Evidence that Lipopolysaccharide-Induced Anorexia Depends upon Central, Rather than Peripheral, Inflammatory Signals

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Systemic inflammatory stimuli cause anorexia and weight loss by disrupting the physiological regulation of energy balance. Mice lacking MyD88, an intracellular mediator of signal transduction activated by Toll-like receptor 4 or IL-1β receptors, are resistant to anorexia induced by the bacterial endotoxin lipopolysaccharide (LPS), despite a significant circulating cytokine response. Thus, we hypothesized that induction of a peripheral inflammatory response is insufficient to cause LPS-induced anorexia when MyD88 signaling in the central nervous system is absent. To test this hypothesis, we used bone marrow transplantation (BMT) to determine if LPS-induced anorexia can be restored to MyD88-deficient mice by reconstituting their bone marrow with wild-type (WT) immune cells. We found that restoring WT circulating immune cells to mice lacking MyD88 conferred only a mild, short-lived anorexia in response to LPS, such that food intake was fully normalized by 20 h post injection (LPS 4.1 ± 0.5 g vs. vehicle 4.3 ± 0.5 g), whereas LPS-induced anorexia was profound and sustained in WT controls after either autologous BMT or sham BMT. Similarly, LPS-mediated induction of hypothalamic mRNA encoding IL-1β and TNFα was robust in both WT control groups but was absent in chimeric MyD88 mice, despite comparable peripheral inflammatory responses across the three groups. We conclude that LPS reduces food intake via a mechanism dependent on MyD88 signaling within brain and/or other tissues and that in the absence of this effect, robust stimulation of circulating immune cells cannot induce sustained anorexia. (Endocrinology 148: 5230–5237, 2007)

Under physiological conditions, a variety of peripheral signals regulate appetite and adjust energy intake to match energy requirements over time (1–3). Systemic inflammatory signals can cause profound anorexia by disrupting the physiological regulation of appetite, an effect that contributes importantly to morbidity and mortality of chronic infectious, neoplastic, and inflammatory diseases (4). After early evidence that cytokine administration reduces appetite in the absence of any bacterial or viral products (5), a large body of work has implicated circulating cytokines as mediators of inflammatory anorexia and weight loss (6–10). Cytokine receptors are abundant in brain (11), supporting a model in which peripheral inflammatory mediators exert behavioral effects via their neuronal actions, and several theories have been forwarded to explain how these large proteins circumvent the blood-brain barrier to access the central nervous system directly (CNS) (12, 13). However, peripheral inflammation also stimulates de novo cytokine production in the CNS (14), and although a critical role for CNS cytokines in inflammatory anorexia and weight loss has been suggested (15, 16), studies have yet to examine anorexia using a paradigm in which inflammatory signaling is selectively disrupted in either circulating immune cells or non-circulating cells. Thus, in the current work, we sought to determine whether peripheral or central cytokine signaling is responsible for inflammatory anorexia by using bone marrow transplantation (BMT) to alter inflammatory signaling selectively in circulating immune cells.

Innate immune receptors for a number of potent inhibitors of food intake rely on the intracellular adaptor protein MyD88 to activate cellular inflammatory responses (17). Accordingly, MyD88 is an important, though not obligatory, inflammatory signaling pathway for lipopolysaccharide (LPS) (which induces cellular inflammation by activating the Toll-like receptor 4) and IL-1β, but not for TNFα (18). Our previous work demonstrated that LPS does not cause anorexia in mice lacking MyD88 (19), despite a robust increase of plasma cytokine levels, including cytokines that do not signal via MyD88. Therefore, we hypothesized that MyD88 signaling in brain or other tissues is required for LPS-induced anorexia, regardless of the magnitude of the peripheral inflammatory response (19). Because the circulating cytokine response induced by LPS in MyD88-deficient mice, though substantial, was not normal, we wished to investigate further the role played by peripheral immune cells in the anorexic response to a systemic inflammatory stimulus. To accomplish this, we asked whether restoring wild-type (WT) inflammatory cells to MyD88-deficient mice through BMT confers sensitivity to LPS-induced anorexia and, conversely, if replacing bone marrow of WT mice with MyD88-deficient...
bone marrow attenuates this response. Our findings demonstrate that MyD88-deficient mice transplanted with WT bone marrow remain largely protected against LPS-induced anorexia, despite a robust peripheral immune response, whereas chimeric WT mice transplanted with MyD88-deficient immune cells remain fully sensitive to the anorexic effect of LPS. In accord with this behavioral data, LPS robustly stimulated hypothalamic cytokine mRNA expression in chimeric WT recipients transplanted with MyD88-deficient bone marrow, but not in chimeric MyD88-deficient recipients transplanted with WT marrow.

Materials and Methods

Animals

Studies were conducted using adult (8–14 wk) male and female MyD88−/− mice and their WT (MyD88+/+) littermate controls that had been backcrossed more than seven generations onto the C57BL/6 strain (generously provided by Dr. R. Winn, University of Washington, Seattle, WA). All animals were housed individually in a temperature-controlled room (23 ± 2°C) and maintained on a 12-h light/dark cycle. All study protocols were approved by the Institutional Animal Care and Use Committee of the University of Washington, Seattle, WA, and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All animals were provided ad libitum access to water and pelleted rodent chow (Test Diet 5015; LabDiet Inc., Richmond, IN), except where otherwise indicated. All food intake studies were performed after a 4-h fast, with food returned at the onset of the dark cycle.

BMT

Pooled bone marrow was harvested from femora and tibiae of three MyD88−/− and five littermate control donor mice, and 8 × 106 cells were injected iv via the retro-orbital venous plexus into each recipient mouse 24 h after lethal irradiation (10 Gy given in two equal fractions 2 h apart), using an established protocol (20). Sham-irradiated animals were placed in the irradiator according to the same protocol but were not irradiated and did not receive donor cells. Accordingly, four groups of experimental animals were generated: 1) sham-irradiated C57Bl/6 mice (sham, n = 10); 2) C57BL/6 mice transplanted with C57BL/6 bone marrow (C57 >C57, n = 10); 3) C57BL/6 mice transplanted with MyD88-deficient bone marrow (MyD88>C57, n = 10); and 4) MyD88-deficient mice transplanted with C57BL/6 bone marrow (C57>MyD88, n = 7). Body weight and cumulative food intake were monitored biweekly in all animals post-BMT and were compared with baseline, pre-BMT values. Body composition was measured by quantitative magnetic resonance (Echo Biomedical Systems, Houston, TX) (21, 22) 4 d before BMT and again 4 d before protocol 1 (described in Study protocols).

Genotyping of splenocyte DNA

As previously described (23), splenic tissue (from half the spleen obtained during the tissue harvest, see below) was dissociated into 5 ml culture medium (RPMI 1640; Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum (Hyclone, Logan, UT). Suspended cells were pelleted, culture medium (RPMI 1640; Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum, and were resuspended in a solution of 0.1% gelatin (dissolved in H2O) and 0.1% trichloroacetic acid precipitated, filtrated by gel chromatography and cryo-irradiated; Sigma-Aldrich, St. Louis, MO) at a dose (50 ng/g ip) that reliably induces anorexia in WT but not MyD88−/− mice (19). All ip injections were given 30 min before dark-cycle onset. Food was returned at dark-cycle onset, and cumulative food intake was measured 2 h into the dark cycle, and again at 6 and 20 h after ip injections. Body weight was measured both before and 20 h after ip injections.

Protocol 2: effects of LPS on serum cytokine concentrations, and on hypothalamic and splenic cytokine mRNA expression. Twenty-one days after the injections in protocol 1, subsets of animals in each group were injected with either VEH (0.3 ml saline ip) or LPS (50 ng/g ip) (n = 3–5 per group) 30 min before the onset of the dark cycle. Food intake and body weight were measured 6 h after injections, after which mice were killed by decapitation after brief exposure to inhaled CO2.

Determination of cytokine mRNA levels by real-time PCR

Splenic and hypothalamic RNA extraction, quantification, and reverse-transcription were performed as previously described (24). PCR was optimized for IL-1β, TNFα, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), using the following primer sequences: IL-1β, fwd 5′-tacaaggagaagaagacagc-3′; IL-1β, rev 5′-gatcactccacactgctgctg-3′; TNFα, fwd 5′-catctctctactagcttcatcatc-3′; TNFα, rev 5'-ggagtagatagggagaacggg-3′; GAPDH, fwd 5′-aagcatccctctgatc-3′; and GAPDH, rev 5′-tccagcaactacactg-3′, as previously described (24) using an ABI prism 7900HT (Applied Biosystems, Foster City, CA) and ABI SYBR Green PCR Master Mix (Applied Biosystems). PCR data were analyzed using the Sequence Detection System software (SDS v2.2; Applied Biosystems). IL-1β and TNFα mRNA expression levels were normalized to GAPDH mRNA content, and nontemplate controls were incorporated into each PCR run.

Statistical methods

Comparisons between group mean values were performed by two-way ANOVA using within-subjects comparison for treatment effect and between-subjects comparisons for genotype effect. The Tukey honestly significant difference post hoc test was used for multiple comparisons. Statistical analyses were performed using Statistica software (version 4.1; StatSoft, Inc., Tulsa, OK). The null hypothesis of no difference between groups was rejected at P < 0.05. All values are presented as the mean ± SEM.

Results

Effect of BMT on food intake, body weight, and body composition in WT and MyD88-deficient mice

At baseline, MyD88−/− and MyD88+/+ littermate mice were comparable with respect to both body weight (MyD88−/− 28.2 ± 1.5 g, MyD88+/+ 27.3 ± 0.3 g; P = 0.4) and daily food intake (MyD88−/− 4.4 ± 0.1 g, MyD88+/+ 4.4 ± 0.1 g; P = 1.0). All mice were irradiated on the same day and transplanted with freshly harvested donor bone
marrow cells 24 h later, generating three groups of chimeric mice: 1) C57Bl/6 mice transplanted with C57Bl/6 bone marrow (C57> C57); 2) C57Bl/6 mice transplanted with MyD88-deficient bone marrow (MyD88>C57); and 3) MyD88-deficient mice transplanted with C57Bl/6 bone marrow (C57>MyD88). A fourth group was comprised of sham-irradiated, nontransplanted C57Bl/6 mice (sham). During the first week post-BMT, MyD88-deficient recipients displayed significantly increased mortality due to sepsis (five of 12 vs. zero of 20 in the two C57 BMT groups), consistent with previous evidence of increased susceptibility to infection in these mutants (25, 26). Data from MyD88-deficient mice that did not survive were excluded from further analysis, and because MyD88<−/− mice that died were heavier than those that survived (30.8 ± 1.1 vs. 25.5 ± 1.7 g, respectively; \( P < 0.05 \)), the baseline body weight of C57>MyD88 mice was reduced relative to other groups after making this adjustment (Fig. 1A).

Weight loss during the first week post transplantation (Fig. 1A) was accompanied by reduced food intake (Fig. 1B) in each of the three transplanted groups but recovered during the second week post transplant such that by the end of the fourth post-transplantation week, body weights were equivalent among all the groups (Fig. 1).

Body composition analysis, evaluated by quantitative magnetic resonance (21) before BMT and again 3 months post-BMT, revealed that mice in each of the three transplanted groups lost a small amount of lean mass (C57> C57 = 0.9 ± 0.6 g, MyD88>C57 = −1.1 ± 0.2 g, and C57>MyD88 = −1.0 ± 0.2 g), relative to the sham-BMT group (0 ± 0.3, \( P < 0.01 \) vs. MyD88>C57 and C57>MyD88, \( P = 0.1 \) vs. C57> C57). In contrast, fat mass increased significantly during the post-BMT period in sham (1.1 ± 0.3 g, C57> C57 (0.7 ± 0.2), and MyD88>C57 (0.9 ± 0.2 g) mice, whereas remaining stable in the C57>MyD88 group (0.0 ± 0.5 g; \( P < 0.05 \) vs. sham). Thus, although MyD88-deficient mice return to baseline weight post-BMT, they do not exhibit the gain of fat mass demonstrated by WT animals.

To evaluate the success of BMT, splenocytes from transplanted animals were harvested at killing, and the MyD88 locus was genotyped to quantify the WT and mutant MyD88 sequence to measure transplantation efficiency. As expected, zero of eight mice in the C57> C57 group had amplification of the MyD88 null gene. In the MyD88>C57 group, only three of 10 mice had detectable amplification of the WT gene, and in those animals, the average transplantation efficiency of MyD88-deficient cells was 93%. The average transplantation efficiency for the entire group was more than 98%. In the C57>MyD88 group, only one of seven mice had detectable amplification of the MyD88 null gene, and transplantation efficiency of WT cells in this mouse was 95%. The average transplantation efficiency for the entire group was more than 99%.

**Effect of ip LPS administration on food intake and body weight in chimeric MyD88-deficient and WT mice**

As expected, cumulative food intake was markedly reduced by LPS administration (50 ng/g ip) in sham mice relative to VEH at both 2 and 20 h (Fig. 2A). LPS reduced food intake comparably in C57> C57 BMT mice, suggesting that the BMT procedure per se does not alter LPS-induced anorexia. In contrast, the inhibitory effect of LPS administration on food intake in C57>MyD88 mice was markedly attenuated relative to the other groups at 2 h (LPS 2 h, 0.8 ± 0.1 g; VEH 2 h, 1.1 ± 0.08 g; \( P < 0.01 \)) and was absent at the later time points (Fig. 2B). By comparison, LPS reduced food intake in MyD88>C57 mice as potently as in the sham and C57> C57 groups. Therefore, neither reconstituting WT mice with MyD88-deficient marrow nor transplanting MyD88-deficient mice with WT marrow substantially alters the ability of LPS to reduce food intake.

The pattern of LPS-induced weight loss across the four groups paralleled the changes of food intake. Compared with VEH, LPS administration caused weight loss in mice from the sham and C57> C57 groups (Fig. 3A) similar to that observed in MyD88>C57 mice (Fig. 3B). In contrast, C57> MyD88 mice did not lose weight 6 h after LPS administration but did demonstrate mild LPS-induced weight loss at 20 h (LPS 20 h, −0.3 ± 0.2 g; VEH 20 h, 0.1 ± 0.2 g; \( P < 0.05 \) vs. VEH) (Fig. 3B), consistent with our previous findings in MyD88-defi-
cient, nontransplanted mice (19). Therefore, the effect of LPS on food intake and body weight in MyD88-deficient mice transplanted with WT bone marrow is mild and transient when compared with the expected and more pronounced weight loss seen in each of the other three groups.

**Effect of ip LPS administration on splenic and hypothalamic cytokine mRNA expression in chimeric MyD88-deficient and WT mice**

To characterize the peripheral and central inflammatory responses to LPS, we measured levels of mRNA encoding IL-1β and TNFα in spleen and mediobasal hypothalamus of chimeric mice killed 6 h after LPS injection (Fig. 4). As expected, relative to VEH, LPS administration induced a robust increase of IL-1β mRNA expression in sham and C57>C57 mice in both hypothalamus (6- to 8-fold) and spleen (10- to 12-fold). Among C57>MyD88 mice, LPS failed to induce IL-1β mRNA expression in the hypothalamus, despite robust IL-1β mRNA induction in the spleen (14-fold), comparable to the splenic response to LPS in sham or C57>C57 mice. Conversely, splenic IL-1β mRNA expression was not induced by LPS in MyD88-deficient mice transplanted with WT bone marrow (C57>MyD88, squares), whereas its hypothalamic expression was robustly increased (7-fold) in a manner equivalent to that of sham and C57>C57 animals.

Similar results were obtained for hypothalamic expression of TNFα. Thus, whereas hypothalamic TNFα mRNA levels were comparable among VEH-injected animals in each of the four experimental groups, LPS induced a 3-fold increase of hypothalamic TNFα mRNA expression in sham, C57>C57, and MyD88>C57 mice (P < 0.05 vs. VEH for all three groups) but was without any effect in C57>MyD88 mice (P = 0.6 vs. VEH). Together, these results indicate that: 1) MyD88-deficient mice transplanted with WT immune cells remain protected from LPS-induced hypothalamic cytokine induction, despite robust...
Each of the groups at each time point. Data presented are means tissue GAPDH mRNA content. There were three to six animals for mined by RT-PCR and is expressed in arbitrary units normalized to (C57 MyD88-deficient mice transplanted with C57Bl/6 bone marrow MyD88-deficient bone marrow (MyD88 black bars) was evaluated 6 h post-LPS (50 ng/g ip) administration or VEH in (C57 MyD88-deficient mice transplanted with WT bone marrow (C57>MyD88) was also equivalent to the effect seen in WT mice, with the exception that levels of the chemokines monocyte chemoattractant protein-1 (MCP-1) and keratinocyte-derived chemokine (KC) were reduced in the C57>MyD88 group (Table 1). Consistent with our previous findings, 6 h post-LPS administration in MyD88-deficient mice (19), plasma concentrations of IL-6, MCP-1, and TNFα in MyD88>57 mice were not significantly different from WT mice, whereas the plasma concentration of the chemokine KC was elevated in these animals. Surprisingly, the LPS-induced plasma IL-1β concentration was not significantly reduced in the MyD88>C57 group relative to WT mice.

**TABLE 1.** Plasma cytokine concentrations in chimeric MyD88-deficient and WT mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Rx</th>
<th>IL-1β</th>
<th>TNFα</th>
<th>IL-6</th>
<th>MCP-1</th>
<th>KC</th>
<th>IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>VEH</td>
<td>2 ± 1</td>
<td>10 ± 1</td>
<td>40 ± 20</td>
<td>0.1 ± 0.02</td>
<td>84 ± 32</td>
<td>11 ± 4</td>
</tr>
<tr>
<td>MyD88&gt;C57</td>
<td>LPS</td>
<td>17 ± 5</td>
<td>23 ± 3</td>
<td>409 ± 204</td>
<td>3.4 ± 0.5</td>
<td>547 ± 124</td>
<td>41 ± 9</td>
</tr>
<tr>
<td></td>
<td>VEH</td>
<td>3 ± 3</td>
<td>8 ± 1</td>
<td>9 ± 3</td>
<td>0.06 ± 0.02</td>
<td>27 ± 5</td>
<td>11 ± 5</td>
</tr>
<tr>
<td>C57&gt;MyD88</td>
<td>LPS</td>
<td>15 ± 2</td>
<td>34 ± 10</td>
<td>646 ± 285</td>
<td>3.1 ± 1.0</td>
<td>978 ± 155a</td>
<td>71 ± 14</td>
</tr>
<tr>
<td></td>
<td>VEH</td>
<td>1 ± 1</td>
<td>9 ± 3</td>
<td>40 ± 27</td>
<td>0.09 ± 0.04</td>
<td>61 ± 31</td>
<td>4 ± 3</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>27 ± 2</td>
<td>17 ± 3</td>
<td>226 ± 76</td>
<td>0.6 ± 0.24a</td>
<td>93 ± 37a</td>
<td>74 ± 38</td>
</tr>
</tbody>
</table>

*Plasma cytokines and chemokine values are shown for MyD88>C57, C57>MyD88, and WT mice (combined data from the C57>57 and sham groups) at 6 h after LPS or saline (VEH) administration. All values are reported as ng/ml except for MCP-1, which is shown as µg/ml. There were three to six animals for each of the groups at each time point. Data presented are means ± SEM. All LPS-stimulated cytokine values were significantly greater than VEH values for all groups. a P < 0.05 relative to the other LPS-treatment groups.

**Discussion**

Circulating cytokines are widely viewed as key mediators of inflammatory anorexia (8–10, 27), and based on this view, several studies have sought to explain how peripheral cytokines enter the brain and subsequently affect neuronal pathways involved in food intake regulation (12, 13). However, we recently reported that despite a robust increase in levels of peripheral cytokines, including those whose action does not require MyD88 signaling, LPS fails to induce either anorexia or CNS cytokine expression in mice lacking MyD88 (19). These findings prompted us to propose an alternative model in which circulating in-
flammary mediator, including cytokines and other circulating factors that do not rely on MyD88-mediated signal transduction, inhibits food intake via a mechanism that ultimately requires activation of MyD88 signaling in target cells within the brain (15).

To test this model more directly, we determined whether: 1) restoring WT circulating cells to mice that otherwise lack MyD88 signaling rescues their ability to mount an anorexic response to LPS, or 2) sensitivity to LPS-induced anorexia is reduced in WT mice transplanted with MyD88-deficient bone marrow. Our results demonstrate that MyD88-deficient mice transplanted with WT circulating cells (C57>MyD88) display transient, markedly attenuated anorexia in response to LPS, despite a robust peripheral inflammatory response, as judged by plasma cytokine concentrations and induction of IL-1β mRNA expression in splenocytes 6 h after LPS administration. Consistent with their protection from anorexia, LPS also failed to induce IL-1β and TNFα mRNA expression in hypothalamus of mice in the C57>MyD88 group. This finding suggests that in the absence of MyD88 in the CNS or other tissues, the capacity of peripheral inflammatory signals to induce neuronal inflammation and anorexia is markedly impaired. This interpretation is consistent with previous evidence that reconstituting the bone marrow of Toll-like receptor 4 signaling-deficient mice with WT immune cells does not restore the ability of LPS to induce IL-1β mRNA expression in the brain (28), and supports the hypothesis that cytokine expression in the CNS is required for peripheral inflammatory mediators to cause sustained anorexia (15).

These results collectively demonstrate that the effect of a potent inflammatory stimulus to induce the release of inflammatory mediators from circulating cells, even TNFα, which signals via MyD88-independent mechanisms, is insufficient to cause anorexia in animals lacking MyD88 signaling in brain and other tissues. Moreover, the circulating IL-1β response to LPS appears to be insufficient to induce either sustained anorexia or hypothalamic cytokine expression in the absence of central MyD88 signaling, despite a putative CNS transport mechanism for plasma IL-1β (12, 13) and known MyD88-independent IL-1β signaling pathways (29). These findings add to growing evidence that anorexia can be dissociated from the circulating inflammatory response induced by LPS, and that MyD88 signaling within the CNS is an essential mediator of inflammatory anorexia because if this pathway cannot be activated, LPS-stimulated circulating cytokines will not alter feeding behavior.

Although LPS-induced food intake inhibition was markedly attenuated in C57>MyD88 mice compared with controls, this finding stands in contrast to our previous evidence that mice lacking MyD88 are completely insensitive to the effect of LPS on food intake. This finding raises the possibilities that: 1) a MyD88-dependent response of WT circulating cells to LPS makes a small but detectable contribution to LPS-induced anorexia, and 2) the CNS mechanism underlying the anorexia does not require MyD88. However, because the current study did not include a MyD88->MyD88 control group, additional study is needed to test this hypothesis. If confirmed in future studies, this outcome would suggest that like fever (30), inflammatory anorexia occurs in phases that involve distinct mechanisms.

Although we detected a robust LPS-induced peripheral inflammatory response in C57>MyD88 mice, one cannot infer that the circulating inflammatory response in these animals was entirely normal without a more complete evaluation of the time course of plasma concentrations of cytokines and other inflammatory mediators, including prostanoids (31, 32). Nevertheless, our finding of markedly elevated plasma cytokine levels and robust splenic cytokine mRNA expression at a single time point (6 h after LPS) suggests that even if the systemic inflammatory response of C57>MyD88 mice was not normal, it would have been expected to cause anorexia in normal mice. Thus, we conclude that an inflammatory response sufficient to cause anorexia in normal mice failed to do so in chimeric C57>MyD88 mice, implying a key role for MyD88 signaling in neuronal and behavioral responses to LPS.

Among various LPS-induced cytokines and chemokines measured in plasma 6 h post-LPS administration, only two, MCP-1 and KC (chemokines that participate in chemotaxis and diapedesis of monocytes into tissues), showed a marked reduction among C57>MyD88 mice compared with WT controls. Because tissue macrophages are likely the initial key source of chemokines induced by LPS (33, 34), and because our study was performed at a time point (3 months after BMT) when radiation-resistant resident macrophages have not been fully replaced by donor cells (35, 36), it is not surprising that levels of these chemokines would be reduced in C57>MyD88 animals.

Interestingly, the plasma cytokine profile of LPS-treated MyD88>C57 resembled that of WT mice, consistent with the ability of LPS to signal through MyD88-independent mechanisms in circulating cells (37). However, LPS-stimulated IL-1β levels in MyD88->C57 mice, despite the demonstrated absence of IL-1β gene expression in circulating cells, suggests that WT noncirculating cells (i.e., endothelial cells, tissue macrophages, adipocytes, etc.) also can contribute significantly to circulating cytokine levels. Thus, caution is warranted in drawing conclusions regarding impairment of the peripheral inflammatory response in MyD88->C57 mice, and additional study is needed to determine whether peripheral inflammatory mechanisms derived from noncirculating cells make a significant contribution to LPS-induced anorexia in MyD88->C57 mice or whether central inflammatory mechanisms alone accounted for the LPS effects in these animals.

Based on our findings, several possibilities can be considered to explain how MyD88 mediates CNS responses to inflammatory stimuli, including that: 1) MyD88 signaling in endothelial cells lining brain capillaries is essential for circulating inflammatory mediators to increase blood-brain barrier permeability and subsequently enter the CNS, 2) MyD88 signaling in neurons or glial cells triggers a local inflammatory response essential for LPS-induced anorexia, or 3) MyD88-dependent signal transduction is a required event within one or more key neuronal subsets that transduce input from inflammatory mediators into a change of feeding behavior. These possibilities are not mutually exclu-
sive, and they justify additional efforts to delineate the role of MyD88 signaling in inflammatory anorexia.

In addition to weight loss caused by anorexia, inflammatory stimuli such as LPS cause catabolism of fat mass and lean mass via mechanisms generally considered to be dependent on humoral factors, including pro-inflammatory cytokines (38). Interestingly, we found that reconstituting MyD88−/− mice with WT bone marrow did not confer protection from the dramatic LPS-induced weight loss seen in WT control animals, suggesting that MyD88-dependent humoral factors produced by circulating cells are not necessary for weight loss to occur. In contrast, mice in the C57>MyD88 group demonstrated only mild LPS-induced weight loss, similar to our previous evidence that MyD88−/− mice are susceptible to LPS-induced weight loss, despite complete protection from anorexia (19). Although further studies are needed to elucidate the specific mechanism of anorexia-independent inflammatory weight loss in MyD88-deficient animals, these results cumulatively imply that humoral factors elaborated from circulating cells are relatively unimportant because the presence or absence of MyD88 signaling in immune cells appears to have had little impact to alter the magnitude of LPS-induced weight loss.

In conclusion, we report that in an acute model of inflammatory sickness, WT circulating immune cells make little, if any, contribution to anorexia in the absence of tissue MyD88 signaling, and that MyD88-dependent signaling in the CNS or other tissues appears to be essential for this anorectic response. These findings constitute additional, direct evidence suggesting a key role for MyD88 signaling in the CNS in the pathogenesis of negative energy balance induced by systemic inflammatory conditions, a concept that may ultimately lead to novel therapies for patients with inflammatory anorexia and wasting illness.

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