Staphylococcal enterotoxin B induces fever, brain c-Fos expression, and serum corticosterone in rats

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Staphylococcal enterotoxin B induces fever, brain c-Fos expression, and serum corticosterone in rats. Am J Physiol Regulatory Integrative Comp Physiol 280: R1434–R1439, 2001.—The paraventricular nucleus of the hypothalamus (PVH) occupies a pivotal point within the network of brain nuclei coordinating critical host-defense responses. In mice, T cell-dependent immune stimuli, including the bacterial superantigen staphylococcal enterotoxin B (SEB), can activate the PVH. To determine whether T cell-dependent immune stimuli activate the PVH in rats, we assessed plasma corticosterone (Cort) levels, fever responses, and c-Fos expression in the PVH in animals treated with intraperitoneal injections of SEB. In animals with previously implanted abdominal thermisters, intraperitoneal injection of 1 mg/kg SEB resulted in a significant rise in body temperature, with a latency of 3.5–4 h. In separate animals, intraperitoneal injection of 1 mg/kg SEB resulted in a significant elevation of plasma Cort and induced c-Fos expression in parvocellular neurons within the PVH. These results support the idea that T cell-dependent immune stimuli activate brain pathways mediating host-defense responses such as fever and neuroendocrine changes.

HOST DEFENSE PRESENTS ONE of the most critical physiological challenges for an animal. The ability to prevail against infection with pathogenic microorganisms requires that animals possess a sensory system capable of detecting pathogens (2, 3). Second, the animal must be able to remove or inactivate the pathogens once detected. In vertebrates, specialized immune cells (e.g., macrophages, granulocytes, and certain T cells) and blood-borne molecules (e.g., complement) directly destroy pathogens. These efforts are supported by endocrine, metabolic, and cardiovascular changes that are coordinated by autonomic and neuroendocrine areas of the brain.

Immune-derived mediators, including cytokines, released following detection of pathogens initiate the activation of a constellation of brain regions collectively involved in homeostatic regulation, including host defense (8). Brain-mediated host-defense mechanisms, components of the “acute phase response” to infection, include fever, somnolence, and elevations of plasma corticosteroids, as well as behavioral alterations such as social withdrawal and hypophagia. In this way, the immune system is able to recruit a wide variety of body-wide defense mechanisms in response to infection.

The immune system relies on two general types of sensory mechanisms tailored to detect different types of pathogenic microorganisms or their products. One pathway is T cell mediated and uses specialized “antigen-presenting” (AP) immune cells (primarily dendritic cells; 1, 30) that phago- or pinocytose toxins or whole microorganisms (bacteria and viruses). Peptide components of these pathogens are bound to major histocompatibility complex molecules that are then presented on the AP cell membrane, allowing binding to specific T cells that express the appropriate receptor. Activated T cells then marshal immune-defense mechanisms via the release of specific cytokines that serve to signal other immune cells. Some of these T cell-derived cytokines, including tumor necrosis factor (TNF) and interleukin-2 (IL-2), are capable of initiating brain-mediated host-defense mechanisms, including the induction of fever and elevation of plasma corticosteroids (11, 19).

In addition to T cell-dependent immune sensory mechanisms, many immune cells, including dendritic cells and macrophages, express specific receptors for bacterial cell wall constituents or viral proteins (24). Lipopolysaccharide (LPS) is believed to be the most important marker for gram-negative bacteria, whereas peptidoglycan biproducts such as muramyl peptides (MDP) serve as salient stimuli from gram-positive bacteria (28, 42). Immune cells activated by binding of these substances release hormone-like mediators such as cytokines and chemokines that activate other immune cells and can also serve to signal the brain. Thus both T cell-dependent and -independent mechanisms of

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pathogen detection lead to release of mediators coordinating both the immune and nervous systems.

Whereas both types of immune sensory transduction mechanisms lead to production of mediators capable of signaling the brain, most of what is known about immune-to-brain communication derives from work using T cell-independent stimuli such as LPS or muramyl dipeptide. However, Shurin et al. (35) and Kusnecov et al. (22) have shown that T cell activation by a superantigen derived from gram-positive bacteria [staphylococcal enterotoxin B (SEB)] activates the hypothalamus-pituitary-adrenal (HPA) axis and initiates anxiety-like behavior in mice. These findings raise interesting issues regarding the specificity of neural responses to immune stimuli. For example, do T cell-dependent antigens such as SEB activate acute phase responses in a similar manner as T cell-independent stimuli?

SEB would seem to be a potentially useful stimulus to investigate the range and mechanisms of afferent immune signaling to the brain. However, whereas most work describing immune signal-transduction mechanisms, both T cell dependent and independent, has been performed with mice, studies investigating neural substrates supporting host-defense responses have been done primarily in rats, with the use of T cell-independent stimuli. Therefore, in an effort to develop a rat model useful for the study of neural signaling of T cell-dependent immune stimuli, the general goal of these experiments was to determine whether SEB induces brain-mediated host-defense responses in rats.

By virtue of its control over the peripheral autonomic nervous system and its pivotal role in the regulation of adrenal function, the paraventricular nucleus of the hypothalamus (PVH) occupies a critical nodal point in the nervous system and its pivotal role in the regulation of adrenal function, the paraventricular nucleus of the hypothalamus (PVH) occupies a critical nodal point in the hypothalamus-pituitary-adrenal (HPA) axis and initiates anxiety-like behavior in mice. These findings raise interesting issues regarding the specificity of neural responses to immune stimuli. For example, do T cell-dependent antigens such as SEB activate acute phase responses in a similar manner as T cell-independent stimuli?

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MATERIALS AND METHODS

Animals. Adult male Sprague-Dawley rats (n = 38; Harlan) were received and maintained under specific pathogen-free conditions. They were housed singly in Plexiglas cages with sawdust bedding and had ad libitum access to food and water. The temperature in the colony room was maintained at 25 ± 1°C, with a 12:12-h light-dark cycle (lights on at 0700). All procedures were performed in accordance with protocols approved by the University of Colorado Boulder Institutional Animal Care and Use Committee.

Thermister implantation. Eighteen animals were implanted with precalibrated intraperitoneal thermisters (Minimitter, Sun River, OR) while under halothane anesthesia, as previously described (11, 40). They were allowed to recover 7 days before the experiments. During this time, the animals were handled daily and received sham (using a blunt syringe hub) injections to habituate them to the injection procedure.

Fever measurement. Rats were injected intraperitoneally with either 1 mg/kg SEB (n = 10; Lot #77H4013, Sigma) or equivalent volume saline (n = 8) at 0.5 mg/kg in 25°C water. The temperature in the colony room was maintained at 25°C, with a 12:12-h light-dark cycle (lights on at 0700). All procedures were performed in accordance with procedures. Animals remained in their home cages during the experiment, while the frequencies emitted by the thermisters were monitored remotely. These frequencies were then converted to temperature values based on each transmitter's calibration data.

Cort levels and Fos expression in the PVH. Rats were injected intraperitoneally with either 1 mg/kg SEB (n = 12) or equivalent volume saline (n = 8) between 2 and 3.5 h after light onset. At 3.5 h after the injection, they were removed from their cages, and blood was collected from the lateral tail vein into microcentrifuge tubes within 3 min. The samples were allowed to clot and were then centrifuged at 3,000 rpm. The supernatants were stored at −20°C until assayed for Cort using a commercially available radioimmunoassay kit (ICN Pharmaceuticals, Costa Mesa, CA) according to the manufacturer's instructions.

Immediately after blood sampling, the rats were anesthetized with 60 mg/kg Nembutal and perfused transcardially with saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB) with pH 7.4. The brains were dissected, postfixed overnight, and stored in 0.1 M PB containing 0.1% sodium azide, at 4°C, before being sectioned at 50 μm on a vibratome. Sections through the hypothalamus were processed for c-Fos immunohistochemistry using a polyclonal antiserum raised in rabbits against the NH2-terminal region of the c-Fos protein (dilution 1:50,000; Ab5, Oncogene Research Products, Lot #60950101), as previously described (14). Specificity of the immunostaining was assessed by omitting the primary antiserum.

Quantification of c-Fos expression. Bright-field images were acquired from an Olympus Vanox II bright-field microscope with a COHU CCD camera coupled to an Apple PowerMac 7200 equipped with National Institutes of Health Image software (version 1.60). Numbers of c-Fos-immunoreactive (c-Fos-IR) profiles in the PVH were counted in serial sections (150 μm apart) bilaterally through the nucleus. The obtained micrographs were labeled using Adobe Photoshop (version 5.0). Except for small adjustments of brightness and contrast, the images were not altered.

Data analysis. The effects of SEB on core body temperature from the baseline temperatures were analyzed across time using a two-way repeated-measures ANOVA (Statview, Berkeley, CA). When appropriate, post hoc analyses were performed at each time point using the Student-Newman-Keuls multiple comparison test. The effects of SEB on plasma Cort levels and c-Fos expression in neuronal nuclei within the PVH were determined using one-way ANOVA. In all tests, an alpha level of P < 0.05 was taken as an indication of statistical significance.
RESULTS

Thermogenic responses to SEB injections. Intraperitoneal injection of 1 mg/kg SEB produced a modest (0.4°C) but significant rise in body temperature compared with injections of equivolume saline vehicle \( F(18,288) = 2.068; P < 0.01 \). This difference appeared by 200 min \( F(1,18) = 7.536; P < 0.02 \) and lasted through the end of the monitoring (at 380 min). The change in body temperature over time is depicted in Fig. 1.

Plasma Cort levels after SEB injections. In SEB-treated rats, Cort levels were elevated (23.2 ± 2.5 mg/dl) at 3.5 h after injection compared with saline-injected control animals (10.5 ± 2.4 μg/dl). This difference was statistically significant \( F(1,18) = 11.6; P < 0.003 \). Mean plasma Cort values are depicted in Fig. 2.

c-Fos expression in the PVH after SEB injections. Concomitant with the rise in plasma Cort, SEB injections induced c-Fos expression in neurons within the PVH (Figs. 3 and 4). c-Fos-positive neurons were located within the parvocellular subdivision that includes dorsomedial neuroendocrine and dorsal and ventral autonomic subnuclei (Fig. 3). SEB-induced c-Fos expression was largely absent in magnocellular neurons of the lateral subdivision of PVH. In SEB-

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**Fig. 1.** Intraperitoneal challenge with staphylococcal enterotoxin B (SEB) (1 mg/kg) induces a rise in core body temperature with an onset ~3 h after injection. *Significant rise in temperature (P < 0.05) in the SEB-treated group (n = 10) compared with the saline-treated group (n = 10).

**Fig. 2.** Intraperitoneal challenge with SEB (1 mg/kg, n = 12) increases corticosterone secretion at 3.5 h following injection compared with saline injection (n = 8).

**Fig. 3.** Photomicrographs depicting the expression of the immediate early gene product Fos in the paraventricular nucleus of the hypothalamus (PVH). Little expression is present following intraperitoneal injection of pyrogen-free saline (A), but many c-Fos-positive nuclear profiles appear 3.5 h following intraperitoneal SEB (B). These c-Fos-positive profiles are found in dorsal (dp), medial (mp), and ventral parvocellular (vp) portions of the PVH, but they are virtually absent in the magnocellular (mg) subdivision of the nucleus.

**Fig. 4.** Quantitative analysis of c-Fos expression in the PVH. The numbers represent cumulative bilateral counts in 3 evenly spaced (150 μm apart) sections through the PVH. Intraperitoneal challenge with SEB (1 mg/kg, n = 12) increases the number of c-Fos-immunoreactive profiles at 3.5 h following injection compared with saline injection (n = 8).
treated animals, the mean number of c-Fos-positive nuclear profiles was 488 ± 104, compared with 165 ± 23 in saline-treated animals (Fig. 4). This difference was statistically significant [F(1,18) = 6.2, P < 0.02].

DISCUSSION

The results of these experiments demonstrate that intraperitoneal injections of SEB, a T cell-dependent antigen, induce thermogenic responses within 3–4 h after injection, concomitant with elevated plasma Cort levels and c-Fos expression in the PVH in rats. T cell-independent immune stimuli such as LPS or MDP are well documented as inducing fever or HPA responses. The finding that SEB treatment also induces thermogenic and Cort responses, concomitant with activation of the PVH, indicates that pathogens detected via different signal-transduction mechanisms can also activate brain-mediated acute phase responses.

The results from previous studies investigating whether T cell-dependent antigens are capable of initiating brain-mediated host-defense responses have been mixed. Besedovsky and Del Rey (2) reported experiments showing HPA axis activation following tumor transplantation and inoculation with sheep red blood cells (SRBCs). These effects were shown to be dependent on T cells. Similarly, our study and those reported by Shurin et al. (35) and Kusnecov et al. (22) report clear evidence of brain activation in response to SEB. However, other studies (34, 37) have not found elevated plasma ACTH or Cort levels following injections of SRBCs. The origins of this inconsistency are unclear but may be related to the saliency of the immune stimulus. SEB is one of a class of compounds known as superantigens, so called because of their very potent ability to activate T cells. Superantigen binding to T cell receptors leads to prolonged T cell activation and, notably, an overabundant secretion of cytokines (20). Thus differences in the ability of T cell-dependent immune stimuli to activate the brain may reflect varying levels of T cell activation and subsequent release of cytokines produced in each experimental paradigm. Furthermore, although Shanks et al. (34) did not observe ACTH or Cort responses to SRBC administration, they did find changes in brain catecholamine turnover, indicating that this treatment does lead to changes in neuronal activities. The lack of HPA response to SRBCs in the study reported by Stenzel-Poore et al. (37) may reflect, as the authors suggest, the fact that under certain immune circumstances, an HPA response may not be adaptive. Thus these findings suggest that brain responses to different immune stimuli may well be tailored to the requirements of immune responses to a particular antigen.

The elevation of plasma levels of Cort following SEB challenge may have functional significance for the immune cell response to this stimulus and other bacterial superantigens. However, the precise effects of glucocorticoids during an immune challenge are not well established and likely depend on tissue concentrations. High, including pharmacological, levels of glucocorticoids are used to inhibit inflammation and are believed to function as a brake, or negative feedback regulation, acting to prevent maladaptive consequences of excessive immune activation (36, 38). However, lower levels of glucocorticoids act to enhance some immune cell functions, especially early in the course of infection or inflammation (41). For some immune functions, glucocorticoids are necessary (12).

The observation that intraperitoneal administration of SEB activates parvocellular neurons in the PVH indicates that the increased secretion of Cort is likely to be centrally driven as opposed to via direct action at the pituitary or adrenal gland. The distribution of c-Fos-labeled nuclear profiles includes the region of the PVH that harbors the corticotropin-releasing hormone (CRH) containing neurons that control the portal release of adrenocorticotropic hormone (CRH) (33). These observations are consistent with previous observations in mice (35).

In addition, SEB induced c-Fos expression in the autonomic portions of the PVH, the dorsomedial and the ventral parvocellular subdivisions (33), indicative of activation of PVH neurons that project to autonomic effector regions that are critical for the induction of fever (4). The present observations that SEB also induces a fever in rats support the notion of a centrally driven rise in core body temperature in response to challenge with SEB.

The question arises as to which neural pathways drive these PVH responses. After systemic challenges with certain immune stimuli, e.g., IL-1 or LPS (7, 9, 10), caudal medullary catecholaminergic neurons drive PVH parvocellular neurons to, via the release of CRH, activate the pituitary-adrenal axis (9, 10). A similar ascending visceral sensory pathway may drive the response of PVH neurons to peripheral SEB challenge (15, 16). Altogether, these observations suggest that, through the PVH, SEB activates neuroendocrine host-defense mechanisms and alters central autonomic control functions that may include thermoregulatory and other, e.g., cardiovascular, changes.

The pathway(s) by which T cell activation leads to activation of the HPA axis and fever is as yet unknown, but it may involve T cell-derived cytokines. After stimulation with SEB, cultured mouse T cells release IL-1β, IL-2, and TNF-α (31). Similarly, T cells in lymph nodes from mice treated with intraperitoneal SEB produced TNF-α within 1 h and IL-2 within 2 h of SEB injection (23) (expression of IL-1β was not reported). All three of these potential T cell-derived mediators have been implicated in immunosensory signaling (3, 5, 19, 38).

Before activation by the antigen, T cells circulate between lymphoid structures (20). It is primarily within the lymph nodes that T cells make contact with the antigen, via the AP cells that make initial contact with pathogens (20). Studies investigating the tissue distribution of either intraperitoneally injected (6, 25) or locally generated (26, 27) substances have shown that these substances are either trapped in lymph nodes or are carried via thoracic lymph vessels to the heart and general circulation. Thus lymph nodes likely...
provide the major source of T cell-derived signal cytokines (or other mediators) released after intraperitoneal SEB administration. These mediators may then signal the brain via sensory nerves derived from dorsal root (29) or vagal (15, 17) ganglia that innervate lymph nodes or via the blood to activate immunosensory brain structures such as vascular endothelium (39) or circumventricular organs (32).

Compared with T cell-independent stimuli such as LPS and MDP, the onset of SEB-induced fever and HPA activation are substantially slower. T cell-independent stimuli activate immune cells via specific receptors and, in general, lead to more rapid onset of effects, typically within 2 h (5, 18). The slower response to SEB may reflect the more elaborate processing required for T cell-dependent stimuli. Alternatively, these differences may reflect species differences as mice respond more rapidly to thermogenic stimuli (21), and HPA axis responses to SEB are faster in mice as well (35).

Another notable difference between the effects of SEB and those of LPS concerns the magnitude of PVH activation. Whereas systemic administration of LPS can lead to the induction of c-Fos expression in thousands of PVH neurons, the effects of SEB are far less dramatic (Fig. 3). This difference may reflect an insensitivity of rats to SEB (e.g., fewer T cells expressing the appropriate receptor). However, the relatively weak induction of c-Fos expression is not as evident in other brain nuclei, including the central nucleus of the amygdala, bed nucleus of the stria terminalis, and lateral parabrachial nucleus (13, Goehler et al. in preparation). In fact, c-Fos induction in the rostral ventral lateral medulla is somewhat greater in animals treated with SEB compared with those treated with LPS. Thus the differences observed between the two stimuli may represent differences in central nervous system processing secondary to the different mechanisms of immune detection (T cell dependent vs. independent) and therefore reflect a kind of parallel processing (15).

In summary, intraperitoneal injections of SEB in rats lead to elevated body temperature and plasma Cort levels. These changes are associated with increased c-Fos expression in parvocellular neurons within the PVN. The findings we report here support the idea that brain-mediated responses contribute to host defense against many if not most types of infectious agents.

**Perspectives**

Whereas hyper- and hypothermic responses are associated with a variety of viral and parasitic diseases (21), the vast majority of systematic studies investigating mechanisms of fever has used T cell-independent bacterial stimuli, notably LPS and MDP. However, clinical symptoms of bacterial illness caused by superantigens, such as toxic shock syndrome, often include high fever. The autonomic and endocrine dysregulation typical of shock syndromes may well involve, at least in part, superantigen-driven activation of central autonomic neurocircuitry. Thus the development of an experimental model to study mechanisms of fever as well as HPA axis responses resulting from superantigen stimulation may be of clinical importance.

Comparing features such as immunosensory signal transduction or peripheral signaling pathways (neural vs. humoral) between T cell-dependent and -independent antigens can provide a comprehensive picture of the mechanisms by which the nervous system responds to immune activation and can provide answers to questions regarding organizing features of immunosensory systems. For instance, does stimulation by both types of antigens lead to the activation of a final common pathway via either converging peripheral or brain signals? Or is the type of antigen a stimulus feature that is maintained throughout immunosensory processing?

Finally, the fact that both T cell-dependent and -independent antigens activate the brain underlines the important role of the nervous system in coordinating physiological and behavioral responses to infection. Clearly, effective host defense can be characterized as a joint venture of the nervous and immune systems.

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