Hyperthermia-enhanced splenic cytokine gene expression is mediated by the sympathetic nervous system

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Ganta, Chanran K., Frank Blecha, Roman R. Ganta, Bryan G. Helwig, Sujatha Parimi, Ning Lu, Richard J. Fels, Timothy I. Musch, and Michael J. Kenney. Hyperthermia-enhanced splenic cytokine gene expression is mediated by the sympathetic nervous system. Physiol Genomics 19: 175–183, 2004. First published August 3, 2004; doi:10.1152/physiolgenomics.00109.2004.—Whole body hyperthermia (WBH) has been used in experimental settings as an adjunct to radiochemotherapy for the treatment of various malignant diseases. The therapeutic effect of WBH has been hypothesized to involve activation of the immune system, although the effect of hyperthermia-induced activation of sympathetic nerve discharge (SND) on splenic immune function is not known. We tested the hypothesis that heating-induced sympathetic sympathectoexcitation would alter splenic cytokine gene expression as determined using gene array and real-time RT-PCR analyses. Experiments were performed in splenic-intact and splenic-denervated anesthetized Sprague-Dawley rats (n = 32). Splenic SND was increased during heating (internal temperature increased from 38° to 41°C) in splenic-intact rats but remained unchanged in nonheated splenic-intact rats. Splenic interleukin-1β (IL-1β), interleukin-6 (IL-6), and growth-regulated oncogene 1 (GRO 1) mRNA expression was higher in heated than in nonheated splenic-intact rats. Splenic IL-1β, IL-6, and GRO 1 mRNA expression was reduced in heated splenic-denervated compared with heated splenic-intact rats, but did not differ between heated sympathetic-nervated and nonheated splenic-intact rats. These results support the hypothesis that hyperthermia-induced activation of splenic SND enhances splenic cytokine gene expression.

whole body hyperthermia; sympathetic nerve discharge; sympatheoctoexcitation; splenic denervation

WBH has been used in experimental settings as an adjunct to radiochemotherapy for the treatment of various malignant diseases (20, 58). The therapeutic effect of WBH in these conditions has been hypothesized to involve activation of the immune system (5, 51, 52). Robins et al. (52) reported elevated plasma levels of granulocyte-colony stimulating factor, interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8, interleukin-10, and tumor necrosis factor-α (TNF-α) when WBH was used in conjunction with chemotherapy in human patients. Atanackovic et al. (5) reported that human patients receiving WBH along with chemotherapy demonstrated immediate and significant increases in peripheral natural killer cells, CD56+ cytotoxic T lymphocytes, and serum concentrations of IL-6 and TNF-α, changes that were not observed in patients receiving chemotherapy alone. These results support the hypothesis that WBH provides a stimulus to the immune system.

The sympathetic nervous system, along with cytokines and the hypothalamic-pituitary-adrenal axis, is thought to play an important role in mediating bi-directional neural-immune interactions (1, 2, 14, 46). A role for the sympathetic nervous system in immune regulation is supported by the innervation of lymphoid organs (including the spleen) by sympathetic nerves (15–19) and the presence of adrenergic receptors on immune cells (8, 34, 39, 42, 48). A role for the immune system in sympathetic nerve regulation is supported by the increased rate of norepinephrine release and turnover in spleen and bone marrow following activation of antigen-specific T cells and B cells by a soluble protein antigen (35) and changes in the level of SND produced by administration of IL-1β antibody (43) and IL-1β (25, 56). As reviewed by Madden (45), complex functional interactions exist between the sympathetic nervous system and the immune system, and it has been shown that the sympathetic nervous system can both enhance and inhibit immune responses, depending on experimental conditions, the type of stress paradigm used, activation state of the sympathetic nervous system, and types of immune cells activated. In addition, immune cell products can influence the functional state of the sympathetic nervous system. For example, Roar gausch et al. (53, 54, 55) demonstrated that locally produced IL-1β inhibits the release of norepinephrine from postganglionic splenic sympathetic nerves, which in turn leads to an increase in splenic blood flow (BF).

Although sympathetic nerves are considered an important component of the communication pathway between the brain and the immune system, the influence of hyperthermia-induced activation of splenic sympathetic nerve outflow on splenic immune function remains poorly defined. In the present study we determined the effect of WBH on splenic SND and splenic cytokine gene expression in urethane-chloralose-anesthetized...
rubs. Because the sympathetic innervation of the spleen provides a direct link between central sympathetic neural circuits and immunocompetent cells in the spleen (15, 19), we tested the hypothesis that hyperthermia-induced activation of splenic SN would alter splenic cytokine gene expression as determined using gene array and real-time RT-PCR analyses. To demonstrate a role for the sympathetic nervous system in mediating splenic cytokine gene expression responses to WBH, experiments were completed in splenic-intact and splenic-denervated rats. The current study provides new findings supporting the idea that hyperthermia-induced activation of splenic SN enhances splenic cytokine gene expression.

METHODS

General procedures. The Institutional Animal Care and Use Committee approved the experimental procedures and protocols used in the present study, and all procedures were performed in accordance with the American Physiological Society’s guiding principles for research involving animals (3). Experiments were performed on Sprague-Dawley rats (381 ± 6 g, n = 32). Anesthesia was induced by isoflurane (3%) and maintained during surgical procedures using isoflurane (1.5%), α-chloralose (80 mg/kg ip), and urethane (800 mg/kg ip) (31, 32, 43). During the experimental protocol, maintenance doses of α-chloralose were infused intravenously (femoral vein; 35-45 mg·kg⁻¹·h⁻¹). The trachea was cannulated with a polyethylene-240 catheter. Femoral arterial pressure was monitored using a pressure transducer connected to a blood pressure analyzer. Heart rate (HR) was derived from the pulsatile arterial pressure output of the blood pressure analyzer. Colonic temperature (Tc) was measured with a thermistor probe inserted ~5 cm into the colon. Tc was maintained between 37.8°C and 38.0°C during surgical procedures by a homeothermic blanket.

Neural recordings. Splenic sympathetic nerve activity was recorded biphaseially with a platinum bipolar electrode after preamplification (bandpass 30–3,000 Hz). In splenic-denervated rats, renal sympathetic nerve activity was recorded using similar recording and preamplification procedures. Using a lateral approach, we dissected splenic and renal sympathetic nerves free of surrounding connective tissue. Nerve-electrode preparations were covered with silicone gel. The filtered neurograms were routed to an oscilloscope and a nerve traffic analyzer for monitoring during the experiment and for subsequent data analysis. Sympathetic nerve potentials were full-wave rectified, integrated (time constant 10 ms), and quantified as volts · seconds (Vs) (29–31, 33). The level of activity in sympathetic nerves was corrected for background noise after administration of the ganglionic blocker trimethaphan camsylate (10-15 mg/kg iv). At the same time, arterial blood pressure was recorded from the carotid artery catheter. After 30 s of blood withdrawal, the carotid artery catheter was disconnected from the pressure transducer, and radioactive microspheres were injected into the aortic arch. Labeled microspheres were 15 ± 3 μm in diameter. The microspheres were suspended in normal saline containing 0.01% Tween 80 with a specific activity ranging from 7–15 μCi/μl. Before each injection, the microspheres, 6–7 × 10⁶, were thoroughly mixed and agitated by sonication to prevent clumping. Microspheres were injected into the ascending aorta in a volume of ~0.10 ml, and the different radioactive labels (46Sc, 85Sr, 113Sn, and 141Ce) were used in random order. At the end of each experiment, the rat was killed with an overdose of methohexital sodium (150 mg/kg iv). The placement of each catheter was verified by anatomical dissection.

Spleens and kidneys were removed, blotted, weighed, and placed immediately into counting vials. The radioactivity of tissue samples was determined on a Packard Cobra II Auto-Gamma spectrometer set to record the peak energy activity of each isotope for 5 min, then analyzed by computer, taking into account the cross-talk fraction between the different isotopes. Tissue blood flow was calculated by the reference sample method (26) and expressed as milliliters per minute per 100 g of tissue. Adequate mixing of the microspheres was verified for each injection by demonstrating a <15% difference in blood flows to the right and left kidneys.

Experimental protocol. After completion of the surgical procedures, anesthetized rats were allowed to stabilize for 60 min. After the stabilization period, a 30-min control period was completed during which Tc was maintained at 38°C in all rats. At the end of the control period, heating experiments were initiated in splenic-intact and splenic-denervated rats by increasing Tc at a rate of 0.1°C/min from 38 to 41°C (30 min), followed by a maintenance phase in which Tc was maintained at 41°C for an additional 90 min. Increases in Tc were produced using a heat lamp (29–33). Nonheated experiments were completed in splenic-intact and spleen-denervated rats by maintaining Tc at 38°C for an additional 120 min beyond the initial 30 min control period. Mean arterial pressure (MAP), HR, and SND were measured continuously during the control periods and the heating and nonheating protocols. Splenic BF measurements were completed at the end of the control period and at 30 and 120 min of the heating and nonheating protocols in nonheated splenic-intact (n = 3), heated splenic-intact (n = 5), and heated splenic-denervated (n = 4) rats.

Spleens were collected at the end of each experiment (with the exception of those used in experiments analyzing splenic BF) for splenic cytokine gene expression analysis and stored at −80°C. Gene expression was determined on spleens collected from four rats in each experimental group (nonheated splenic-intact, heated splenic-intact, and heated splenic-denervated). To validate the gene array results, TaqMan probe-based real-time RT-PCR analysis was performed on spleens used for gene array analysis (n = 4 for each group) and spleens from additional experiments in each group (nonheated splenic-intact, n = 2; heated splenic-intact, n = 3; heated splenic-denervated, n = 3).

RNA isolation. Frozen spleens were homogenized in liquid nitrogen and the total RNA was isolated using the TRI-Reagent RNA isolation kit according to the manufacturer’s protocol (Sigma Chemical, St. Louis, MO). RNA purity and concentration were determined spectrophotometrically by calculating the ratio between the absorbances at 260 nm and 280 nm. The absorbance ratio for all samples ranged between 1.8 and 2.0. The quality of RNA for all samples was confirmed by resolving them on a 1.5% formaldehyde agarose gel (47).

Gene array analysis. Splenic cytokine gene expression was evaluated using a mouse inflammatory cytokine cDNA array system from SuperArray Biosciences (Bethesda, MD) as reported earlier (44). The cDNA array blot contained 23 inflammatory cytokine and chemokine gene fragments spotted in duplicate wells. In addition, β-actin and...
GAPDH were included as positive controls, and pUC18 DNA was included as a negative control. Biotin-labeled cDNA probes were synthesized from total RNA by reverse transcription using an RT-Labeling Kit (SuperArray Biosciences). The labeled probes were hybridized to gene-specific cDNA fragments spotted on the gene array membranes. Membranes were washed to remove any unincorporated probe and incubated with alkaline phosphatase conjugated streptavidin (AP-streptavidin). Relative expression levels of specific genes were detected from signals generated by chemiluminescence from the alkaline phosphatase substrate, CDP-Star. The luminizing blots were used to expose X-ray films and quantified by spot densitometry with the aid of AlphaEase v5.5 software (Alpha Innotech, San Leandro, CA). The relative gene expression levels were estimated by comparing the signal intensity of the target gene to the signal intensity derived from β-actin.

**Real-time RT-PCR analysis.** To validate the gene-array results, TaqMan probe-based real-time RT-PCR analysis was performed. Total RNA (2 µg) was reverse-transcribed in a 20-µl volume containing 1 µM of oligo(dT) primers, 0.5 mM of each dNTP, 0.5 U/µl of RNase inhibitor, and 0.2 U/µl of Omniscript reverse transcriptase (Qiagen, Valencia, CA) in RNase-free water. The reaction was carried out for 60 min at 37.0°C, and the cDNA mixture was used for the real-time PCR analysis of specific cytokine gene expression.

Gene-specific PCR primer pairs and a TaqMan probe for growth-regulated oncogene 1 (GRO 1) were obtained from Applied Biosystems (Foster City, CA). The primers and probes for β-actin, IL-6, and IL-1β genes were custom synthesized using published sequences (7, 38). TaqMan probes were labeled with 6-carboxyfluorescein (FAM) as the reporter dye molecule at the 5’ end and 6-carboxytetramethylrhodamine (TAMRA) as the quencher dye molecule at the 3’ end. Real-time PCR reactions were performed with 2 µl of cDNA using Universal PCR Master Mix (Applied Biosystems, Foster City, CA), containing 0.9 µM each of the forward and reverse primers and 0.25 µM TaqMan probes in a 25-µl reaction. Real-time PCR analysis was performed in a Smart Cycler (Cepheid, Sunnyvale, CA) with the following PCR conditions: one cycle each of 50°C for 2 min and 95°C for 5 min, followed by 45 cycles of 95°C for 15 s and 60°C for 1 min.

The threshold cycle (Ct) value for each gene was defined as the PCR cycle at which the emitted fluorescence rose above a background level of fluorescence, i.e., 30 fluorescence units. Gene expression levels were calculated as fold change relative to the gene expression of nonheated splenic-intact rats. The PCR amplification efficiencies of β-actin and the target genes were calculated using the following formula: PCR efficiency = \((10^{\text{1/Slope}}) - 1\) where S is the slope (21). The amplification efficiency was greater than 90% for all genes. The comparative Ct method \((2^{-\Delta \Delta Ct}}\) was used to quantify the results obtained by real-time RT-PCR (41). Data were normalized by determining differences in Ct values between the target gene of interest and β-actin, defined as ΔCt (Ct of target gene — Ct of β-actin gene). The fold change was calculated as \(2^{\text{S AvgΔCt} - \text{CAvgΔCt}}\) where S AvgΔCt = CAvgΔCt is the difference between the sample (heated-intact/heat-denervated/nonheated-denervated) ΔCt and the control (nonheated intact) ΔCt. For nonheated intact samples, ΔCt equaled zero and 2^0 equaled one, so that the fold change in gene expression relative to the nonheated intact samples equaled one. For the treated samples, evaluation of \(2^{-\Delta \Delta Ct}}\) was defined as the fold change in gene expression relative to nonheated intact samples.

**RESULTS**

**SND, MAP, and HR responses to WBH.** Figure 1 shows SND traces from three representative experiments. Tc was maintained at 38°C during the control period in each rat. In the

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Fig. 1. A: traces of splenic sympathetic nerve discharge (SND) recorded during control and at 30 min and 120 min after cessation of control in a nonheated rat with intact splenic nerves whose colonic temperature was maintained at 38°C. B: traces of splenic SND recorded before (control) and during (30 min and 120 min) whole body heating that increased colonic temperature (Tc) from 38°C to 41°C in a rat with intact splenic nerves. C: traces of splenic and renal SND recorded before (control) and during (30 min and 120 min) whole body heating that increased Tc from 38°C to 41°C in a splenic-denervated rat. Horizontal calibration is 500 ms.
nonheated splenic-intact rat (Fig. 1A), Tc was held constant at 38°C for 120 min after control, and splenic SND was unchanged from control at 30 and 120 min. In the heated splenic-intact rat (Fig. 1B), Tc was increased from 38 to 41°C during the first 30 min after control and was maintained at 41°C for an additional 90 min (120 min total heating time). Splenic SND was increased from control during heating at 30 and 120 min in the splenic-intact rat (Fig. 1B). In the heated splenic-denervated rat (Fig. 1C), no measurable splenic SND was detected during control or heating (30 and 120 min), although renal SND was increased during heating. Renal SND was recorded in the splenic-denervated rat to demonstrate specificity in the denervation procedure.

Figure 2 summarizes splenic SND, MAP, and HR responses in nonheated (n = 4) and heated (n = 4) splenic-intact rats and heated splenic-denervated (n = 4) rats. In nonheated splenic-intact rats, Tc was held constant at 38°C for 150 min (30 min of control, −30 to 0 min, and 120 min after control), and splenic SND, MAP, and HR remained unchanged from control in these rats. In heated splenic-intact and splenic-denervated rats, Tc was increased from 38 to 41°C during the first 30 min (0 to 30 min) after the control period (−30 to 0 min) and was maintained at 41°C for an additional 90 min (30 to 120 min). During heating, splenic SND was increased significantly from control in splenic-intact rats and was undetectable in splenic-denervated rats. MAP was increased significantly from control during the first 60 min of heating in splenic-intact rats and during the first 30 min of heating in splenic-denervated rats. HR was significantly increased from control during heating in splenic-intact and splenic-denervated rats.

Splenic BF responses to WBH. Figure 3 summarizes splenic BF responses in nonheated splenic-intact (n = 3), heated splenic-intact (n = 5), and heated splenic-denervated (n = 4) rats. Tc was maintained at 38°C during a 30 min preheating control period (control) in all rats. In nonheated splenic-intact rats, Tc was held constant at 38°C for 120 min after the control period, and splenic BF was unchanged from control values at 30 and 120 min following cessation of the control period. In heated splenic-intact and splenic-denervated rats, Tc was increased from 38–41°C during the first 30 min after control and was maintained at 41°C for an additional 90 min. Splenic BF in splenic-intact and splenic-denervated rats was significantly increased from preheating control values at 30 min of heating but decreased to control values in both groups of rats at 120 min of heating. Splenic BF responses during heating did not differ between splenic-intact and splenic-denervated rats.

Gene array analysis of splenic gene expression responses to WBH. Figure 4 shows splenic gene array results from three representative experiments. β-Actin and GAPDH served as internal control genes. Expression of IL-1β, IL-6, and GRO1 genes was increased in the heated splenic-intact rat (marked by broken lines) compared with the nonheated splenic-intact rat and heated splenic-denervated rat. Expression of transforming growth factor-β1 (TGF-β1), IL-2, and IL-16 was observed in each experiment but did not consistently differ between groups.

Quantitative values relative to β-actin for splenic IL-1β, IL-6, GRO1, TGF-β1, IL-2, and IL-16 mRNA expression in nonheated splenic-intact (n = 4), heated splenic-intact (n = 4), and heated splenic-denervated (n = 4) rats are summarized in Fig. 5. Expression of IL-1β, IL-6, and GRO1 genes was significantly higher in heated splenic-intact compared with nonheated splenic-intact and heated splenic-denervated rats. Expression of TGF-β1, IL-2, and IL-16 genes did not differ between groups.
Real-time RT-PCR analysis of splenic gene expression responses to WBH. Real-time RT-PCR analysis was performed for four genes (β-actin, IL-1β, IL-6, and GRO 1) from three experimental groups of rats: nonheated splenic-intact, heated splenic-intact, and nonheated splenic-denervated rats. Representative amplification plots are shown in Fig. 6. β-Actin mRNA expression did not differ between groups. As indicated by the lower Ct values (a reflection of increased mRNA levels), expression of IL-1β, IL-6, and GRO 1 mRNA was higher in the heated splenic-intact rats compared with nonheated splenic-intact and heated splenic-denervated rats.

Ct values in heated splenic-intact rats for IL-1β, IL-6, and GRO 1 genes were significantly lower than those in nonheated splenic-intact and heated splenic-denervated rats (Table 1). Ct values did not differ between nonheated splenic-intact and heated splenic-denervated rats (Table 1). When the Ct values were translated to fold change, mRNA expression of IL-1β (2.3- to 4.0-fold), IL-6 (4.7- to 16.2-fold), and GRO 1 (3.9- to 9.1-fold) was higher in heated splenic-intact rats (n = 7) than in nonheated splenic-intact rats (n = 6). The fold change for mRNA expression of IL-1β (2.3- to 4.0-fold), IL-6 (2.7- to 9.4-fold), and GRO 1 (3.2- to 9.5-fold) was lower in heated splenic-denervated rats (n = 7) compared with heated splenic-intact rats.
intact rats and did not differ between heated splenic-denervated and nonheated splenic-intact rats.

The role of the sympathetic nervous system in regulation of splenic gene expression under basal conditions was determined by comparing gene expression responses in nonheated splenic-intact (n = 6) and nonheated splenic-denervated rats (n = 3). Ct values for IL-1β (nonheated splenic-intact, 24.13 ± 0.45; nonheated splenic-denervated, 27.2 ± 0.3) and GRO 1 (nonheated splenic-intact, 29.51 ± 0.53; nonheated splenic-denervated, 31.0 ± 0.6) were significantly higher in nonheated-denervated compared with nonheated-intact rats. Ct values for β-actin (nonheated splenic-denervated, 19.29 ± 0.33; nonheated splenic-intact, 19.6 ± 0.13) and IL-6 (nonheated splenic-denervated, 30.20 ± 0.44; nonheated splenic-intact, 31.1 ± 0.4) did not differ between groups. When the Ct values were translated to fold change, mRNA expression of IL-1β (5.2- to 8-fold) and GRO 1 (1.4- to 2.8-fold) was lower in nonheated splenic-denervated compared with nonheated splenic-intact rats.

DISCUSSION

We present three new findings concerning the effect of WBH on sympathetic-immune interactions in anesthetized rats. First, splenic SND was increased during heating in splenic-intact rats but remained unchanged in nonheated splenic-intact rats. Second, splenic IL-1β, IL-6, and GRO 1 mRNA expression was higher in heated than in nonheated splenic-intact rats. Third, splenic IL-1β, IL-6, and GRO 1 mRNA expression was significantly less in heated splenic-denervated rats than in heated splenic-intact rats, but did not differ between heated splenic-denervated and nonheated splenic-intact rats. The observed differences in splenic cytokine gene expression to WBH between splenic-intact and splenic-denervated rats were not
dependent on differences in splenic BF during heating in these groups of rats. These results support the hypothesis that hyperthermia-induced activation of splenic SND enhances splenic cytokine gene expression.

Changing the level of efferent sympathetic nerve activity is a primary strategy used by mammals to respond to acute physical stress. WBH is an acute stressor that substantially changes the level of activity in efferent sympathetic nerves (10–13, 22, 27, 29–31, 33, 36, 50). Hyperthermia-induced changes in sympathetic outflow alter blood flow distribution profiles to enhance heat dissipation while maintaining arterial blood pressure and vital organ perfusion pressure. In addition to the thermoregulatory and cardiovascular consequences of heating-induced sympathoexcitation, the current results support a role for the sympathetic nervous system in immune regulation as demonstrated by the fact that the enhanced expression of splenic cytokine and chemokine genes in response to WBH was abrogated by splenic denervation.

Because the sympathetic nervous system plays an important role in mediating cardiovascular responses to heating (30, 32, 37), differences in splenic gene expression to heating in splenic-intact and splenic-denervated rats may have resulted from altered splenic BF responses to heating after splenic denervation. However, this was not the case in the present study, as splenic BF responses to heating did not differ between splenic-intact and splenic-denervated rats. As demonstrated in a series of studies by Rogausch et al. (53, 54, 55), splenic BF regulation in the rat involves complex mechanisms. In this regard, the current study provides little insight concerning the role of the sympathetic nervous system in modulating splenic BF responses to WBH; however, the primary focus of the blood flow studies was not to discern mechanisms regulating splenic BF responses to heating. Rather, we wanted to assess whether heating-induced changes in splenic gene expression could be ascribed to substantial changes in blood flow to this organ following splenic sympathetic denervation.

WBH has been used in experimental settings as an adjunct to cytotoxic therapy in the treatment of various malignant diseases, and it is hypothesized that the beneficial effect of WBH in these conditions may relate to heating-induced expression of cytokines (23, 28, 52). The current finding that hyperthermia-induced increases in splenic IL-1β, IL-6, and GRO 1 mRNA expression were abrogated in splenic-denervated rats suggests that splenic sympathoexcitation to acute heating may contribute to the induction of cytokines observed during chemotherapy plus WBH protocols. Consistent with this idea, ThyagaRajan et al. (60) reported reduced splenic norepinephrine concentrations, splenic IL-2 and interferon-γ levels, and hypothalamic dopaminergic activity in saline-treated, mammary tumor-bearing rats. In contrast, administration of L-deprenyl (a monoamine oxidase inhibitor) to rats with mammary tumors was associated with tumor regression, enhanced splenic production of IL-2, interferon-γ, and natural killer cell activity, increased splenic norepinephrine concentrations, and increased hypothalamic dopaminergic activity. These findings demonstrate interactions between the sympathetic nervous system and splenic immune function and suggest that increased central and peripheral catecholaminergic activity produced by deprenyl administration may enhance anti-tumor immunity (60).

The physiological relevance of the enhanced expression of splenic IL-1β, IL-6, and GRO 1 genes to WBH is presently unknown, although numerous functional effects of these molecules have been established. For example, IL-1β is a proinflammatory cytokine that induces lymphocyte proliferation, fever, and production of other cytokines (6, 62). IL-6 is a multifunctional cytokine that is considered a factor in hematopoietic colony stimulation (63), cytolysis T lymphocyte differentiation (49, 59), B cell differentiation (24), and T cell activation (24). In addition, IL-6 is involved in the acute phase reaction (9, 40) and hematopoiesis (61) and has been shown to inhibit the growth and metastasis of autologous human cancers (59) and inhibit the growth of carcinoma cell lines (57). GRO 1 is a growth-related oncogene and melanoma growth stimulatory factor, and expression of the GRO family of genes is important in IL-1-induced inflammatory responses in fibroblasts (4).

The current results are applicable to splenic tissue only, and application to other lymphoid or secondary lymphoid organs remains to be established. The current study used genomic level of analyses and may not represent the influence of WBH on splenic protein expression. The present results provide insight concerning the role of splenic sympathetic nerves in splenic immune regulation in response to a specific experimental intervention (WBH) using an in vivo preparation and cannot necessarily be applied to other experimental interventions or preparations. However, within the constraints of the current experimental protocols and analyses, the present results strongly support a role for splenic sympathetic nerves in increasing the expression of selective splenic cytokine genes in response to WBH.

GRANTS

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