# Marked Antiinflammatory Effects of Decentralization of the Superior Cervical Ganglia

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## Summary

Intravenous challenge with parasite antigens in *Nippostrongylus brasiliensis*–sensitized rats resulted in anaphylactic shock and, in some animals, death. Surviving animals showed significant drop in mean arterial blood pressure, cardiac output, and blood flow to the trachea, bronchioles, and mesentery. After anaphylaxis, changes in the cellular and protein composition in bronchoalveolar lavage fluids (BALF) were assessed. 8 h after antigen challenge, there was significant influx of inflammatory cells and an increase in the levels of histamine and serum-derived immunoglobulins (IgG and IgM) in BALF. Chemotactic activity for neutrophils was also present in BALF.

Once we established this anaphylaxis-induced model of pulmonary inflammation, we sought to determine whether or not the superior cervical ganglia (SCG) modulate this inflammation. We performed bilateral superior cervical ganglionectomy or decentralization of the SCG. Our results show that decentralization significantly reduced mortality (by 68%) after anaphylaxis. Furthermore, the increases in levels of serum-derived proteins, histamine, and influx of cells (especially neutrophils) observed in BALF after anaphylaxis were attenuated by both decentralization and ganglionectomy. By contrast, hemodynamic parameters in the respiratory tract and the presence of neutrophil chemotactic activity in BALF were not influenced by decentralization. Thus, the severity of pulmonary inflammation initiated by systemic anaphylaxis is depressed by bilateral ganglionectomy or decentralization of SCG.

Infection with the nematode *Nippostrongylus brasiliensis* induces marked pulmonary inflammation, mastocytosis, and development of reaginic antibodies in rats (1–6). When infected rats are challenged intravenously with antigens prepared from adult worms (6), there is severe systemic anaphylactic shock and death of many animals (7–9). However, changes in the cellular and protein composition of bronchoalveolar lavage fluids (BALF)[1] after anaphylaxis have not been studied. Therefore, to understand the involvement of airways in anaphylaxis in this model, we quantitated (*a*) bronchoalveolar cellular response; (*b*) total proteins, Igs, and histamine in BALF and serum; (*c*) histopathological changes in lungs; and (*d*) hemodynamic parameters such as mean arterial blood pressure, regional blood flow, cardiac output, and heart rate in parasite-sensitized rats after antigen challenge.

Pulmonary injury during anaphylaxis is thought to involve one or more immunologic pathways including release of leukotrienes (10, 11), immune complex–mediated injury (12), lymphocyte activation, IgE-mediated histamine release, and activation of mast cells (13–16). Earlier studies show that some of these changes may be closely related to similar changes in the draining lymph nodes in the cervical region (17, 18).

Noradrenergic fibers from the sympathetic trunk innervate these cervical lymph nodes, thymus, and salivary glands (19, 20), and these nerves can modulate lymphocyte function within the cervical lymph nodes and thymus (21–25). Moreover, the submandibular glands contain immunosuppressive and antiinflammatory agents (26, 27), and recently, nerve growth factor (NGF), which is produced in abundance by these glands, has been shown to be a potent antiinflammatory factor (28). Therefore, the inflammatory responses in the respiratory tract after anaphylaxis could be modulated by sympathetic innervation to cervical lymphoid structures and/or submandibular glands. Thus, we have assessed the role of cervical sympathetic trunk in pulmonary inflammation after anaphylaxis by surgically denervating the superior cervical ganglia (SCG) by bilateral decentralization or bilateral ganglionectomy. Anaphylaxis was induced by intravenous challenge with worm antigens in *N. brasiliensis*–sensitized rats 8 d after surgical manipulation.

Therefore, our objective was to study inflammatory re-

[1] *Abbreviations used in this paper:* BALF, bronchoalveolar lavage fluids; CO, cardiac output; H, heart rate; MABP, mean arterial blood pressure; NGF, nerve growth factor; 6-OHDA, 6-hydroxydopamine; PFC, plaque-forming cell; SCG, superior cervical ganglia; TPR, total peripheral resistance; WE, worm equivalent.

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sponses in vivo in an organ not directly innervated by the SCG but influenced by tissues innervated by the postganglionic nerve fibers of the SCG. Moreover, the SCG do not directly influence the pulmonary vasculature, a critical target structure we wished to avoid in our modulation of the nervous system because of its quintessential role in inflammation. However, SCG innervate crucial lymphoid and endocrine structures in the cervical region thought to be important in inflammation and immune function.

After surgical manipulation and the induction of anaphylaxis, cellular and protein changes in the lung were assessed using bronchoalveolar lavage. Our initial results showed reduced neutrophil migration into BALF after bilateral ganglionectomy or decentralization of SCG. Therefore, we measured the neutrophil chemotactic activity in BALF. Because mast cell activation and histamine secretion are central to allergic reactions, we have also quantitated mast cell numbers and cell-associated and free histamine levels in BALF and peritoneal fluids.

## Methods

*Animals and Infection.* Outbred male Sprague-Dawley rats (Charles River Inc., St. Constant, Canada) weighing 300–400 g were used. Rats were sensitized to the nematode *N. brasiliensis* by subcutaneous infection with 3,000 third-stage larvae (4).

*Surgical Procedure.* Parasite-sensitized rats were subjected to bilateral decentralization or ganglionectomy of the SCG, 30–42 d after infection. Briefly, rats were anesthetized with halothane; their cervical region was shaved and sterilized; and a longitudinal middle incision of ~2.5 cm was made. After reflecting the sternohyoideus muscles, the vagus nerve was located on either side and, using a dissection microscope, the cervical sympathetic trunk was carefully isolated. For bilateral ganglionectomy, the SCG were identified (29) on both sides and carefully removed. For decentralization of SCG, ~3 mm of the sympathetic trunk was removed bilaterally between the middle and superior cervical ganglia (hereafter designated as decentralized animals). The wound was sutured and the rats were allowed to recover for 1 wk. Success of the operation was determined by the development of ptosis and miosis in animals ganglionectomized or decentralized. A third group of sensitized rats was surgically manipulated in a similar manner, but their sympathetic trunks were left intact, and thus served as sham-operated animals. A fourth group of infected rats received no surgical manipulation and represented the unoperated animals. A fifth group of uninfected, unoperated animals were normal controls.

Studies on the bronchoalveolar response showed that bilateral ganglionectomy of SCG gave similar results as those with bilateral decentralization of SCG. Hence, we used the decentralization model for the majority of experiments because of the ease in the operative procedures and the anticipation that decentralization would provide an easier lesion to investigate the underlying defect than ganglionectomy.

*Anaphylaxis.* Anaphylactic shock was induced in rats 8 d after surgery, by intravenous challenge into the easily accessible penile vein, with 125–150 worm equivalents (WE) (6) of adult *N. brasiliensis* homogenate in 0.1 ml saline (hereafter designated as challenged animals). Rats were anesthetized in a $CO_2$ chamber before challenge. Sensitized animals which did not receive the antigen challenge were given an equal volume of PBS intravenously (hereafter designated as unchallenged animals).

*Collection and Analysis of BALF and Cells.* Cells and proteins from the bronchoalveolar spaces of rats were recovered 8 h after antigen challenge using bronchoalveolar lavage (5). Briefly, animals were given an overdose of $CO_2$, their trachea exposed, and using a polypropylene catheter, their lungs were washed 10 times with warm (≈35°C) PBS ($Ca^{++}$, $Mg^{++}$ free). Total fluid recovery was 95 ± 4% of that instilled and all BALF values are expressed as the total recovery per animal. Cells from BALF were separated by centrifugation at 200 *g* for 20 min and a total cell count was made. Differential cell counts were made on cytocentrifuge smears stained with May Grunwald-Giemsa (BDH, Toronto, Canada).

Levels of albumin, IgG, IgA, and IgM in BALF and serum were measured by a specific and sensitive (2–9 ng/ml) double-antibody ELISA (5). Ratios of Ig to albumin in BALF and serum were calculated to assess the source of proteins in the BALF. The principles underlying these ratios have been more extensively discussed elsewhere (5, 30). Briefly, under normal conditions, the amount of serum-derived albumin in BALF is greater than that of serum-derived Igs because of its lower molecular mass. However, the concentration of albumin in BALF is significantly lower than that in serum. Therefore, given that there is secretion of locally produced Igs in the respiratory tract, the ratio of Igs to albumin in BALF is significantly different from that of serum. However, when there is local vascular and epithelial damage, both albumin and Igs readily leak from the vasculature into bronchoalveolar space. Such leakage of serum-derived proteins into the bronchoalveolar space causes the ratio of Igs to albumin in BALF to approach that of the serum range.

*Collection and Analysis of Peritoneal Lavage Fluids and Cells.* Cells and fluids from the peritoneum were recovered by instilling 15 ml of cold (≈4°C) PBS ($Ca^{++}$, $Mg^{++}$ free) into the peritoneum using a 21G × 1½" needle. After gentle massage of the abdomen, the fluid was retrieved and the cells sedimented by centrifugation at 200 *g* for 5 min. Total cell numbers and differential counts were made as above.

*Histamine Analysis.* Levels of histamine in unconcentrated fluids and cell pellets from BALF and peritoneal lavage were measured using a radioenzymatic assay (1). Recovery of a known amount (10 ng) of exogenously added histamine to several samples was assessed simultaneously to determine whether the samples contained substances that were degradative for histamine, or other factors that influenced the measurement of histamine.

*Blood Flow Studies.* Vascular hemodynamics and blood flow to various tissues and organs were studied (31) at 5, 60, and 240 min after intravenous challenge in separate groups of rats. Rats were anesthetized before surgery with intraperitoneal pentabarbital sodium (30 mg/kg body weight) and maintained with supplemental injections as required. To assess hemodynamic values and collect reference blood samples, the right femoral artery was cannulated with polyethylene tubing (PE50; Clay Adams, Parsippany, NJ). The left ventricle was then cannulated through the right carotid artery with PE50 tubing. Verification of the ventricular cannula placement was obtained by monitoring the blood pressure until a characteristic left ventricular pressure tracing appeared (i.e., ~120 mm Hg systole and 0 mm Hg diastole). Ventricular cannula placement was also verified postmortem. Heart rate (H), mean arterial blood pressure (MABP), and mean left ventricular pressure were recorded using a Statham P23Db (Grass Instruments, Quincy, MA) pressure transducer connected to a Beckman Dynograph (Summit Technology, Toronto, Canada).

Regional blood flow and cardiac output (CO) were determined with $^{95}$Nb-labeled microspheres (15 ± 3 μm, specific activity 10 mci/g; New England Nuclear, Boston, MA). The microspheres were suspended in 0.9% NaCl containing 0.05% Tween-80 by vig-

orous shaking for 15 s and then passed repeatedly through a 27-gauge needle connected to a 1-ml syringe immediately before administration. This procedure assured that the microspheres were not aggregated. The microspheres (~80,000–100,000) were injected into the left ventricle in a total volume of 0.5 ml followed by 0.8 ml of saline flush. Starting 15 s before the microsphere injection, the reference blood sample was drawn into a motor-driven syringe at a rate of 0.68 ml/min for 75 s.

Upon completion of the experiment, the animals were killed with an overdose of urethane. Tissues and organs were removed, packed into tared tubes, and their wet weight was recorded; and radioactivity counted in a gamma counter (LKB Instruments, 1282 Compugamma, Turku, Finland). Although blood flow to numerous tissues was examined, only flows to the trachea, conducting airways (bronchioles), and mesentery are reported in this paper. These tissues exhibit typical changes in blood flow 5, 60, and 240 min after antigen administration to sensitized animals. The trachea was removed between the larynx and bronchial bifurcation; pieces of whole lung were sampled for the blood flow to the bronchi and bronchioles. The mesentery was removed from the intestine and separated from the pancreas. Blood flow, CO, and total peripheral resistance (TPR) were calculated with the program described by Flaim et al. (32). Blood flow was expressed as ml/min per g wet tissue. CO was calculated as follows: CO (ml/min) = radioactivity injected (cpm)/reference sample radioactivity (cpm) × 0.68 (ml/min). Total peripheral resistance was calculated according to the formula (33) where: TPR (dyn·s·cm-5) = MABP (mm Hg) × 80/CO (ml/min). Several requirements, as defined by Hadengue et al. (33), were met before using the data from an individual animal: (*a*) a difference of <10% between left and right kidneys—indicative of adequate microsphere mixing, and (*b*) stability in MABP and H between the pre- and postmicrosphere injections. All data are presented as the mean ± SEM.

*Chemotactic Assay.* Neutrophil chemotactic activity of BALF was measured by a microchemotaxis assay (34). Briefly, peripheral blood neutrophils from five uninfected rats were separated by differential centrifugation in Polyprep (Cederlane Labs, Ontario, Canada; reference 35). The separated cells were washed, counted, and resuspended in complete RPMI. Viability of the cells was determined by trypan blue dye exclusion. The cells were subsequently labeled with $^{111}$Indium (Amersham, Ontario, Canada; reference 36) for 30 min at RT, washed once, and resuspended in RPMI at a concentration of 5 × $10^6$ cells/ml. For the chemotaxis assay, 45 μl of BALF were loaded into the lower compartment of a 48-well microchemotaxis chamber (Neuroprobe Inc., Bethesda, MD). *N*-FMLP (Sigma Chemical Co., St. Louis, MO), a peptide chemotactic for neutrophils, was used as a positive control at $10^{-5}$ M (a predetermined optimal concentration) in RPMI. 35 μl of radiolabeled cell suspension was placed in the upper compartment. The lower and upper compartments were separated by two polycarbonate membrane filters (3-μm pore diameter; Nuclepore Corp., Pleasanton, California). After incubation of the chamber at 37°C for 90 min, the filters were removed and fixed in methanol for 5 min. After fixation, the regions corresponding to each well were cut out from the bottom filters and counted in a gamma counter (LKB Instruments). Neutrophil chemotaxis was expressed as follows: Chemotactic index = $10^6$ × (number of counts in the lower membrane/total number of counts loaded into upper compartment).

*Histopathological Studies.* Lungs from infected rats subjected to either sham operation or decentralization and subsequently challenged or unchallenged were fixed in situ by intratracheal inflation with Zamboni's fixative (37) at 25 cm of $H_2O$ for 15 min. Portions of lung and trachea were processed for light microscopy and stained with hematoxylin and eosin. Serial sections were also stained with avidin–biotin complex (38) and alcian blue–safranin (39) for mast cells. Our earlier observations in intestine show that both these techniques stain connective tissue and mucosal mast cell subpopulations.

*Statistical Analysis.* Results of the experiments are expressed as mean (± SEM) values. Statistical differences were assessed among the groups using one-way analysis of variance. The Student-Neuman-Keul procedure (40) was used to determine significance at $p \leq 0.05$ level.

## Results

*Mortality.* After intravenous challenge with antigen, both unoperated and sham-operated animals exhibited marked respiratory distress resulting in the death of 8 of 19 (42.1%) unoperated animals and 12 of 27 (44.4%) sham-operated animals within 4 h of challenge. After challenge in decentralized rats, fewer animals exhibited symptoms of anaphylactic shock and mortality was markedly reduced (14.3%, 3 of 28).

*Total Protein and Immunoglobulin Levels in BALF.* Com-

**Table 1.** *Effect of Decentralization of SCG on the Amount of Proteins in BALF of N. brasiliensis-sensitized Rats**

| | Unoperated | | Sham operated | | Decentralized | |
|---|---|---|---|---|---|---|
| Proteins in BALF | Antigen | Saline | Antigen | Saline | Antigen | Saline |
| | $n$ = 11 | $n$ = 7 | $n$ = 15 | $n$ = 7 | $n$ = 25 | $n$ = 7 |
| Total protein (mg) | 6.9 ± 2.1* | 1.8 ± 0.4 | 6.6 ± 1.2* | 1.8 ± 0.6 | 2.4 ± 1.1‡ | 1.9 ± 0.7 |
| IgG (μg) | 652.9 ± 75.7* | 146.4 ± 18.5 | 564.3 ± 29.6* | 129.8 ± 32.6 | 187.8 ± 67.4‡ | 139.5 ± 24.4 |
| IgA (μg) | 60.1 ± 13.1* | 34.3 ± 11.4 | 84.5 ± 18.7* | 28.7 ± 4.7 | 22.5 ± 9.0‡ | 36.6 ± 6.4 |
| IgM (μg) | 63.5 ± 29.1* | 18.6 ± 9.0 | 99.1 ± 3.9* | 23.5 ± 1.8 | 29.2 ± 2.4‡ | 15.8 ± 8.5 |
| Albumin (μg) | 1,053.1 ± 412.6* | 770.5 ± 212.6 | 1,045.2 ± 326.6* | 618.1 ± 218.4 | 574.3 ± 196.5‡ | 697.5 ± 218.6 |

Values represent mean ± SE per animal. $p \leq 0.05$ compared with either decentralized group or respective saline controls.
* 8 h after intravenous challenge with 125 WE antigen or saline.
‡ Not significant compared with saline controls.

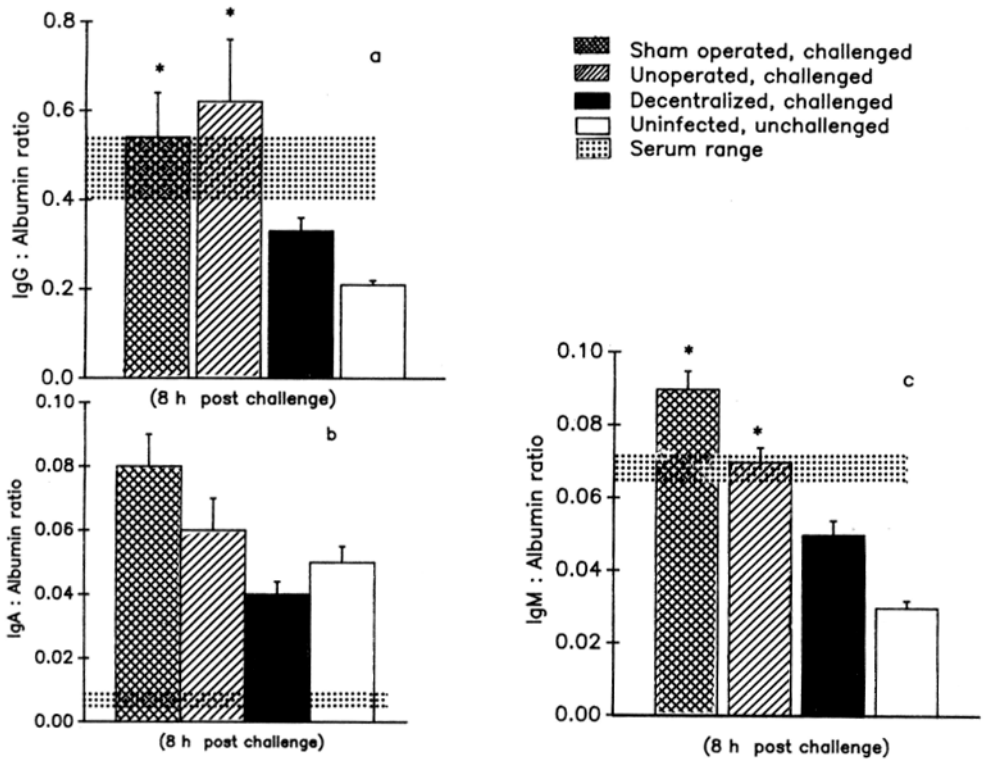


**Figure 1.** Effects of decentralization of SCG on (*a*) IgG/albumin, (*b*) IgA/albumin, and (*c*) IgM/albumin ratios in BALF of *N. brasiliensis*-sensitized rats 8 h after intravenous antigen challenge. The values represent means for 7–25 animals in the indicated groups. Mean serum ranges for the groups are represented in the dotted areas (*Serum range*). $p \leq 0.05$ (*) when compared with the uninfected, unchallenged controls (□).

pared to unchallenged rats, 8 h after antigen challenge, there was a significant increase in the total protein concentration in the BALF of sham and of unoperated animals. However, after challenge in decentralized animals this increase in total protein was not evident (Table 1).

Similarly, concentrations of total albumin, IgG, IgA, and IgM in BALF of sham and unoperated groups were significantly increased 8 h after antigen challenge compared to normal controls. Decentralization of SCG, however, prevented the increase in the levels of these proteins in BALF after antigen challenge (Table 1). Serum levels of albumin, IgG, IgA, or IgM showed no significant differences among the groups (data not shown).

*Ig/Albumin Ratios.* To assess the source of increased proteins in BALF, Ig/albumin ratios were compared between serum and BALF (Fig. 1). The results show that IgG/albumin and IgM/albumin ratios in BALF of sham and unoperated animals were within serum ranges 8 h after antigen challenge. However, these ratios were below the serum range in decentralized challenged animals. Interestingly, IgA, which is nor-

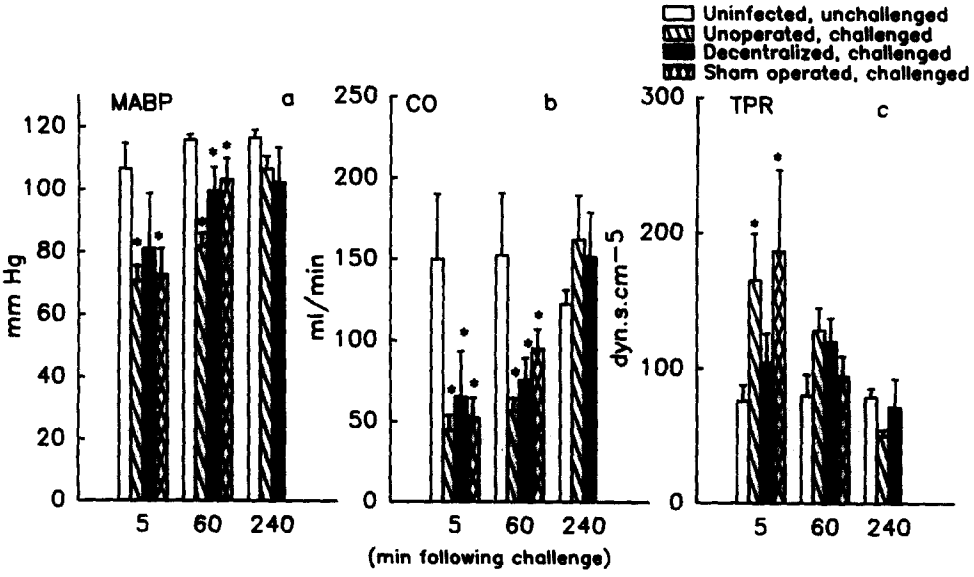


**Figure 2.** Changes in MABP, CO and TPR in four groups of rats at different time periods after intravenous challenge with 150 WE. The values represent means ± SEM for 5–8 animals. $p \leq 0.05$ (*) when compared with the uninfected antigen-challenged group. There was no sham-operated group for 240 min.

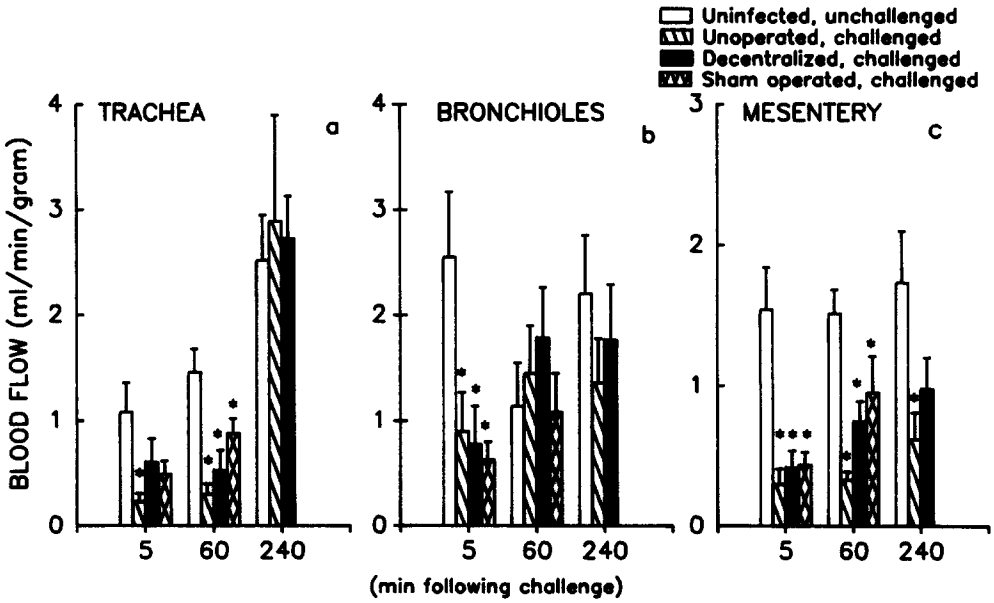


**Figure 3.** Effects of intravenous antigen challenge on blood flow to the trachea, bronchioles, and mesentery in the same groups of animals described in Fig. 2. The values represent means ± SEM for 5–8 animals. $p \leq 0.05$ (*) when compared with the uninfected, antigen-challenged group.

mally present in higher concentrations in the lower respiratory tract than in serum, showed no significant difference among groups.

*Microcirculatory Changes.* Analysis of the microcirculatory events after antigen challenge showed that in *N. brasiliensis*–sensitized rats, there was a pronounced fall in MABP within 5 min which persisted for up to 60 min. By 240 min, however, MABP had returned to prechallenged values (Fig. 2 *a*). Cardiac output showed a similar decrease after antigen challenge (Fig. 2 *b*). In rats subjected to bilateral decentralization of SCG the pattern of changes in MABP and CO were similar to that in unoperated and sham-operated animals. By contrast, in unoperated and sham-operated rats but not in decentralized rats, TPR was significantly increased at 5 min after challenge (Fig. 2 *c*). By 60 min, TPR was similar to prechallenged values in all groups.

Blood flow studies showed that compared to normal control animals, there was a pronounced decrease in blood flow to the trachea, bronchi, and the mesentery of animals in all sensitized groups within 5 min after antigen challenge (Fig. 3). This marked reduction in blood flow persisted for up to 60 min in the trachea and up to 240 min in the mesentery. However, blood flow to the bronchioles had returned to prechallenge levels by 60 min. By 240 min, blood flow to the trachea was markedly elevated in all groups. Thus, although antigen challenge significantly altered hemodynamic parameters, there were no significant differences between sympathetic decentralized and sham-operated groups.

*Histamine Levels in BALF and Peritoneal Fluids.* Compared with normal controls, levels of histamine in BALF of sham-operated and unoperated animals were increased significantly 8 h after intravenous antigen challenge (Fig. 4 *a*). However, in decentralized animals, similar antigen challenge did not produce a significant increase in histamine levels. Since we recovered over 93% of the externally added histamine from BALF of all sensitized animals, it appeared that there were no histamine-catabolizing substances or substances that influenced the assay in the samples.

Similarly, the histamine levels in the peritoneal fluids were also decreased after decentralization (Fig. 4 *a*). In contrast, the peritoneal cell pellets contained an increased histamine level in decentralized animals compared with sham-operated animals (Fig. 4 *b*). A differential count on the peritoneal cell suspension showed that after systemic anaphylaxis, in *N. brasiliensis*–infected rats, there were $3.1 \times 10^6 \pm 0.2$ mast cells per animal. However, sham operation or decentralization of SCG did not alter the mast cell numbers significantly ($2.9 \pm 0.7$ and $2.3 \pm 0.8 \times 10^6$, respectively). Given that the major histamine-containing cells in peritoneum are mast cells, the histamine levels were expressed as the histamine content per mast cell (Fig. 4 *b*). Whereas, decentralized animals had $3.3 \pm 0.2$ pg of histamine per peritoneal mast cell, sham-operated and unoperated animals had lower histamine content per cell ($2.1 \pm 0.3$ and $1.7 \pm 0.2$ pg, respectively; $p \leq 0.5$). However, the bronchoalveolar cell pellets (which had < 0.05% mast cells in cytospin smears) contained only very low concentrations of histamine ($0.05 \pm 0.02$ μg/animal) and showed no significant differences among the groups whether challenged or unchallenged (data not shown).

*Cells in BALF.* Fig. 5 illustrates the total and differential cell populations in the bronchoalveolar space of rats sensitized with *N. brasiliensis*. In unchallenged animals, alveolar macrophages represented 97% of the total number of cells (Fig. 6 *A*). Other cells included neutrophils (0.3%), eosinophils (0.5%), and lymphocytes (2%). After intravenous challenge with antigen there was a significant increase in the total bronchoalveolar cells in sham-operated and unoperated animals. In particular, there was a 20-fold increase in neutrophils (Fig. 5 and 6 *B*) compared with normal controls. By contrast, bilateral decentralization of SCG significantly re-

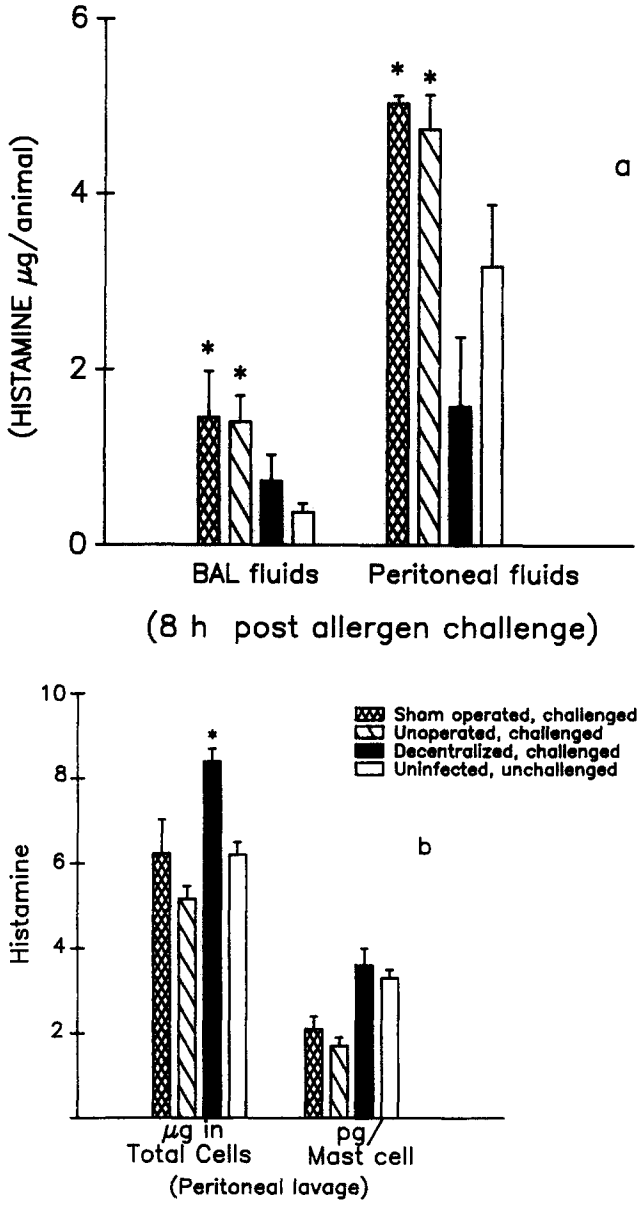


**Figure 4.** Effect of decentralization of SCG on histamine levels in bronchoalveolar and peritoneal lavage fluids (*a*) and peritoneal cell pellets (*b*) of sensitized animals. The values represent means ± SEM of 3–6 animals. $p \leq 0.05$ (*) when compared with normal controls.

duced this increase in the number of bronchoalveolar cells, 8 h after antigen challenge. Differential analysis of the cells showed significantly fewer alveolar macrophages and neutrophils after decentralization. Ganglionectomy also resulted in a similar decrease in total cells as well as neutrophils (data not shown). Interestingly, there was a significant increase in lymphocytes in decentralized animals (Figs. 5 and 6 *C*). Although eosinophil number was significantly increased after anaphylaxis in *N. brasiliensis*–sensitized rats, there was no significant difference in the eosinophil numbers between sham-operated and decentralized groups.

*Chemotactic Activity of Neutrophils towards BALF.* BALF from sensitized animals contained components chemotactic for normal blood neutrophils (data not shown). However, neither antigen challenge nor decentralization of SCG altered the presence or levels of this chemotactic activity in bronchoalveolar spaces.

*Histopathological Changes in Lungs.* Sections of lungs from sham-operated animals taken 8 h after antigen challenge showed mild perivascular interstitial edema with prominent margination and emigration of PMN from the pulmonary arterioles (Fig. 7 *a*). The periarteriolar connective tissues contained large numbers of neutrophils and few eosinophils. Neutrophils were also seen within the bronchiolar epithelium and within the air spaces adjacent to the arterioles. Some of the alveoli contained fibrinous exudate indicative of early alveolar edema (Fig. 7 *b*). By contrast, lungs of animals decentralized and challenged with antigen were for the most part indistinguishable from unchallenged animals. Margination and emigration of only a few polymorphs were evident in some of the small arterioles of decentralized, challenged animals.

Mast cell stains showed increased number of mast cells in the interstitium and mucosa of lungs and tracheas of unchallenged sensitized animals. 8 h after antigen challenge, there was a marked reduction in stainable mast cells in sham-operated, challenged animals. The mast cells that were discernible were partially degranulated (Fig. 8 *A*). This effect was much less pronounced in decentralized, challenged animals. Moreover, only a few mast cells in these animals had extracytoplasmic granules nearby (Fig. 8 *B*).

## Discussion

We have demonstrated that intravenous antigen challenge in *N. brasiliensis*–sensitized rats results in pronounced pulmonary inflammation and a drop in hemodynamic parameters such as MABP, CO, blood flow, and TPR. Furthermore, anaphylaxis-induced pulmonary inflammation was attenuated after bilateral ganglionectomy or decentralization of SCG.

Rats infected with *N. brasiliensis* develop high reaginic antibody titers and when subsequently challenged intravenously with worm antigen, undergo systemic anaphylaxis (7–9). During anaphylactic shock, many animals may develop respiratory distress and die (7, 11, 15). In our experiments there was 42% mortality in unoperated challenged animals. Although the primary organ that is functionally compromised after anaphylaxis in rats is the intestine, symptoms of anaphylactic shock such as vascular congestion, edema, and petechial hemorrhages are seen in many internal organs, including lungs, after a single sensitization (7).

Histopathological changes in the lungs of sham-operated animals 8 h after antigen challenge showed that there was lymphatic edema, increased cellularity of the pulmonary parenchyma, presence of fibrinous exudates, and granulocytes within alveoli and margination of neutrophils in the pulmonary blood vessels (Fig. 7). This was further confirmed by our observation that after anaphylaxis, there was a significant increase in inflammatory cells in the air spaces and increased

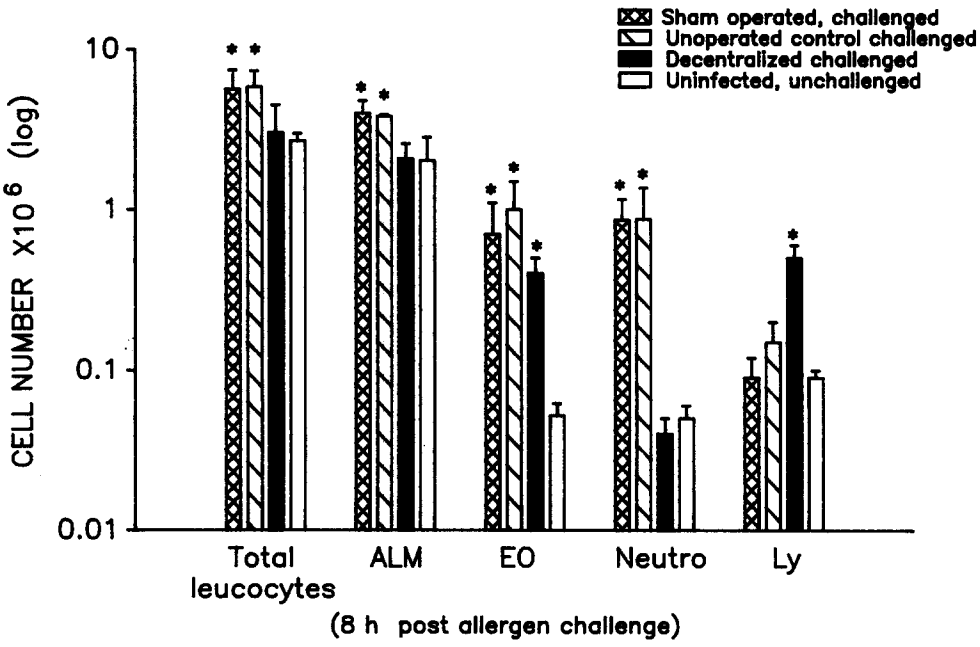


**Figure 5.** Effect of decentralization of SCG on bronchoalveolar lavage cells 8 h after challenge with antigen. The values represent means ± SEM for 7–25 animals in the indicated groups. $p \leq 0.05$ (*) when compared with uninfected, unchallenged normal controls (□).

recovery of serum-derived proteins in BALF. Similarly, the MABP, CO, TPR, and blood flow to the trachea and conducting airways dropped precipitously within 5 min after antigen challenge and persisted for at least 60 min, but returned to prechallenged levels by 4 h.

Prior sensitization is a prerequisite for the development of anaphylaxis, as unsensitized antigen challenged animals do not develop anaphylaxis. The role of IgE antibodies, activated lymphocytes, and mast cells is well documented (9, 11, 13, 16) in this anaphylactic model. Similarly, the central nervous system also plays a significant role in the development of anaphylaxis (41). Although neuroendocrine pathways have received considerable study in immune and inflammatory responses (42), the interactions between the autonomic nervous system and immune system in the development and modulation of anaphylaxis are largely unknown. After decentralization, any one or all of these pathways could be modified.

It is well established that lymphoid tissues are richly innervated by noradrenergic nerve fibers derived from ganglia in the sympathetic chain (23, 43). Functional studies suggest that this innervation modulates antigen-specific responses in vivo by influencing the lymphocyte proliferation (21–25), migration (44), and differentiation (19, 20, 23, 34). It is possible that our sympathetic decentralization modulated lymphocyte function in the cervical lymph nodes. Interestingly, we recovered more lymphocytes in BALF of decentralized animals (Fig. 6). Currently we are investigating the phenotype of these lymphocytes. Similarly, studies elucidating the stress-induced release of NGF from submandibular glands of mouse (46, 47) demonstrate that neuroendocrine pathways are involved in the release of this factor with antiinflammatory properties (28). Thus, given that the post-ganglionic fibers from SCG innervate the thymus and lymph nodes in the cervical region and submandibular glands (19, 20), we studied the effects of ganglionectomy or decentralization of SCG on pulmonary inflammation after systemic anaphylaxis.

Our surgical procedures were designed not to directly influence neural control of pulmonary vasculature. Although integrity of the sympathetic innervation to lungs was not assessed after neural manipulation in our experiments, existing knowledge of the local neuroanatomy and ongoing studies have established that hemodynamic parameters and respiratory functions were unaltered after the surgical procedures we used. Our results showed that decentralization significantly reduced anaphylaxis-induced mortality (by 68%), attenuated serum transudation in lungs, and reduced migration of inflammatory cells into the bronchoalveolar spaces.

Several earlier studies addressed the role of the sympathetic nervous system in inflammation, allergy, and immune responses (48–50) by using chemical sympathectomy using 6-hydroxydopamine (6-OHDA). This procedure resulted in either enhanced (23, 24), depressed (48, 49), or unaltered (50) antibody responses in spleen or lymph nodes. The reasons for this spectrum of functional changes may be due to species, strain, or age differences of animals used; to differences in protocol of 6-OHDA administration (49); or to the complexity of systemic effects that chemical sympathectomy induces (51).

Studies of the effects of more targeted surgical sympathectomy, rather than chemical sympathectomy, have identified alterations on immune responsiveness (22, 25, 52). Besedovsky et al. (25) demonstrated that surgical denervation of the spleen significantly increased splenic plaque-forming cell (PFC) responses to SRBC. However, they resected all the nerve and arterial supply to the spleen and the complexity of possible effects is difficult to analyze. In another study, Alito et al. (22) showed that unilateral superior cervical ganglionectomy

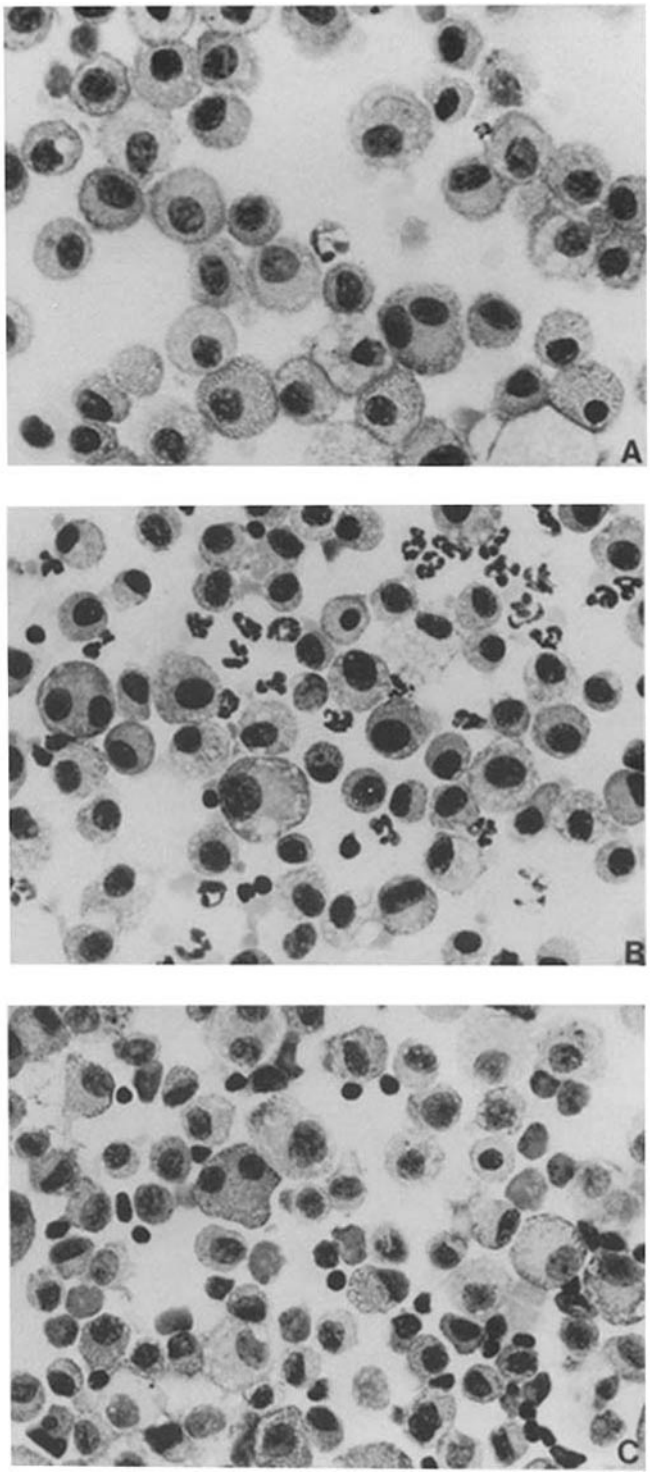


**Figure 6.** Bronchoalveolar lavage cells. Cytospin smears stained with May Grunwald-Giemsa. (*A*) BAL cells from normal, uninfected rats (>95% of the cells are alveolar macrophages). Intravenous challenge with 125 WE of worm antigens in sensitized rats resulted in significant neutrophilia. (*B*) However, similar challenge in SCG-decentralized, sensitized animals resulted in significantly reduced neutrophils in BALF (*C*).

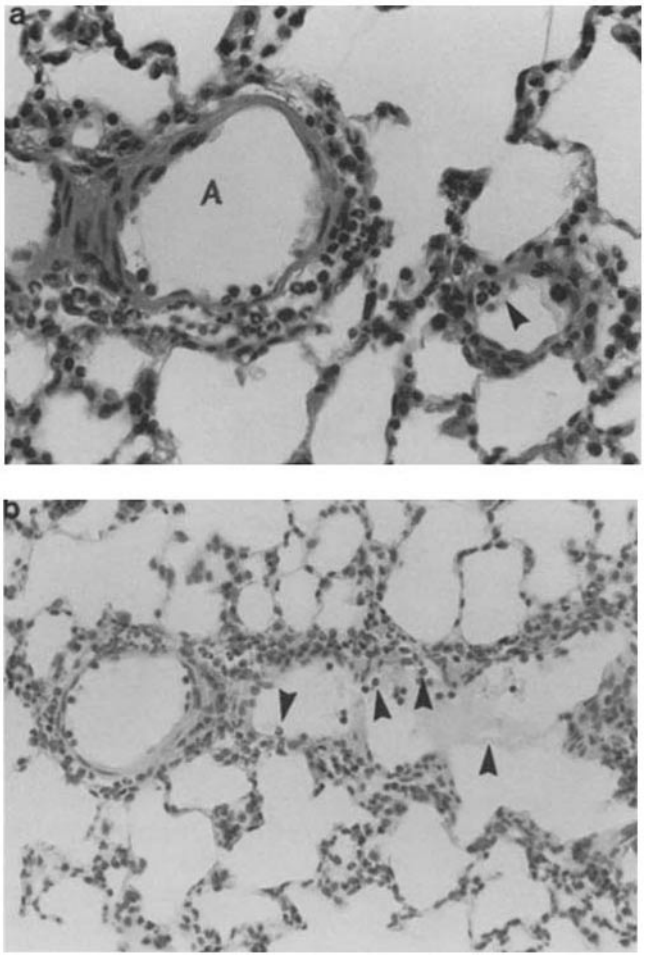


**Figure 7.** Sections of rat lungs 8 h after antigen challenge in sham-operated animals showing (*a*) accumulation of neutrophils and eosinophil with mild edema of the perivascular space of a small artery (*A*). Note the margination and emigration of neutrophils in an adjacent arteriole (*arrowhead*). (*b*) Air spaces adjacent to the arterioles also contained neutrophils and fibrinous material (*arrows*); hematoxylin and eosin. ×400.

in mice enhanced the PFC response to SRBC in submaxillary lymph nodes, contact hypersensitivity, and allogeneic delayed-type reaction in the ipsilateral cutaneous region and GVH reaction. By contrast, our bilateral ganglionectomy or decentralization of SCG suppressed inflammatory cellular responses and extravasation of serum proteins in the bronchoalveolar space, a site not directly innervated by SCG. Coderre et al. (53) observed a similar decrease in plasma extravasation into the synovial joints, induced by pharmacologic activation, after surgical excision of the lumbar sympathetic chain.

Melvin et al. (52) showed that superior cervical ganglionectomy resulted in decreased responsiveness to $\beta$-adrenergic stimulation in adult rat parotid glands, although the $\beta$-adrenergic receptor density was unaltered 7 d after ganglionectomy. This study suggests the possibility that alternations in physiological responsiveness of such target tissues after denervation may play an important role in anaphylaxis in our model. We postulate that SCG modulate pulmonary inflammation through

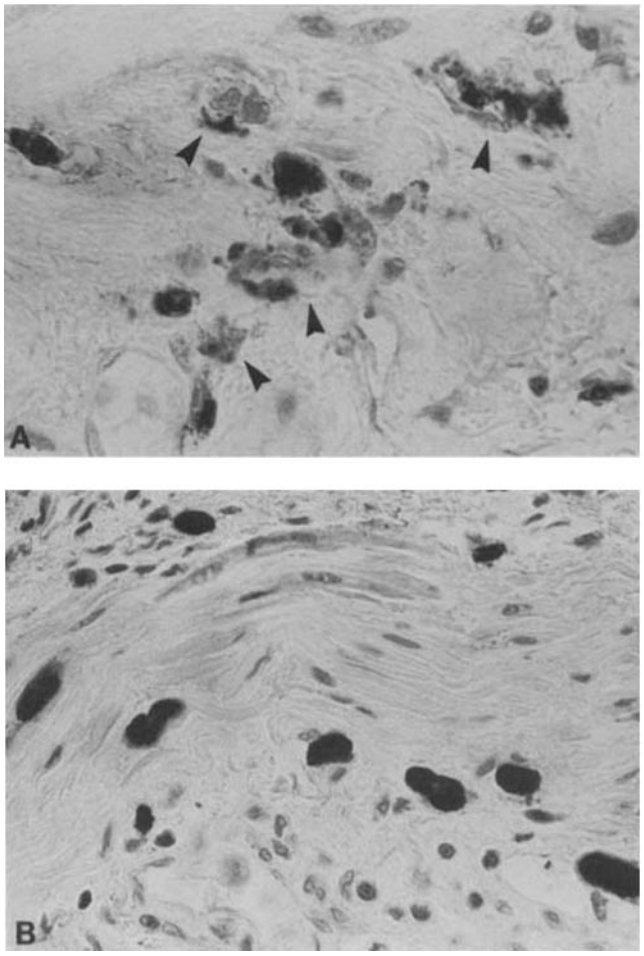

**Figure 8.** Sections of trachea 8 h after challenge in (*A*) sham-operated or (*B*) decentralized animal. Note (*arrowheads*) the partially degranulating mast cells in sham-operated animal compared with more compact granules within the mast cells from decentralized animal. Similar changes were seen in the lungs as well; avidin-biotin-peroxidase complex stain. ×1,000.

innervation of one or more target tissues and we are presently systematically investigating the role of different tissues or organs. One of our studies has suggested an important role for the submandibular glands (54) which are known to be a rich source for several cytokines and growth factors.

Although serum protein levels in BALF were modulated by decentralization, studies of the microcirculation after anaphylaxis showed that the blood flow to conducting airways was unaltered 1 h after antigen challenge in sensitized animals. Concomitant with the transudation of serum proteins in antigen-challenged sensitized rats, there was a marked migration of inflammatory cells into the alveolar spaces. Decentralization significantly attenuated these cellular responses, particularly that of neutrophil influx (Fig. 5 and 6 *C*). Several mediators such as platelet-activating factor, complement fragments, neuropeptides, and mast cell or macrophage-derived factors present in the lower respiratory tract can potentiate neutrophil migration and activation. However, our study indicates that the total chemotactic activity in BALF is unaltered after decentralization. This reduced infiltration of neutrophils into the alveolar space after decentralization may suggest that there are functional alterations in the ability of neutrophils to respond to chemotactic stimuli. Alternatively, the endothelial properties may have changed, thus modifying neutrophil margination. We are investigating the functional status of neutrophils after decentralization of SCG.

Infection with *N. brasiliensis* induces marked eosinophilia in BALF (4), but decentralization did not alter the eosinophil numbers in BALF of sensitized animals. Given that eosinophils may play a significant role in the pathology associated with late-phase allergic reactions (55, 56), it is possible that the eosinophil function was modulated by decentralization, but this was not investigated.

Using mast cell–deficient W/W$^v$ mice, Martin et al. (14) demonstrated that the cardiopulmonary changes and mortality associated with anaphylaxis are mediated by mast cells. Several studies have established that there is a close association between mast cells and sympathetic nerves (57). Furthermore, various neurotransmitters are shown to regulate release of mediators from mast cells (58). After intraarterial administration of *Ascaris suum* antigens into the bronchus of allergic dogs, Garrity et al. (59) showed that sympathetic stimulation inhibited, whereas parasympathetic stimulation augmented (60), the secretion of histamine from pulmonary mast cells. Moreover, $\beta$-adrenergic blockade before sympathetic stimulation increased histamine secretion from mast cells (61). Coderre et al. (53) suggested that mediators like prostaglandins may be involved in this sympathetic regulation of histamine release by mast cells. Our histopathological studies also showed a decreased mast cell degranulation in trachea and lungs of decentralized animals (Fig. 8 *A* and *B*). However, since this effect on mast cells in our study was also seen in an area distant from the site of decentralization, mechanisms other than direct sympathetic or parasympathetic modulation of mast cell secretion must be involved.

Despite evidence that free histamine is rapidly catabolized (62), we found increased levels of histamine in BALF (Fig. 4 *a*) and degranulating mast cells in trachea (Fig. 8, *A*) and lungs of unoperated and sham-operated animals 8 h after-antigen challenge. Factors such as the continued presence of histamine-releasing activity (63) or the initiation of a late-phase reaction (64) could explain these elevated histamine levels at 8 h after challenge. However, after decentralization, factors contributing to increased histamine levels in BALF appear to be absent or attenuated. Alternatively, decentralization may enhance catabolism of histamine. Perhaps a similar mechanism is functioning in the peritoneum, a site distant from the area of decentralization, because peritoneal mast cells from decentralized animals contained more histamine at 8 h after challenge than sham-operated or unoperated rats. This later observation, as well as our earlier studies on attenuated subcutaneous granuloma formation in decentralized animals (65), attests to this widespread antiinflammatory effect of the decentralization of SCG.

In summary, we have established that the cervical sympathetic trunk is part of a bidirectional communication network between the nervous system and the immune system.

Bilateral decentralization or ganglionectomy of SCG markedly depressed pulmonary inflammation and had widespread systemic effects as well. We postulate that these effects involve antiinflammatory pathways driven by tissues or organs innervated by SCG such as the submandibular glands (54).

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