessed for severity of colitis.

**Assessment of colitis.** The severity of TNBS-induced colitis was assessed by measuring changes in body weight at each day after TNBS. In addition, at death, colons were removed, opened along the mesenteric border, and examined for macroscopic damage. Briefly, colons were scored by an experimenter who was blinded to the neonatal treatment for the presence and severity of adhesions or diarrhea, the maximum thickness of the colonic wall (mm), and the extent of inflammation, ulceration, and hyperemia. These criteria have been previously described in detail (44).

**Assessment of plasma corticosterone and TNF-α.** A standard TNF-α ELISA kit (Bioscience, Camarillo, CA) was used to assess plasma concentrations of TNF-α. The interassay variability for this assay was 3.5–4.3% coefficient of variation (CV), intraassay variability was 2.6–2.7% CV, and the lower limit of detection was 4 pg/ml. A corticosterone ELISA kit (R&D Systems, Minneapolis, MN) was used to assess plasma corticosterone. The interassay variability for this ELISA was 7.8–13.1% CV, intraassay variability was 6.7–8.4% CV, and lower limit of detection was 27 pg/ml. For each assay, all samples were assayed together and in duplicate.

**Data analysis.** Changes in body weight, chow consumed, and fecal pellets defecated for each day were compared with a two-way ANOVA with repeated measures (RM), with neonatal treatment and adult treatment as between factors and time from treatment (day) as the repeated measure. Where a significant effect of treatment was found, one-way ANOVAs with Student-Neumann-Keuls post hoc comparisons were conducted, comparing the groups at each day. Where a significant effect of time was found, one-sample t-tests were used for each group, comparing the change at each day with zero (i.e., treatment day). Total change in body mass, chow and water consumed, feces and urine excreted, total tissue scores, osmolality, plasma corticosterone concentrations, and plasma TNF-α concentrations were compared with two-way ANOVAs, with neonatal treatment and adult treatment as between factors, followed by a one-way ANOVA with Student-Neumann-Keuls post hoc comparisons as appropriate.

Temperature data were calculated as a temperature index (°C × h) for 24 h starting at 9:00 AM the day after administration of TNBS or saline. Thus the temperature at each 15-min interval from 9:00 AM to 10:00 AM for the two preceding days was used to calculate a baseline temperature. The mean change from this baseline was then calculated per hour for each animal, and these data were summed for 24 h to produce an area under the curve value (temperature index). Temperature indexes were then compared with a two-way ANOVA, with neonatal treatment and adult treatment as between factors, followed by a one-way ANOVA with Student-Neumann-Keuls post hoc comparisons. Additional analyses indicated the same differences between groups when these data were separated into light and dark cycle temperature changes; thus only the 24-h data are presented.

In each case, data were considered statistically significant when P < 0.05. Data are presented as means ± SE.

**RESULTS**

**Neonatal immune challenge exacerbates weight loss during colitis.** Despite differences in the treatment received as neonates, no differences in body weight were seen between the neonatally (P14) saline-treated (nSal; 422.6 ± 10.2 g, n = 21) and neonatally LPS-treated (nLPS; 411.8 ± 16 g, n = 21) animals before TNBS administration. Body weights did differ,
however, in the 4 days after adult administration of saline or TNBS depending on neonatal and adult treatment as was revealed by a two-way RM-ANOVA ($F = 3.975, P = 0.01$). Both nSal ($n = 8$) and nLPS ($n = 11$) rats given intracolonic saline as adults demonstrated a slight but significant gain in body weight in the days subsequent to treatment that was similar for each group (Fig. 1, A and B). Treatment with TNBS resulted in a significant loss (by day 3) of body weight, irrespective of neonatal treatment (Fig. 1, A and B). However, the magnitude of this weight loss was different depending on whether or not the rats had received LPS at P14. nLPS rats lost a significant amount of body weight as early as 1 day after treatment (Fig. 1, A and B).
TNBS \((t = 4.676, P = 0.003, n = 10)\) and continued to lose weight (Fig. 1A). By 4 days after TNBS their weight had still not recovered \((t = 6.169, P = 0.009)\). This decrease in body weight was significantly different from that seen in the nSal animals after TNBS \((F = 10.622, P = 0.005)\) at each of the 4 days \((P < 0.05\) for each day). In the nSal group a significant loss was seen only at day 3 \((t = 3.027, P = 0.01)\), and this had recovered by day 4 \((t = 0.366, P = 0.73, n = 13)\). The maximal weight loss of the nSal animals was also much less than that of the nLPS group (Fig. 1B).

**Consumption and excretion changes do not account for the differences in weight loss.** In an attempt to account for the exacerbation in weight loss during colitis in the nLPS rats we assessed food and water intake as well as excretion in a subgroup of the animals. Both nLPS and nSal animals \((n = 8–13)\) treated with saline as adults consumed \(-112\) g of chow \((-18\) g on the 1st day and \(-20–25\) g thereafter) and drank \(-215\) ml of water \((-35\) ml on the 1st day and \(-45–55\) ml thereafter) in the 4 days after treatment. No baseline differences were seen between groups in any of these parameters measured. However, analysis of the change in chow consumed from the day before treatment indicated a significant effect of adult treatment \((F = 3.528, P = 0.018)\) that was apparent at day 2 \((P < 0.05;\) Fig. 1C). Surprisingly, given the marked loss of body weight, especially in the nLPS animals, no significant effect of neonatal treatment was seen in the amount of chow (Fig. 1C) or water (Fig. 1D) consumed after adult TNBS \((n = 9–14)\), either in total or at any of the individual days examined (daily data not shown for water consumption). We did, however, observe a trend toward a reduction in chow consumption 2 days after treatment in nLPS rats given TNBS as adults (Fig. 1C). In addition, the amount of feces \((-4.3\) g on the 1st day and \(-4.5–6.0\) g thereafter) and urine \((-17.0\) ml daily) excreted, measured for the 3 days after adult treatment, were nearly identical between the four groups (Fig. 1, E and F; daily data not shown for urine excretion).

As a further index of fluid consumption and excretion we also assessed plasma osmolality in a subgroup of animals. Plasma osmolalities on the final experimental day indicated that all groups were slightly dehydrated, but there were no differences between the nLPS and nSal groups. [nSal-adult (a)Sal: 317.4 ± 1.2 mosmol/kgH\(_2\)O, \(n = 8\); nLPS-aSal: 319.9 ± 2.2 mosmol/kgH\(_2\)O, \(n = 8\); nSal-aTNBS: 318 ± 1.6 mosmol/kgH\(_2\)O, \(n = 9\); nLPS-aTNBS: 318.9 ± 1.0 mosmol/kgH\(_2\)O, \(n = 10\)].

**Neonatal immune challenge enhances the severity of colitis.** Macroscopic damage score measurements were used to assess the degree of colitis. In rats treated with saline as adults, no significant differences between the nSal \((n = 13)\) and nLPS \((n = 11)\) groups were seen in the macroscopic appearance of the colon (Fig. 2). This finding is reflected in scores that were essentially zero, with only the colonic wall thickness, and in some cases the presence of mild adhesions resulting from the abdominal temperature monitors, contributing to the score.

All animals, irrespective of neonatal treatment, displayed severe damage in response to TNBS treatment compared with their respective saline-treated controls \((F = 5.422, P = 0.03)\). However, the extent of this damage depended on the neonatal treatment to which the animal had been exposed. nLPS animals \((n = 11)\) administered TNBS sustained significantly more macroscopic damage than did their nSal counterparts \((n = 13; \text{Fig. 2})\). The length of TNBS-induced severe damage \((P < 0.05;\) Fig. 2A). Investigation of specific components of the macroscopic damage score revealed that nLPS animals had significantly increased length of TNBS-induced damage \((P < 0.05;\) Fig. 2B). In addition, all of the nLPS rats showed some degree of hemorrhage associated with the TNBS, whereas 23\% of nSal rats showed no TNBS-related hemorrhage \((P < 0.05)\). Eleven other parameters assessed, including, for example, edema and presence of fecal blood, were all significantly greater after TNBS treatment but were not different between neonatal treatments (data not shown).

**Neonatal immune challenge alters circadian temperature regulation during colitis.** Before the adult treatment, temperatures and circadian temperature rhythms of the nLPS rats were identical to those from the nSal group (Fig. 3A). Adult treatment with saline caused a slight \((-0.25°C)\) elevation of the daytime temperature on the day after the intracolonic saline, and this did not differ between nLPS and nSal treatments. A similar change \((-0.5°C)\) was seen in the nSal animals given TNBS in adulthood. However, \(-10\) h after adult TNBS treatment, at the beginning of the dark cycle, temperatures of nLPS
rats (n = 12) became consistently lower than those of the nSal rats (n = 13) and of the adult saline-treated animals (nSal, n = 13; nLPS, n = 11). While circadian rhythmicity was maintained in nLPS rats treated with TNBS, the amplitude of the temperature rhythms was reduced. Although data are only shown for 60 h after adult treatment, this effect was maintained until the end of the experiment (Fig. 3B).

Calculation of temperature indexes for 24 h beginning at 9:00 AM on the day after TNBS administration revealed a significant difference between groups (F = 6.683, P = 0.01), indicating that nLPS rats given TNBS as adults had significantly lower body temperatures for this time period than did nSal rats or either of the aSal groups (P < 0.05; Fig. 3C). Additional analyses indicated the same differences between groups when these data were separated into light and dark cycle temperature changes; thus only the 24-h data are presented.

Postneonatal immune challenge does not alter the severity of colitis or body temperatures during colitis. To determine whether the changes we observed were due to LPS exposure during the neonatal period or merely to prior exposure to LPS at any time, rats were treated as juveniles (P28) with LPS or saline and then examined as adults. All parameters measured, including body weight (Fig. 4, A and B), macroscopic damage score (Fig. 4C), and body temperatures (Fig. 4D), were nearly identical between animals given LPS at P28 and those given saline at P28 (and P14). Temperature indexes for the 24 h beginning at 9:00 AM on the day after TNBS administration were, respectively, 11.9 ± 1.6 and 13.3 ± 2 for P28Sal and P28LPS adult saline rats and 15.2 ± 1.7 and 17.3 ± 1.6 for P28Sal and P28LPS adult TNBS-treated rats, values identical to those of the P14 saline-treated rats for the appropriate adult treatment (P28Sal-aSal, n = 7; P28Sal-aTNBS, n = 8; P28LPS-aSal, n = 3; P28LPS-aTNBS, n = 5). Thus there was a developmental window associated with the LPS treatment at P14 but absent by P28 that was responsible for the changes we observed in adulthood.

Neonatal immune challenge elevates plasma TNF-α but not corticosterone during adult colitis. To explain the greater severity of colitis and altered body temperature after neonatal immune challenge, we measured corticosterone and TNF-α levels as markers of HPA axis and immune activation, respectively. No significant differences in plasma corticosterone concentrations were seen between any of the groups (n = 7–10), at least at the time of blood sample collection 4 days after TNBS administration (Fig. 5A). Corticosterone levels observed here were similar to previously reported baseline corticosterone levels (20). Similar plasma TNF-α levels were seen in nSal and nLPS rats given saline in adulthood (Fig. 5B), and TNF-α levels were not affected in nSal animals by exposure to TNBS, at least not 4 days after treatment. nLPS rats, however, displayed significantly elevated plasma TNF-α levels (F = 4.774, P = 0.031) that were increased relative to all other groups (P < 0.05; n = 7–10).

DISCUSSION

In the present investigation, we have demonstrated that an immune challenge experienced during the neonatal period, and not later in life, enhances the severity of TNBS-induced colitis in adulthood. Although a neonatal immune challenge had no effect on any of the baseline parameters we measured in adults, neonatally LPS-treated animals had significantly greater colonic damage after TNBS, as well as displaying a more pronounced reduction in body weight and an increased inflammatory response, as measured by plasma TNF-α levels. These animals also displayed a reduction in body temperature in the
days after TNBS treatment compared with neonatally saline-treated controls. Our neonatally LPS-treated rats are clearly more severely affected by colitis in adulthood, a finding that may have implications for our understanding of the development of colitis in humans.

Neonatal immune challenge amplifies alterations in body mass and other indicators of illness during adult colitis. Neonatally saline-treated rats given TNBS as adults lost a small amount of body weight in the days after treatment. The weight loss was accompanied by a slight and nonsignificant hypophagia that possibly accounts for this. TNBS is known to transiently elevate levels of leptin in rats, and this leads to appetite suppression, anorexia, and subsequent weight loss (7). We therefore hypothesized that the neonatally LPS-treated rats would simply show a much more pronounced reduction in food consumption or increased excretion representing more pronounced sickness behavior following a greater elevation of leptin in response to TNBS, and that this would account for the severe loss in body weight seen in these animals. Indeed, increases in macromolecule excretion have been seen after colitis by other groups (8). This did not, however, prove to be the case. Neonatally LPS-treated rats ate only marginally less than their saline-treated counterparts, and they did not excrete more in total volume, although it is possible that there was greater secretion of unabsorbed macromolecules.

The most likely explanation for how these rats could be losing weight without substantially altering their input or output is an increase in metabolic activity. Changes in protein metabolism have been described previously in association with weight loss by other models of colitis (31). Large changes in metabolic activity have also been identified as a response to a severe immune challenge, such as with sepsis, and this is associated with loss of lean body mass and muscle wasting (14). To help ascertain whether neonatally LPS-treated rats with TNBS exposure did develop the increased metabolic activity that would explain the reduction in weight, we measured their body temperatures continuously after TNBS treatment. Somewhat unexpectedly, neonatally LPS-treated rats displayed a consistently lower body temperature, rather than an elevated body temperature after TNBS treatment compared with their neonatally saline-treated controls. This finding would suggest that the neonatally LPS-treated animals were in a state of reduced metabolic activity at the same time they were demonstrating increased weight loss in the face of unaltered food intake. To attempt to understand this finding, we measured plasma TNF-α.

Cytokines, particularly TNF-α, are known to play an important role in the weight loss that is induced by an extreme immune challenge. For instance, TNF-α stimulates protein degradation via a ubiquitin-proteasome proteolytic pathway that results in a loss of skeletal muscle (14, 33). Studies have shown an elevation of TNF-α with colitis in some humans and animal models (36), and anti-TNF-α agents have shown promise in the treatment of colitis in humans (43). Here we show...

Fig. 4. Postneonatal immune challenge does not alter adult colitis: effects of aTNBS on rats treated at postnatal day (P)28 with saline (P28Sal) or LPS (P28LPS). A: change in body mass for the days subsequent to adult treatment. B: change in body mass at day 3. C: tissue damage scores. D: body temperature for the day of and 2 days after adult treatment (filled bar). Open bars, lights on. n = 3–8 per group.
that TNF-α is elevated in neonatally LPS-treated rats as late as 4 days after induction of colitis. It is possible that chronically elevated levels of the cytokine could alter protein metabolism more markedly in these animals, leading to the pronounced loss of weight that we see.

TNF-α has also been associated with thermoregulation during inflammation (26). A significant increase in serum TNF-α levels is known to occur just before the development of hypothermia (19), and it has therefore been proposed that TNF-α released from peripheral macrophages may trigger a hypothermic response during an inflammatory challenge (18, 19, 25). The mechanism for this is thought to be the inhibition of extracellular signal-regulated kinase-regulated peroxisome proliferator-activated receptor-γ-induced activation of uncoupling protein-1 in brown adipose tissue mitochondria, and thus the inhibition of thermogenesis (34, 42). Because the initial inflammatory stimulus (LPS) is a potent activator of TNF-α release (26), it is possible that this early exposure was sufficient to cause an amplification of the TNF-α production in the adult inflamed animal.

It is therefore likely that both the hypothermic core temperatures we have seen in our neonatally LPS-treated TNBS rats and the enhanced weight loss are due to the effects of the persistently elevated TNF-α. In this investigation, we examined plasma TNF-α levels 4 days after TNBS treatment only, and it would be interesting to determine whether the elevated TNF-α indeed preceded the hypothermia and weight loss in these animals. It was interesting to note that plasma TNF-α levels were not altered in neonatally saline-treated colitic rats. This is, however, consistent with other studies in which no alterations in TNF-α protein levels after TNBS treatment were recorded (12), and it is likely that it is the neonatal LPS affecting this system in the long term.

The mechanism by which neonatal LPS might alter responses to adult colitis (in particular the amplification of the TNF-α response) is still unknown. It is known that maternal behavior can alter the development of the pups (see, e.g., Refs. 5, 16). Because of this, we specifically implemented an experimental protocol whereby each dam had a litter that was equally divided between LPS- and saline-treated neonates. We have considerable evidence that these dams do not treat the LPS- and the saline-injected neonates differently. For example, the pups do not have altered body mass in the days after LPS at various neonatal ages, indicating that they are still suckling properly and still undergoing anogenital grooming by the mother to stimulate excretion. We have also demonstrated that the dams do not demonstrate a pup preference in retrieval tests of LPS- and saline-treated pups (for discussion, see Ref. 41). The alterations that we see in responses to adult colitis are therefore unlikely to be due to changes in maternal attention.

These alterations are also unlikely to be related to changes in HPA axis activation, at least not long-lasting changes, given that no change was seen in corticosterone levels. One suggestion, however, is that alterations in the expression or signaling of the Toll-like receptor (TLR) pathways in response to LPS during development may lead to changes in TLR activation in the gut and could in this way confer a lasting susceptibility to colitis. LPS is known to interact primarily with TLR-4 (27), and, despite the fact that the gut abounds in gram-negative bacteria, intestinal epithelia are generally hyporesponsive to LPS because of the low TLR-4 expression and because epithelial tight junctions usually restrict the passage of LPS or bacteria from the lumen (35). It is possible that a neonatal LPS challenge may upregulate TLR-4 in the gut, leading to the increase in TNF-α that we see with TNBS colitis. It was demonstrated previously that TLR-4 expression is upregulated under inflammatory conditions and polymorphisms in TLR can result in IBD (1), and at least one study has reported increased TLR-4 expression in the intestinal epithelia of humans with IBD (15). Further investigation will be necessary to elucidate the actual mechanism by which neonatal LPS has such a pronounced effect on the severity of adult colitis; however, TLR-4 provides an excellent target for future investigation.

Conclusions. We have demonstrated that a single neonatal immune challenge can have serious detrimental effects on the animals’ physiology after TNBS colitis in adulthood, as well as increasing the severity of the colitis. These findings may have important implications for the understanding and treatment of IBD in humans, in particular the use of anti-TNF-α therapy in those patients who suffered from increased bacterial infections early in life or during childhood, because this study would suggest a poorer disease prognosis in these individuals. (2–5, 9, 16, 22, 29, 41)

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Fig. 5. Neonatal immune challenge elevates plasma TNF-α during adult colitis: plasma corticosterone (A) and TNF-α (B) concentrations at day 4 in nSal and nLPS animals after either aSal or aTNBS. *P < 0.05, n = 7–10 per group.
REFERENCES


