Published in final edited form as:

J Neuroimmune Pharmacol. 2011 December ; 6(4): 585–596. doi:10.1007/s11481-011-9284-5.

The Role of IL-1 β in Nicotine-induced Immunosuppression and Neuroimmune Communication¹

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Abstract

Although a number of inflammatory cytokines are increased during sepsis, the clinical trials aimed at down-regulating these mediators have not improved the outcome. These paradoxical results are attributed to loss of the “tolerance” phase that normally follows the proinflammatory response. Chronic nicotine (NT) suppresses both adaptive and innate immune responses, and the effects are partly mediated by the nicotinic acetylcholine receptors in the brain; however, the mechanism of neuroimmune communication is not clear. Here, we present evidence that, in rats and mice, NT initially increases IL-1 β in the brain, but the expression is downregulated within 1–2 wk of chronic exposure, and the animals become resistant to proinflammatory/pyrogenic stimuli. To examine the relationship between NT, IL-1 β , and immunosuppression, we hypothesized that NT induces IL-1 β in the brain, and its constant presence produces immunological “tolerance.” Indeed, unlike wild-type C57BL/6 mice, chronic NT failed to induce immunosuppression or downregulation of IL-1 β expression in IL-1 β -receptor knockout mice. Moreover, while acute intracerebroventricular administration of IL-1 β in LEW rats activated Fyn and protein tyrosine kinase activities in the spleen, chronic administration of low levels of IL-1 β progressively diminished the pyrogenic and T cell proliferative responses of treated animals. Thus, IL-1 β may play a critical role in the perception of inflammation by the CNS and the induction of an immunologic “tolerant” state. Moreover, the immunosuppressive effects of NT might be at least partly mediated through its effects on the brain IL-1 β . This represents a novel mechanism for neuroimmune communication.

Keywords

Nicotine; IL-1 β ; immunosuppression; neuroimmune communication; Fyn

¹This work was supported in part by grants from the US Army Medical Research and Materiel Command (GW093005), National Institutes of Health (RO1 DA017003, RO1 DA04208-17, and RO1DA04208S) and the Defense Threat Reduction Agency (HDTRA1-08-C-002).

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Disclosures

The authors have no financial conflict of interest.

Introduction

Cigarette smoking is a major health risk factor and contributes to over three million premature deaths annually worldwide. Many adverse health effects of cigarette smoke might stem from its immunosuppressive effects (Holt and Keast, 1977; Sopori, 2002). To that end, we and others have shown that chronic inhalation of cigarette smoke suppresses the immune system in humans and experimental animals; reviewed in (Sopori et al., 1998a; Stampfli and Anderson, 2009). Nicotine (NT), the major immunosuppressive compound in cigarette smoke, may affect immune and inflammatory responses through the central and peripheral mechanisms (Sopori et al., 1998b; Sopori, 2002; Wang et al., 2003; van Westerloo et al., 2005; Mishra et al., 2008). We have reported that while acute NT increases intracellular calcium $[Ca^{2+}]_i$ in T cells (Razani-Boroujerdi et al., 2007), chronic exposure of animals to NT causes T cell anergy through constitutive activation of protein tyrosine kinase (PTK) activity, including Fyn, production of inositol-1,4,5-trisphosphate, and depletion of inositol-1,4,5-trisphosphate-sensitive Ca^{2+} stores, leading to loss of TCR-mediated elevation in $[Ca^{2+}]_i$ (Geng et al., 1996; Kalra et al., 2000). The immunosuppressive effects of chronic NT treatment in vivo are, at least partially mediated through the nicotinic acetylcholine receptors in the CNS, and the effects are independent of the hypothalamus-pituitary-adrenal (HPA) axis (Sopori et al., 1998b; Singh et al., 2000).

The mechanism by which NT modulates the immune/inflammatory system through the CNS is not clear. There is evidence that cytokines in the brain play an important role in the regulation of the inflammatory and anti-inflammatory responses. For example, neuroinflammation may damage neurons, but it also confers neuroprotection (Stoll et al., 2002). Increasing evidence suggests that proinflammatory cytokines such as IL-1 β also provide neuroprotection and dampen neuroinflammation (Stoll et al., 2002; Jin et al., 2009; Pinteaux et al., 2009). Similarly, head trauma/injury, usually associated with a pyrogenic response (Badjatia, 2009), also leads to systemic inflammatory response syndrome followed by a compensatory anti-inflammatory response syndrome (Lenz et al., 2007; Lu et al., 2009). Proinflammatory cytokines such as TNF- α , IL-1 β , and IL-6 have been implicated in acute inflammatory responses (Dinarello, 2000); however, there is increasing evidence that early presence of these cytokines might have a protective role in sepsis (Torre et al., 1994; del Rey and Besedovsky, 2000; Kox et al., 2000). Similarly, pretreatment with sublethal doses of endotoxin increases proinflammatory cytokine production, but protects against septic peritonitis through a mechanism independent of adaptive immunity (Urbaschek and Urbaschek, 1987; Varma et al., 2005); these results can be achieved by pretreatment with IL-1 β and TNF- α (Urbaschek and Urbaschek, 1987). In this communication, we present evidence that (a) acute NT exposure induces IL-1 β in the brain, but the response is lost through continued exposure to NT, leading to an immunologic “tolerant” state, and (b) while an acute administration of IL-1 β in the brain activates splenic T cells, continued exposure suppresses the pyrogenic response and T cell function.

Materials and Methods

Animals

Male pathogen-free Lewis (LEW) rats were purchased from Harlan Sprague-Dawley Farms (Branchburg, NJ, USA), and IL-1 receptor knockout (IL-1R KO) mice and wild-type (WT) control (C57BL/6) mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA). Rats and mice were housed individually in class 100 air quality rooms with 12-h light/dark cycle; food (Teklad certified diet) and water were provided ad libitum. Animals were maintained at the thermo-neutral temperatures, i.e., $25\pm 1^{\circ}\text{C}$ (rats) and $30\pm 1^{\circ}\text{C}$ (mice). Three- to 4-mo-old animals were used in these experiments. The Lovelace Respiratory Research Institute IACUC reviewed and approved these studies.

Reagents

Monoclonal antibodies to the rat $\alpha\beta$ -TCR and the appropriate isotype control antibodies were purchased from PharMingen (San Jose, CA, USA), and the anti-p59^{fyn} antibody was purchased from Upstate Biotechnology (Lake Placid, NW, USA). Other reagents were obtained from the following vendors: recombinant rat IL-1 β (R & D Systems, Inc., Minneapolis, MN, USA), guinea pig complement (Cedarlane Laboratories, Ltd, Burlington, Ontario, Canada), chlorisondamine (Tocris Cookson, Ltd., Ellisville, MO, USA), ($\pm$) NT base (MP Biomedicals, Inc., Santa Ana, CA, USA), and P³²-ATP (MP Biomedicals). Unless mentioned otherwise, all other reagents, including acetyl-methyl ester of indo-1 and Con A were obtained from Sigma-Aldrich (Saint Louis, MO, USA).

IL-1 β treatment

For constant administration, IL-1 β (100 ng/kg/day) was given through a subcutaneous (s.c.) implanted Alzet miniosmotic pumps (Alzet Corp., Cupertino, CA, USA) that delivered a volume of 0.25 $\mu\text{l/h}$. The pumps were implanted as described (Geng et al., 1996). Briefly, rats were anesthetized with isoflurane-oxygen and shaved at the base of the neck. A pocket of approximately 2 cm was made by a transverse incision under the skin, and the pumps were placed in the pocket with the delivery end of the pump facing toward the bottom of the pocket. For intracerebroventricular (ICV) delivery, a 5-mm long, 28-gauge stainless steel cannula was placed stereotactically into the rat, and connected to a s.c. -implanted miniosmotic pump (ALZET Brain Infusion Kit, Alzet Corp) as described (Sopori et al., 1998b). Rats were anesthetized with a mixture of ketamine (85 mg/kg) and xylazine (15 mg/kg). For ICV placement of cannulas, the coordinates were 1 mm posterior to the bregma and 1.4 mm lateral to the midline. Miniosmotic pumps were filled with IL-1 β in artificial cerebrospinal fluid (aCSF) containing 0.2% BSA or aCSF-BSA only (control). In some rats, a single injection of 50 ng of IL- β was given either by a s.c. injection in 0.1 ml saline or administered in 6 μl of aCSF in ICV cannulas placed surgically 1 wk prior to IL-1 β injection.

NT treatment

Nicotine was administered as ($\pm$) NT base through s.c.-implanted miniosmotic pumps that delivered 2 mg of the NT/day/kg body wt in C57BL/6 mice and LEW Rats. In rats, this

amount of NT raises the blood cotinine (the main metabolic byproduct of nicotine) level equivalent to humans smoking 1 packet of cigarettes/day (Geng et al., 1995). To study acute effects of NT, some mice received a single intraperitoneal (i.p.) injection of 7.5 µg of NT, while LEW Rats received a single i.p. injection of 62.5 µg of NT.

Immunizations

To determine the antibody-forming cell (AFC) response, 4 days prior to sacrifice, animals were injected with 5×10^8 SRBC intravenous (i.v.) and i.p. into rats and mice, respectively (Sopori et al., 1989).

Collection of tissues and purification of T cells

Rats were scarified by isoflurane inhalation; some animals were perfused through the heart with endotoxin-free saline prior to the removal of the brain. Brains were collected, dissected longitudinally into halves, and placed in tubes containing Tri Reagent (Molecular Research Center, Inc., Cincinnati, OH, USA), snap frozen in liquid nitrogen, and stored at -80°C for later RNA extraction. Spleens were harvested and cell suspensions made as described (Razani-Boroujerdi et al., 1994a). Briefly, spleens were pressed through stainless steel mesh and treated with buffered NH_4Cl solution to lyse red blood cells. Cells were washed 3 times with PBS and resuspended in complete tissue culture medium (RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 50 µM 2-mercaptoethanol, 1 mM sodium pyruvate, and 1% penicillin/streptomycin). Purified splenic T cells were obtained by MACS separation (Miltenyi Biotec, Inc., Bergisch Gladbach, Germany). Spleen cells were suspended in PBS containing 2 mM EDTA and 0.5% BSA, and incubated with Rat Pan T cell Micro beads (Miltenyi Biotec) for 15 min at 4°C , washed with the same buffer, and loaded onto magnetized columns. The column was moved out of the magnetic field, and the retained cells (purified T cells) were eluted from the column with the buffer.

Assay for AFC Response

The primary direct AFC response was determined by the Cunningham and Szenberg method as described (Sopori et al., 1989). Briefly, spleen cells were mixed with 2% SRBC and 20 µl of guinea pig complement (pre-absorbed on SRBC) in a final volume of 140 µl. Aliquots were distributed in duplicates into Cunningham slide chambers, incubated for 45 min at 37°C , and counted under low-power microscope. Results were expressed as AFC/ 10^6 spleen cells.

Assay for T cell proliferation

Proliferative responses were performed as described (Geng et al., 1995). Briefly, in a final volume of 0.2 ml of complete medium, 2×10^5 spleen cells were cultured in triplicate in flat-bottomed, 96-well microtiter plates in the presence and absence of indicated concentrations of Con A or anti- $\alpha\beta$ -TCR monoclonal antibody. Unlike the mouse and human T cells, rat T cells are activated to proliferate with anti-TCR antibodies without anti-CD28. Plates were incubated at 37°C in a 5% CO_2 atmosphere. After 48 h, culture wells were labeled with 0.5 µCi of [^3H]-thymidine (New England Nuclear Corp, Newton, MA, USA) and harvested 24 h later by a Skatron cell harvester (Molecular Devices Inc,

Sunnyvale, CA, USA). Samples were counted in a liquid scintillation counter and results expressed as the mean cpm $\pm$ SEM of triplicate cultures.

Assay for Intracellular Ionized Calcium in Lymphocytes

The intracellular calcium level ($[Ca^{2+}]_i$) of splenocytes was measured using acetyl-methyl ester of indo-1 acetyl-methyl ester of indo-1 under the conditions described (Razani-Boroujerdi et al., 1994a). Briefly, cells (5×10^6 /ml) were incubated at 37°C for 30 min with 5 μ M acetyl-methyl ester of indo-1 in loading medium (PBS containing, 2 mM $CaCl_2$, 1mM $MgCl_2$, and 3% FCS). After washing, cells were suspended in loading medium and incubated at 37°C for 30 min in a 5% CO_2 atmosphere. Cells were kept in the dark on ice until the assay. Before each measurement, 1 ml of the cell suspension was washed and resuspended in 2 ml of the loading medium. The $[Ca^{2+}]_i$ concentration of cells was determined by spectrofluorometry in a PTI Deltascan fluorometer (Photon Technology International, Inc., Birmingham, NJ, USA) at 37°C with constant, gentle stirring. Recording of cell fluorescence was started 60 s before the addition of the $\alpha\beta$ anti-TCR and the secondary antibody in rats, and anti-CD3 plus anti-CD28 antibodies in mice. $[Ca^{2+}]_i$ was calculated as we have described (Razani-Boroujerdi et al., 1994b).

Assay for PTK and Fyn activities

Protein tyrosine kinase activity was determined by immunoblotting using anti-phosphotyrosine antibodies (Geng et al., 1996). Briefly, purified spleen cells were suspended in the complete medium and incubated with anti-TCR antibody (2 μ g/ml) or an equivalent amount of isotype control antibody for 2 min at 37 °C. The reaction was stopped by the addition of ice-cold PBS, and the cell pellet was lysed in cold RIPA buffer (50 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 0.25 % sodium deoxycholate, 1% Nonidet P-40, 1 mM EDTA, 50 mM NaF, 1 mM activated Na_3VO_4 , and protease inhibitors: 1 mM PMSF, and 1 μ g/ml each of aprotinin, leupeptin, pepstatin). The lysates were clarified by centrifugation and aliquots of the lysate boiled in Laemmli sample buffer (60 mM Tris-HCl pH 6.8, 2% SDS, 10% glycerol, 10 mM DTT, and 0.001% bromophenol blue) for 5 min. Protein concentration was determined by the bicinchoninic acid method (Thermo Fisher Scientific Inc., Rockford, IL, USA), and equal amounts of proteins (10–20 μ g) were electrophoresed on 7.5% SDS-PAGE and transferred on polyvinylidene difluoride (PVDF) membranes. The blots were blocked with 5% dry skim milk protein (Upstate Biotech. Inc.) in 10 mM Tris-HCl pH 7.4 containing 150 mM NaCl for 1.5 h at room temperature. The blots were washed and probed with anti-phosphotyrosine monoclonal antibody and developed with HRP-conjugated second antibody. Fyn kinase activity of the lysates was determined essentially by the method of Gould and Hunter (Gould and Hunter, 1988). Briefly, 10 μ l of anti-p59^{fyn} antibody was added to 500 μ l of cell lysate (1 mg protein/ml) and incubated overnight at 4°C on a rocker. Protein-AG (50 μ l) was added to the sample, and the mixture was incubated at 4°C for 3 h. The immunoprecipitates were washed 3X with RIPA buffer and 2X with the kinase buffer (50 mM Tris-HCl, pH 7.4, 3 mM $MnCl_2$, 0.1 mM Na_3VO_4), and resuspended in the kinase buffer. The kinase activity of the immunoprecipitate was assayed by incubating the immunoprecipitates with 0.2 μ g of acid-treated enolase (Sigma-Aldrich) and γ -³²P-ATP (10 μ Ci) for 20 min at room temperature (Cooper et al., 1984; Gould and Hunter, 1988). The reaction was stopped by the addition of Laemmli sample buffer, boiled for 5 min, and the

samples analyzed on 10% SDS-PAGE. The gel was dried, and the phosphorylated enolase was visualized by autoradiography.

Real-time PCR (qPCR) for IL-1 β mRNA expression

Total RNA was isolated from the frozen brain as described (Razani-Boroujerdi and Sopori, 2007). Briefly, tissues were homogenized in the presence of TRI reagent (Molecular Research Center Inc., Cincinnati, OH, USA). Total RNA was isolated using the BCP phase separation reagent (Molecular Research Center Inc.). RNA was precipitated by 2-propanol and washed with 75% ethanol. The RNA pellet was dried for a short time, resuspended in RNase-free water, and quantitated spectrophotometrically. The IL-1 β and GAPDH primers and probes were purchased from ABI. qPCR was performed on the Prism 7900 HT sequence Detection System (Life Technologies Corp., Carlsbad, CA, USA), using the one-step RT-PCR master mix (Life Technologies Corp.), and standard protocols. PCR was performed for 40 cycles with denaturation (95°C for 15 s) and annealing (60 °C for 60 s). All results were derived from the linear amplification curve and normalized to GAPDH. The CT method was used to calculate the fold change in IL-1 β expression.

Assay for corticosterone

Serum corticosterone (CORT) levels were measured using the instructions and reagents provided with a ³H-CORT radioimmunoassay kit (ICN Pharmaceuticals Inc., Costa Mesa, CA, USA)

Measurement of the deep body temperature (Tb)

One week before the implantation of IL-1 β delivering miniosmotic pumps, rats were implanted intra-abdominally with biotelemeters (model VM-FH; Mini-Mitter Co., Bend, OR, USA) as reported (Sopori et al., 1998a; Sopori et al., 1998b). After the implantation, the animals were housed individually in plastic cages in special temperature-controlled rooms at 25°C $\pm$ 0.1°C. Signals for Tb were collected at 5-min intervals with a peripheral processor (Dataquest III system) connected to a personal computer. To test the pyrogenic properties of IL-1 β contained in the miniosmotic pumps after 14 days of implantation, the contents of the pumps were harvested, pooled, and injected into biotelemeters implanted rats i.v. Tb was recorded following the injection of the contents of miniosmotic pumps or vehicle.

Statistical analysis

Statistical comparisons between three or more experimental groups were performed using a one-way analysis of variance. The Scheffe *post hoc* test was used to determine the significance among groups, and the Student's *t* test was to compare the means between two groups (CON and IL-1 β -treated). These statistical procedures were performed using ABSTAT (Anderson-Bell Corp., Arvada, CO, USA).

Results

Acute exposure to NT transitorily increases IL-1 β expression in the brain

We and others have shown that NT is anti-inflammatory (Sopori, 2002; Scott and Martin, 2006; Ulloa and Wang, 2007) and reduces the pyrogenic response to turpentine-induced sterile abscess (Sopori et al., 1998a; Sopori et al., 1998b; Razani-Boroujerdi et al., 2004). Because the brain IL-1 β is an important cytokine for the pyrogenic response to turpentine and LPS (Horai et al., 1998; Kozak et al., 1998), we ascertained whether NT affected the expression of IL-1 β in the brain. C57BL/6 mice were treated with a single i.p. injection of 7.5 μ g of NT, and the brain IL-1 β expression was determined by qPCR analysis at various times after NT injection. Surprisingly, within 1 h after NT treatment, the expression of IL-1 β in the brain increased significantly and peaked at around 24 h post-NT administration (Fig. 1A). Similar changes in the brain IL-1 β expression were observed when LEW rats were challenged with 62.5 μ g of NT via single i.p. injection (Fig. 1B). Chronic treatment of C57BL/6 mice with NT through Alzet miniosmotic pumps increased IL-1 β mRNA that peaked at 4d and returned to the baseline around 8d (Fig. 1C); a similar increase in IL-1 β was also observed after chronic NT treatment of LEW rats that peaked at one wk but reverted to the baseline levels around 3 wks after chronic treatment (Fig. 1D). Thus, both acute and chronic NT treatments increase the brain expression of IL-1 β in both mice and rats; however, even in the continued presence of NT, the cytokine expression dropped within 2 to 3 wk of chronic NT exposure (Fig. 1C/D). Interestingly, i.m. administration of small quantities of turpentine (a model for sterile abscess) increased IL-1 β expression in the brain of naïve control rats; however, animals treated chronically with NT for 2–3 wk, failed to increase IL-1 β after the turpentine treatment (Fig. 2). The suppression of cytokine expression by chronic NT-treatment was not absolute, and larger doses of LPS broke the state of unresponsiveness and induced production of proinflammatory cytokines, including IL-1 β and TNF- α (not shown). These results suggest that following the early increase in IL-1 β , chronic NT induces a state of unresponsiveness (“tolerance”) to proinflammatory/pyrogenic stimuli; however, the tolerance is not total and is broken by stronger proinflammatory stimuli.

Unlike WT mice, IL-1R KO mice do not exhibit NT-induced “tolerance”

To ascertain whether the early increase in IL-1 β and its interaction with IL-1R were obligatory for the induction of NT-induced “tolerance,” we compared the brain IL-1 β expression in WT and IL-1R KO C57BL/6 mice after a 3-week exposure to NT. Although the IL-1R KO mice did not exhibit as robust NT-induced IL-1 β expression as WT control mice, the brain IL-1 β expression remained significantly higher than WT mice even after 3-wk NT exposure (Fig. 3). Thus, unlike normal mice, where brain IL-1 β expression returned to baseline after 1–3 wks exposure, increased IL-1 β -induced expression persisted in IL-1R KO mice. Therefore, it is likely that production of IL-1 β and the interaction between IL-1 β and IL-1R during the early phase of NT treatment are critical for the induction of cytokine-induced suppression during the chronic phase of NT treatment.

IL-1R KO mice are resistant to the immunomodulatory effects of NT

Nicotine inhibits the T-cell-dependent antibody response, T cell proliferation, and inflammatory responses (Sopori et al., 1989; Sopori et al., 1998a; Wang et al., 2003; Razani-Boroujerdi et al., 2004; Mishra et al., 2008). To examine whether the immunosuppressive effects of NT were related to IL-1R-mediated responses, IL-1R KO and WT mice were chronically exposed to NT for 3 wk, and spleen cells were examined for the AFC response to SRBC, Con A-induced T cell proliferation, and anti-TCR/CD28-stimulated rise in $[Ca^{2+}]_i$. Although IL-1R KO mice exhibited a significantly lower anti-SRBC AFC response than WT mice, NT inhibited the AFC (Fig. 4A) and Con A (Fig. 4B) responses in WT but not in IL-1R KO mice. Similarly, NT significantly blunted the anti-TCR/CD28-induced rise in $[Ca^{2+}]_i$ in WT but not IL-1R KO spleen cells (Fig. 5). These data indicate that IL-1R KO mice are resistant to the immunosuppressive effects of NT, and IL-1 β may play an important role in mediating the NT-induced immunosuppression.

ICV but not s.c.exposure to IL-1 β inhibits the AFC response

To ascertain whether chronic exposure to IL-1 β is immunosuppressive, rats were given IL-1 β (100 ng/day/Kg) either centrally (ICV) or peripherally (s.c.) via miniosmotic pumps for 1 and 2 wk. The animals were immunized with SRBC 4 days before the sacrifice, and spleen cells were tested for the anti-SRBC AFC response. Until 1 wk after IL-1 β treatment, there was no significant difference in the AFC response between controls and IL-1 β -treated animals (data not shown); however, at 2 wk after IL-1 β treatment, ICV administration of IL-1 β led to a significant decrease in the anti-SRBC AFC response (Fig. 6). On the other hand, s.c. administration of IL-1 β did not cause significant changes in the AFC response (Fig. 6). ICV administration of IL-1 β also inhibited T cell proliferation in response to Con A (Fig. 7A) and anti-TCR/CD28 (Fig. 7B). Moreover, the increase in $[Ca^{2+}]_i$ in response to TCR ligation was also attenuated by the IL-1 β treatment (Fig. 7C). None of these parameters were affected by s.c. administration of IL-1 β (not shown). These results suggest that chronic presence of low levels of IL-1 β in the brain, but not in the periphery, suppresses T cell responses.

Chronic IL-1 β exposure does not increase serum CORT levels

Acute administration of IL-1 β activates the HPA axis and increases the production of glucocorticoids (Skurlova et al., 2006). Therefore, it was possible that chronic low-dose IL-1 β also stimulated CORT production that in turn suppressed T cell function. Blood CORT levels were determined in rats at 4 h after acute ICV IL-1 β (50 ng/animal) administration and at 14 days after chronic ICV IL-1 β (100 ng/day/kg body wt) exposure. While acute bolus exposure significantly increased the serum CORT level, chronic exposure to IL-1 β did not cause significant change in CORT level on days 14 (Fig. 8). Therefore, as with chronic NT treatment (Singh et al., 2000), it is unlikely that the immunosuppressive effects of the chronic IL- β administration resulted from the increased serum CORT levels through activation of the HPA axis.

Chronic ICV administration of IL-1 β reduces its pyrogenic activity—Chronic NT treatment blunts the turpentine-induced fever response in rats (Razani-Boroujerdi et al.,

2004). To evaluate whether chronic ICV administration of IL-1 β altered the pyrogenic response of animals, rats were intra-abdominally implanted with biotelemeters to monitor Tb. Rats were also implanted with ICV cannulas for chronic administration of aCSF (control) or IL-1 β (100 ng/day/kg body wt) through s.c.-implanted miniosmotic pumps. Tb was recorded every 5 min, and average Tb traces for controls and IL-1 β -treated rats during the light/dark cycle of the day (where the rats are at rest or active, respectively) are shown in Fig. 9. It is clear that during the early phase, IL-1 β increased the Tb significantly and skewed the Tb circadian rhythm; however, continued presence of IL-1 β blunted the Tb response and normalized its circadian rhythm. By day 10, IL-1 β -treated rats were essentially indistinguishable from the aCSF-treated animals. To ascertain whether the IL-1 β contained in the miniosmotic pumps retained its biological (pyrogenic) activity, pumps were removed from several animals on day 14, and the pooled material (equivalent to approximately 100 ng of IL-1 β /kg body wt) was injected i.v. into naïve rats. Compared to aCSF-treated rats, the pooled sample caused a significant rise in Tb within 60 min of the injection (not shown), thus indicating that the IL-1 β within osmotic pumps was biologically active at 2 wk after the implantation. These results suggest that the constant presence of IL-1 β in the brain blunts its pyrogenic activity.

Acute ICV administration of IL-1 β activates Fyn and PTK activities in splenic T cells

—One of the earliest effects of TCR ligation is the activation of PTK, including Src-like kinases Fyn and Lck (Salmond et al., 2009). NT exposure activates PTK and Fyn in T cells (Geng et al., 1996; Kalra et al., 2004). To determine whether IL-1 β stimulates PTK activities, rats were surgically implanted with ICV cannulas, and 1 wk after the surgery a single administration of 50 ng of IL-1 β in 5 μ l of aCSF or aCSF alone was injected into the cannulas. In another group of rats, 50 ng of IL-1 β in 50 μ l of PBS was injected i.v. Animals were sacrificed 2 h later, and splenic T cells were isolated. After culturing with anti-TCR or isotype control antibodies for 2 min, T cell lysates were prepared, run on gels, and probed with anti-phosphotyrosine antibodies to detect total PTK activity. Extracts were also treated with anti-Fyn monoclonal antibody, and the immunoprecipitated Fyn was assayed for the kinase activity. Prior to anti-TCR treatment, the basal PTK activity in T cells from control rats (aCSF-treated) was low; the activity increased significantly after the anti-TCR treatment (Fig. 10A). On the other hand, the PTK activity in T cells from ICV IL-1 β -treated rats was high even before the anti-TCR treatment, and this activity did not increase significantly after the anti-TCR treatment. Similarly, the ICV IL-1 β administration also increased the Fyn activity (Fig. 10B). These results suggest that within 2 h a single ICV but not i.v. exposure of IL-1 β activates intracellular signaling in splenic T cells.

Discussion

We have previously shown that chronic ICV exposure to very small amounts of NT (human equivalent of < 0.5 cigarettes/day) caused immunosuppression that was blocked by the non-selective nAChR antagonist mecamylamine (Singh et al., 2000). This suggested that central nicotinic acetylcholine receptors are involved in the NT-induced immunosuppression. Moreover, NT treatment also blocked the pyrogenic response of turpentine (an inducer of brain IL-1 β levels), suggesting a possible link between IL-1 β (the major pyrogen) and

immunosuppression. Similar effects of NT on immunosuppression and pyrogenic activity were also observed when NT was chronically administered s.c. at higher doses (human equivalent of about 1 pack/day) (Sopori et al., 1998a; Sopori et al., 1998b). Therefore, we expected that NT treatment would inhibit formation of the pyrogenic cytokine IL-1 β in the brain. Surprisingly, however, the data presented herein clearly indicate that acute NT exposure actually increased IL-1 β expression in the brain, and it is only after chronic NT exposure that IL-1 β expression decreased to control levels. Although not as strongly, NT moderately increased the expression of TNF- α and IL-6 (not shown). Moreover, approximately 2 wk into NT exposure, when challenged with an inflammatory stimulus such as turpentine or cryptococcal extracts (unpublished observation), the animals failed to elicit a proinflammatory cytokine response in the brain. Thus, NT induces a biphasic response in the brain: acutely it induces the expression of proinflammatory cytokines, particularly IL-1 β and, chronically, the anti-inflammatory phase replacing the initial proinflammatory phase, where continued presence of nicotine is unable to sustain the initial rise in IL-1 β levels.

Proinflammatory cytokines such as TNF- α , IL-1 β , and IL-6 are produced during acute inflammatory responses (Dinarello, 2000); however, IL-1 β has also been implicated in neuroprotection (Stoll et al., 2002; Pinteaux et al., 2009). Increasing evidence shows that the early presence of these cytokines promotes protection against inflammation and sepsis (del Rey and Besedovsky, 2000; Kox et al., 2000; Jin et al., 2009). In fact, when given prophylactically, IL-1 β was protective in a mouse model of acute lung injury (Torre et al., 1994). Similarly, pretreatment with sublethal doses of endotoxin increases proinflammatory cytokine production, but it also protects against septic peritonitis through a mechanism independent of the adaptive immunity (Urbascsek and Urbascsek, 1987; Varma et al., 2005); similar results are achieved by pretreatment with IL-1 β and TNF- α (Urbascsek and Urbascsek, 1987). Thus, in general, early presence of proinflammatory cytokines ushers in an anti-inflammatory phase, and it is possible that the early induction of IL-1 β is critical for the subsequent immunosuppression and the anti-inflammatory response seen in chronically NT-treated animals.

Although, NT induces the expression of other proinflammatory cytokines (e.g., TNF- α , IL-6) in the brain, the predominant response is that of IL-1 β , which is also implicated in the protection from neuroinflammation (Jin et al., 2009). To ascertain whether IL-1 β is critical for the NT-induced immunosuppression, we used IL-1R KO mice. These mice tend to be immunologically hyporesponsive such as antibody response, clearance of pathogens (Lebeis et al., 2009), and pyrogenic effects of turpentine (Kozak et al., 1998); however, unlike the WT mice, chronic exposure of IL-1R KO mice to NT did not cause the progressive decline in the IL-1 β expression in the brain. Moreover, IL-1R KO mice were refractory to the immunosuppressive effects of chronic NT treatment, such as antibody response to SRBC and anti-TCR/CD28-induced T cell proliferation. Although the KO animals also expressed TNF- α , it did not replace the function of IL-1 β in inducing the anti-inflammatory phase of chronic NT treatment. Thus, the interaction between IL-1 β and its receptor is critical for induction of NT-induced immunosuppression.

To prove that brain IL-1 β modulates the immune/inflammatory responses, we examined the effects of ICV administration of IL-1 β on various parameters of inflammation and TCR

signaling. Initially, IL-1 β increased fever and the serum concentration of CORT; however, continued presence of IL-1 β prompted a progressive decrease in Tb and serum CORT levels, and at around 10 days after IL-1 β treatment, Tb and CORT were comparable to control animals. The decrease did not reflect the loss of IL-1 β biological activity in the implanted pumps; as the IL-1 β collected on day 14 from the implanted pumps induced a fever response in naïve animals. In addition to downregulation of the acute phase response (i.e., activation of the HPA axis and fever response), chronic IL-1 β exposure also suppressed the T cell function including the mitogen and TCR-induced proliferative responses, and production of antibodies to SRBC. Thus, chronic exposure to IL-1 β attenuates the innate and adaptive immune responses.

Under our conditions, unlike ICV administration, chronic s.c. administration of low concentrations of IL-1 β did not significantly affect the immune responses. This was surprising because both IL-1 β and TNF α are protective in the mouse sepsis model (Urbaschek and Urbaschek, 1987). It is possible that, similar to NT (Sopori et al., 1998b), the concentration of IL-1 β required to elicit a biological response through peripheral routes is much higher than the concentration needed to produce a similar response via the ICV route, and it is likely that in our experiments the concentration of IL-1 β required to affect immune responses was not attained through s.c. administration.

Chronic NT failed to suppress T cell responses in IL-1R KO mice. Given that these KO mice lack a functional IL-1 receptor, the loss of IL-1 function in these animals is understandable; however, IL-1R KO mice have a functional TNF- α receptor and exhibit a moderate increase in TNF in response to NT (not shown). Thus, TNF- α is not as efficient as IL-1 β in inducing the immunosuppressive phase in these mice. There are two potential but not necessarily mutually exclusive explanations for the failure of IL-1R KO mice to respond to elevated levels of TNF- α after NT treatment: (a) the immune/inflammatory responses in IL-1R KO are relatively weak and the concentration of TNF- α elicited by NT in KO mice was not sufficient to affect the immune response. Moreover, IL-6 and TNF- α are considered to be IL-1-responsive genes and are significantly reduced in IL-1R KO glial cells (Parker et al., 2002); (b) the efficacy of a cytokine to induce the protective response depends on the mouse strain. Thus, IL-1 is far superior in providing radioprotection in C57BL/6 than C3H mice, and TNF is better than IL-1 in C3H than C57BL/6 (Neta et al., 1988). Because IL-1R KO mice are on a C57BL/6 background, it is likely that IL-1 β is a superior inducer than TNF- α in these mice. Although we directly tested only IL-1 β in this model, NT also induced IL-1 α and, because both cytokines activate the same receptor, it is possible that either of them could be effective in dampening the immune response.

A bidirectional communication exists between the brain and the immune system (Blalock, 1994), and chronic presence of IL-1 β in the brain may modulate this communication. However, it is clear that even the acute presence of IL-1 β affected the interaction between the brain and the immune system and ICV, but not i.v., administration of relatively small concentrations of IL-1 β -induced Fyn and PTK activities in splenic T cells within 2 h of the administration. Thus, changes in the brain milieu are immediately transmitted to the immune system.

The above results, we believe, provide the first direct demonstration that a neuroactive substance such as NT invokes a proinflammatory response in the brain to control the immune system. A number of human conditions are associated with upregulated expression of IL-1 in the brain that eventually leads to immunosuppression. For example, head trauma stimulates an inflammatory response followed by a compensatory anti-inflammatory response (Lenz et al., 2007; Lu et al., 2009). Similarly, the proinflammatory phase of neuroinflammation is essential for limiting subsequent inflammation (Kox et al., 2000; Varma et al., 2005), and we suggest that change in the cytokine milieu in the brain is an important mechanism for limiting the immune/inflammatory responses. Furthermore, we postulate that the brain senses inflammation through proinflammatory cytokines, in particular IL-1, and, because inflammation can have severe adverse consequences in the CNS, the brain has developed a mechanism(s) to limit the immune and inflammatory responses. The precise mechanism by which the chronic presence of IL-1 limits immune/inflammatory responses is not clear. It is possible that the chronic presence of IL-1 downregulates the expression of IL-1 receptors and/or the production of the soluble IL-1R antagonist; however, these possibilities remain to be explored.

Acknowledgments

The authors would like to thank Mr. Steve Randock and Ms. Paula Bradley for their help with graphics and editing, respectively.

Abbreviations

$[Ca^{2+}]_i$	intercellular calcium concentration
NT	nicotine
PTK	protein tyrosine kinase
HPA	hypothalamus-pituitary-adrenal
LEW	Lewis
KO	knockout
WT	wild-type
ICV	intracerebroventricular
aCSF	artificial cerebrospinal fluid
AFC	antibody-forming cell
qPCR	real-time PCR
CORT	corticosterone

References

- Badjatia N. Hyperthermia and fever control in brain injury. *Critical care medicine*. 2009; 37:S250–257. [PubMed: 19535955]

- Blalock JE. The syntax of immune-neuroendocrine communication. *Immunol Today*. 1994; 15:504–511. [PubMed: 7802919]
- Cooper JA, Esch FS, Taylor SS, Hunter T. Phosphorylation sites in enolase and lactate dehydrogenase utilized by tyrosine protein kinases in vivo and in vitro. *The Journal of biological chemistry*. 1984; 259:7835–7841. [PubMed: 6330085]
- del Rey A, Besedovsky HO. The cytokine-HPA axis circuit contributes to prevent or moderate autoimmune processes. *Z Rheumatol*. 2000; 59(Suppl 2):II/31–35.
- Dinarello CA. Proinflammatory cytokines. *Chest*. 2000; 118:503–508. [PubMed: 10936147]
- Geng Y, Savage SM, Razani-Boroujerdi S, Sopori ML. Effects of nicotine on the immune response. II. Chronic nicotine treatment induces T cell anergy. *J Immunol*. 1996; 156:2384–2390. [PubMed: 8786295]
- Geng Y, Savage SM, Johnson LJ, Seagrave J, Sopori ML. Effects of nicotine on the immune response. I. Chronic exposure to nicotine impairs antigen receptor-mediated signal transduction in lymphocytes. *Toxicology and applied pharmacology*. 1995; 135:268–278. [PubMed: 8545837]
- Gould KL, Hunter T. Platelet-derived growth factor induces multisite phosphorylation of pp60c-src and increases its protein-tyrosine kinase activity. *Mol Cell Biol*. 1988; 8:3345–3356. [PubMed: 2463476]
- Holt PG, Keast D. Environmentally induced changes in immunological function: acute and chronic effects of inhalation of tobacco smoke and other atmospheric contaminants in man and experimental animals. *Bacteriol Rev*. 1977; 41:205–216. [PubMed: 405003]
- Horai R, Asano M, Sudo K, Kanuka H, Suzuki M, Nishihara M, Takahashi M, Iwakura Y. Production of mice deficient in genes for interleukin (IL)-1alpha, IL-1beta, IL-1alpha/beta, and IL-1 receptor antagonist shows that IL-1beta is crucial in turpentine-induced fever development and glucocorticoid secretion. *The Journal of experimental medicine*. 1998; 187:1463–1475. [PubMed: 9565638]
- Jin W, Wang H, Yan W, Zhu L, Hu Z, Ding Y, Tang K. Role of Nrf2 in protection against traumatic brain injury in mice. *J Neurotrauma*. 2009; 26:131–139. [PubMed: 19125683]
- Kalra R, Singh SP, Savage SM, Finch GL, Sopori ML. Effects of cigarette smoke on immune response: chronic exposure to cigarette smoke impairs antigen-mediated signaling in T cells and depletes IP3-sensitive Ca(2+) stores. *The Journal of pharmacology and experimental therapeutics*. 2000; 293:166–171. [PubMed: 10734166]
- Kalra R, Singh SP, Pena-Philippides JC, Langley RJ, Razani-Boroujerdi S, Sopori ML. Immunosuppressive and anti-inflammatory effects of nicotine administered by patch in an animal model. *Clin Diagn Lab Immunol*. 2004; 11:563–568. [PubMed: 15138183]
- Kox WJ, Volk T, Kox SN, Volk HD. Immunomodulatory therapies in sepsis. *Intensive care medicine*. 2000; 26(Suppl 1):S124–128. [PubMed: 10786969]
- Kozak W, Kluger MJ, Soszynski D, Conn CA, Rudolph K, Leon LR, Zheng H. IL-6 and IL-1 beta in fever. Studies using cytokine-deficient (knockout) mice. *Annals of the New York Academy of Sciences*. 1998; 856:33–47. [PubMed: 9917862]
- Lebeis SL, Powell KR, Merlin D, Sherman MA, Kalman D. Interleukin-1 receptor signaling protects mice from lethal intestinal damage caused by the attaching and effacing pathogen *Citrobacter rodentium*. *Infection and immunity*. 2009; 77:604–614. [PubMed: 19075023]
- Lenz A, Franklin GA, Cheadle WG. Systemic inflammation after trauma. *Injury*. 2007; 38:1336–1345. [PubMed: 18048040]
- Lu J, Goh SJ, Tng PY, Deng YY, Ling EA, Moochhala S. Systemic inflammatory response following acute traumatic brain injury. *Front Biosci*. 2009; 14:3795–3813.
- Mishra NC, Rir-Sima-Ah J, Langley RJ, Singh SP, Pena-Philippides JC, Koga T, Razani-Boroujerdi S, Hutt J, Campen M, Kim KC, Tesfaigzi Y, Sopori ML. Nicotine primarily suppresses lung Th2 but not goblet cell and muscle cell responses to allergens. *J Immunol*. 2008; 180:7655–7663. [PubMed: 18490768]
- Neta R, Oppenheim JJ, Douches SD. Interdependence of the radioprotective effects of human recombinant interleukin 1 alpha, tumor necrosis factor alpha, granulocyte colony-stimulating factor, and murine recombinant granulocyte-macrophage colony-stimulating factor. *J Immunol*. 1988; 140:108–111. [PubMed: 2447166]

- Parker LC, Luheshi GN, Rothwell NJ, Pinteaux E. IL-1 beta signalling in glial cells in wildtype and IL-1RI deficient mice. *British journal of pharmacology*. 2002; 136:312–320. [PubMed: 12010781]
- Pinteaux E, Trotter P, Simi A. Cell-specific and concentration-dependent actions of interleukin-1 in acute brain inflammation. *Cytokine*. 2009; 45:1–7. [PubMed: 19026559]
- Razani-Boroujerdi S, Sopor ML. Early manifestations of NNK-induced lung cancer: role of lung immunity in tumor susceptibility. *American journal of respiratory cell and molecular biology*. 2007; 36:13–19. [PubMed: 16873770]
- Razani-Boroujerdi S, Savage SM, Sopor ML. Alcohol-induced changes in the immune response: immunological effects of chronic ethanol intake are genetically regulated. *Toxicology and applied pharmacology*. 1994a; 127:37–43. [PubMed: 8048051]
- Razani-Boroujerdi S, Partridge LD, Sopor ML. Intracellular calcium signaling induced by thapsigargin in excitable and inexcitable cells. *Cell Calcium*. 1994b; 16:467–474. [PubMed: 7712540]
- Razani-Boroujerdi S, Singh SP, Knall C, Hahn FF, Pena-Philippides JC, Kalra R, Langley RJ, Sopor ML. Chronic nicotine inhibits inflammation and promotes influenza infection. *Cellular immunology*. 2004; 230:1–9. [PubMed: 15541713]
- Razani-Boroujerdi S, Boyd RT, Davila-Garcia MI, Nandi JS, Mishra NC, Singh SP, Pena-Philippides JC, Langley R, Sopor ML. T cells express alpha7-nicotinic acetylcholine receptor subunits that require a functional TCR and leukocyte-specific protein tyrosine kinase for nicotine-induced Ca²⁺ response. *J Immunol*. 2007; 179:2889–2898. [PubMed: 17709503]
- Salmond RJ, Filby A, Qureshi I, Caserta S, Zamoyska R. T-cell receptor proximal signaling via the Src-family kinases, Lck and Fyn, influences T-cell activation, differentiation, and tolerance. *Immunol Rev*. 2009; 228:9–22. [PubMed: 19290918]
- Scott DA, Martin M. Exploitation of the nicotinic anti-inflammatory pathway for the treatment of epithelial inflammatory diseases. *World J Gastroenterol*. 2006; 12:7451–7459. [PubMed: 17167832]
- Singh SP, Kalra R, Puttfarcken P, Kozak A, Tesfaigzi J, Sopor ML. Acute and chronic nicotine exposures modulate the immune system through different pathways. *Toxicology and applied pharmacology*. 2000; 164:65–72. [PubMed: 10739745]
- Skurlova M, Stofkova A, Jurcovicova J. Exogenous IL-1beta induces its own expression, but not that of IL-6 in the hypothalamus and activates HPA axis and prolactin release. *Endocr Regul*. 2006; 40:125–128. [PubMed: 17201586]
- Sopor M. Effects of cigarette smoke on the immune system. *Nat Rev Immunol*. 2002; 2:372–377. [PubMed: 12033743]
- Sopor ML, Cherian S, Chilukuri R, Shopp GM. Cigarette smoke causes inhibition of the immune response to intratracheally administered antigens. *Toxicology and applied pharmacology*. 1989; 97:489–499. [PubMed: 2609346]
- Sopor ML, Kozak W, Savage SM, Geng Y, Kluger MJ. Nicotine-induced modulation of T Cell function. Implications for inflammation and infection. *Advances in experimental medicine and biology*. 1998a; 437:279–289. [PubMed: 9666281]
- Sopor ML, Kozak W, Savage SM, Geng Y, Soszynski D, Kluger MJ, Perryman EK, Snow GE. Effect of nicotine on the immune system: possible regulation of immune responses by central and peripheral mechanisms. *Psychoneuroendocrinology*. 1998b; 23:189–204. [PubMed: 9621398]
- Stampfli MR, Anderson GP. How cigarette smoke skews immune responses to promote infection, lung disease and cancer. *Nat Rev Immunol*. 2009; 9:377–384. [PubMed: 19330016]
- Stoll G, Jander S, Schroeter M. Detrimental and beneficial effects of injury-induced inflammation and cytokine expression in the nervous system. *Advances in experimental medicine and biology*. 2002; 513:87–113. [PubMed: 12575818]
- Torre D, Minoja G, Maraggia D, Chiaranda M, Tambini R, Speranza F, Giola M. Effect of recombinant IL-1 beta and recombinant gamma interferon on septic acute lung injury in mice. *Chest*. 1994; 105:1241–1245. [PubMed: 8162754]
- Ulloa L, Wang P. The neuronal strategy for inflammation. *Novartis Foundation symposium*. 2007; 280:223–233. discussion 233–227. [PubMed: 17380797]

- Urbaschek R, Urbaschek B. Tumor necrosis factor and interleukin 1 as mediators of endotoxin-induced beneficial effects. *Rev Infect Dis.* 1987; 9(Suppl 5):S607–615. [PubMed: 3317753]
- van Westerloo DJ, Giebelen IA, Florquin S, Daalhuisen J, Bruno MJ, de Vos AF, Tracey KJ, van der Poll T. The cholinergic anti-inflammatory pathway regulates the host response during septic peritonitis. *J Infect Dis.* 2005; 191:2138–2148. [PubMed: 15898001]
- Varma TK, Durham M, Murphey ED, Cui W, Huang Z, Lin CY, Toliver-Kinsky T, Sherwood ER. Endotoxin priming improves clearance of *Pseudomonas aeruginosa* in wild-type and interleukin-10 knockout mice. *Infection and immunity.* 2005; 73:7340–7347. [PubMed: 16239532]
- Wang RD, Tai H, Xie C, Wang X, Wright JL, Churg A. Cigarette smoke produces airway wall remodeling in rat tracheal explants. *American journal of respiratory and critical care medicine.* 2003; 168:1232–1236. [PubMed: 12958058]

FIGURE 1.

NT treatment induces IL-1 β expression in the brain. A: C57BL/6 mice were injected with single i.p. injection of 7.5 μ g NT and, at various times following NT administration, the brain mRNA was analyzed by qPCR for IL-1 β expression. B: Brain IL-1 β qPCR analysis of LEW Rats injected with single i.p. injection of 62.5 μ g NT at various time points after administration. C: C57BL/6 mice were chronically exposed to NT (2 mg/kg/day) through Alzet miniosmotic pumps, and brain tissues were analyzed by qPCR for IL-1 β expression at 2 days, 4 days and 8 days post NT treatment. D: qPCR analysis of IL-1 β expression in the brain of LEW Rats chronically exposed to NT (2 mg/kg/day) 1-3 wk post NT treatment. Bar graphs represent mean $\pm$ SEM from 5 rats/group. * $P < 0.05$.

FIGURE 2.

Chronic NT exposure blocks turpentine-induced expression of IL-1 β in brain. Rats were exposed to saline (CON) or NT for 3 wk and then injected i.m. with turpentine (Turp). At 24 h after turpentine injection, brain mRNA was analyzed for IL-1 β expression by qPCR. Results are mean $\pm$ SEM from five rats/group * $P < 0.05$.

FIGURE 3.

Chronically NT-treated IL-1R KO mice have higher brain expression of IL-1 β mRNA than WT C57BL/6 mice. Mice (IL-1R KO and WT) were treated with NT (1 mg/kg/day) via Alzet miniosmotic pumps, and brain tissues were analyzed for IL-1 β expression by qPCR after 3 wk of NT treatment. Bar graphs represents mean $\pm$ SEM from five mice/group, * P 0.05.

FIGURE 4.

Chronic NT treatment does not affect the immune responses of IL-1R-KO mice. WT and IL-1R-KO mice (n=6/group) were implanted with NT (2 mg/kg/day)- or PBS-containing Alzet miniosmotic pumps for 3 wk. Four days prior to sacrifice, animals were immunized with SRBC, and the spleen cells were analyzed: (A): AFC/10⁶ spleen cells; (B): Con A-induced proliferation as described in Materials and Methods. Bar graphs represents mean $\pm$ SEM. * P = 0.05.

FIGURE 5.

Chronic NT treatment does not affect the TCR-mediated $[Ca^{2+}]_i$ responses of IL-1R-KO spleen cells. WT and IL-1R-KO mice ($n=6$) were treated with (NT) or PBS (CON) as described in Fig. 4, and the anti-CD3/CD28-induced $[Ca^{2+}]_i$ response of splenocytes was measured by fluorometry. A representative set of $[Ca^{2+}]_i$ responses is shown in the figure after subtracting the basal (unstimulated) values for $[Ca^{2+}]_i$

FIGURE 6.

Chronic ICV IL-1 β treatment, but not s.c. IL-1 β inhibits the anti-SRBC AFC response. Rats (n=6/group) were given IL-1 β (100 ng/day/kg), aCSF (CON) through ICV-implanted cannulas, or IL-1 β (s.c.; 100 ng/day/kg) via s.c. miniosmotic pumps for 2 wk, and immunized with SRBC 4 days prior to sacrifice. The anti-SRBC AFC response of spleen cells was determined as described in Fig. 4. The graph represents mean $\pm$ SEM of AFC/10⁶ spleen cells. * P < 0.05.

FIGURE 7.

Chronic ICV exposure to IL-1 β causes immunosuppression. Rats (n=6/group) were treated with IL-1 β or aCSF(CON) for 2 wk as described in Fig. 6. Spleen cells were evaluated for Con A (A) and anti-TCR (B)-induced proliferative responses. (C) A representative profile of the spleen cell anti-TCR-induced $[\text{Ca}^{2+}]_i$ response is shown as described in Fig. 5. Bar graphs are mean $\pm$ SEM. * $P < 0.05$.

FIGURE 8.

Changes in serum CORT levels after ICV IL-1 β administration.

Rats were treated ICV with IL-1 β (50 ng/day/kg) or aCSF (CON) for 4 h or 12 days. The 10:00 AM serum CORT levels were determined as described in Materials and Methods. Bar graphs represent mean $\pm$ SEM of 4 animals/group. * $P < 0.05$.

FIGURE 9.

Changes in Tb after ICV IL-1 β or aCSF (CON) exposure. Rats (n=12/group) were implanted with biotelemeters to monitor Tb as described in Materials and Methods. Seven days post-implantation, animals were given IL-1 β (100 ng/d/kg) or aCSF (CON) via ICV cannulas in the morning. Average changes in Tb are shown for CON and IL-1 β -treated animals 1 day before IL-1 β /aCSF administration and 1, 7, and 10 days post IL-1 β /aCSF ICV exposure. The horizontal bar over the X-axis indicates the dark (active) phase of the light/dark cycle.

FIGURE 10.

Acute ICV administration of IL-1 β activates Fyn and PTK activities in splenic T cells. Rats (3/group) were implanted with ICV cannulas 1 wk prior to single injection of 5 μ l of aCSF (CON) or IL-1 β (50 ng/kg). Spleen cells were isolated and the extracts were resolved by Western blotting and visualized by anti-phosphotyrosine antibodies for PTK activity (A). The extracts were also immunoprecipitated with an anti-Fyn antibody, and the immunoprecipitates were used to determine Fyn kinase activity as described in Materials and Methods. Representative blots are shown in the figures.