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CATECHOLAMINE INFLUENCES AND SYMPATHETIC NEURAL MODULATION OF IMMUNE RESPONSIVENESS

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ABSTRACT

Primary and secondary lymphoid organs are innervated extensively by noradrenergic sympathetic nerve fibers. Lymphocytes, macrophages, and other cells of the immune system bear functional adrenoreceptors. Norepinephrine fulfills criteria for neurotransmission with cells of the immune system as targets. In vitro, adrenergic agonists can modulate all aspects of an immune response (initiative, proliferative, and effector phases), altering such functions as cytokine production, lymphocyte proliferation, and antibody secretion. In vivo, chemical sympathectomy suppresses cell-mediated (T helper-1) responses, and may enhance antibody (T helper-2) responses. Noradrenergic

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innervation of spleen and lymph nodes is diminished progressively during aging, a time when cell-mediated immune function also is suppressed. In animal models of autoimmune disease, sympathetic innervation is reduced prior to onset of disease symptoms, and chemical sympathectomy can exacerbate disease severity. These findings illustrate the importance of the sympathetic nervous system in modulating immune function under normal and disease states.

INTRODUCTION

During the past two decades, evidence has accumulated from a variety of experimental approaches that the immune system is regulated by signaling from the central nervous system (CNS): (*a*) Classical behavioral conditioning paradigms that require CNS processing can condition alterations in immune function (reviewed in 1); (*b*) stress and other psychosocial factors can induce alterations in immune function (2, 3); (*c*) lesions of specific areas or nuclei within the CNS, especially regions associated with limbic (affective) or hypothalamic (autonomic and neuroendocrine control) circuitry, can alter peripheral measures of immune function (4); and (*d*) experimental manipulation of the hypothalamo-pituitary axis and the autonomic nervous system, the two visceral output pathways from the CNS to the periphery, can alter immune function (reviewed in 1). The hypothalamo-pituitary axis communicates with the periphery through the release of anterior and posterior pituitary hormones into the circulation. The autonomic nervous system provides "hard wiring" from the CNS to visceral target tissues via the sympathetic and parasympathetic nervous systems. In this review, we summarize the anatomical, biochemical, and functional evidence for modulation of immune function by the sympathetic nervous system (SNS) and its principal neurotransmitter, norepinephrine (NE). SNS innervation of primary and secondary lymphoid organs and the presence of adrenoceptors on cells of the immune system provide the means for noradrenergic signaling of lymphocytes and macrophages from sympathetic nerves. Functionally, we discuss noradrenergic influences on immune reactivity in a hierarchical fashion: (*a*) individual cellular functions, (*b*) collective interactions of lymphoid cells, and (*c*) host responses to challenges requiring immune reactivity for expression or resolution (Figure 1). Finally, evidence for communication from the immune system to the nervous system is discussed, completing a bidirectional interconnection between the nervous and immune systems.

NORADRENERGIC INNERVATION OF LYMPHOID ORGANS

The autonomic nervous system is composed of preganglionic neurons, whose cell bodies are located in the brain stem and spinal cord and whose axons exit the

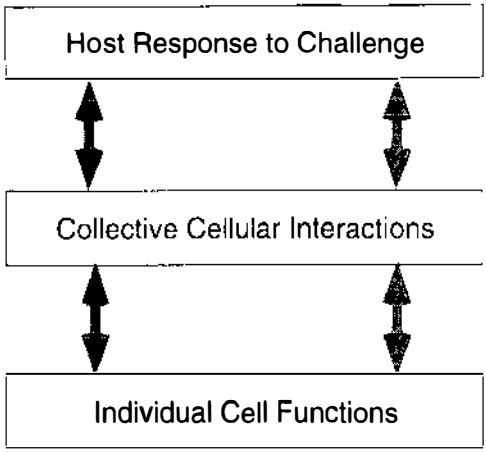

Figure 1 General hierarchy of immunologic activity that reflects the various levels of investigation of catecholamine influences and sympathetic neural modulation of immune responsiveness. Individual cell functions (e.g. proliferation, differentiation, receptor expression, cell adhesion molecule expression, cytokine synthesis and release, second messenger production, etc), often studied in vitro, can lead to predictions regarding neurotransmitter influence on collective cellular interactions (e.g. primary and secondary antibody responses, cytotoxic T lymphocyte activity, delayed hypersensitivity, NK cell activity), studied both in vitro and in vivo, which in turn can lead to predictions about how specific viral, bacterial, tumor, or autoimmune challenges should be responded to by the host. This permits predictive hypothesis testing to span the gap between in vitro cellular and molecular studies, and physiological studies in a challenged host with variables such as age, gender, past learning, nutrition, etc. This hierarchy also has been used in the opposite direction, such that psychosocial or physiological effects on immunologic reactivity or disease outcome in a challenged host can lead to predictions about what aspect of humoral or cellular immunity is altered, which in turn can lead to specific hypothesis testing about specific molecular mechanisms of action through which the initial phenomenon might have occurred. Such a hierarchy serves to integrate studies at various levels.

CNS and synapse in peripheral autonomic ganglia. These ganglion cells send postganglionic fibers to terminate in target tissues. The targets (effector tissues) of the autonomic nervous system include cardiac muscle, smooth muscle, secretory glands, metabolic cells (liver, brown fat), and cells of the immune system (5, 6). For the SNS, the preganglionic cell bodies are located in the lateral horn (intermediolateral cell column) and other regions of intermediate gray of the thoraco-lumbar spinal cord (T1-L2). The preganglionic axons exit via the ventral roots, and synapse in sympathetic chain ganglia (paravertebral) or collateral ganglia (prevertebral), the latter mainly associated with plexuses along the great vessels of the thorax, abdomen, and pelvic region. The postganglionic nerve fibers of the SNS, mainly noradrenergic (NA) nerve fibers, give rise to widespread arborizations, and release NE as the major neurotransmitter. NA

nerve fibers can be detected by means of fluorescence histochemistry for catecholamines, or by immunocytochemistry (ICC), using antibodies specific for the rate-limiting enzyme in NE biosynthesis, tyrosine hydroxylase (TH). These histological techniques are supplemented by chemical assay to substantiate the nature and quantity of the catecholamine neurotransmitter. Using these techniques, we have demonstrated that primary and secondary lymphoid tissues are target tissues for NA sympathetic nerve fibers (7, 8).

The compartmentation of sympathetic NA innervation of rat and mouse spleen has been studied extensively. NA postganglionic innervation of rat spleen originates mainly in the superior mesenteric/coeliac ganglion (11, 12) and the nerve fibers enter the spleen at the hilar region with the vasculature. Some fibers arborize with the capsular/trabecular system. Extensive arrays of NA nerve fibers also travel with the central artery and its arteriolar branches, and extend into the parenchyma of the periarteriolar lymphatic sheath (PALS). NA fibers are present among cells in the T-dependent areas, including OX-19$^+$ (pan T cells, Figure 2), W3/25$^+$ (T helper cells), and CD8$^+$ (cytotoxic) T cells (10, 13). ED3$^+$ macrophages and sIgM$^+$ B cells residing in the marginal zone and the marginal sinus, the site of lymphocyte entry into the spleen, also receive NA innervation. Innervation of the B cell–containing follicles is sparse, although some NA fibers course along the edges of the follicles. The red pulp contains scattered fibers, primarily associated with the plexuses along trabeculae and surrounding the sinuses. This NA innervation of the lymphoid components of the white pulp is present at birth in rats, and the fibers form close associations with incoming lymphoid cells several days prior to the first appearance of smooth muscle cells in this organ (14).

Electron microscopic immunocytochemical studies of TH$^+$ nerve fibers have shown direct contacts of nerve terminals with lymphocytes and macrophages in the white pulp of the spleen (9, 10). The associations between TH$^+$ nerve terminals and lymphocytes consist of parallel membrane appositions as close as 6 nm. No cell processes (e.g. Schwann cell, interdigitating cell) are interposed between these neuro-effector junctions, unlike the ubiquitous intervening basement membrane and frequent cell processes between TH$^+$ nerve terminals and other targets of NA sympathetic nerves, such as smooth muscle cells. Thus, TH$^+$ nerve terminals can form very close appositions with lymphocytes and macrophages. In the spleen, these close contacts have been found along the outer edge of the adventitial zone along the central artery, throughout the PALS, along the marginal sinus, and scattered within the marginal zone. In addition to signaling from NA terminals in close contact with lymphocytes and macrophages, NE also can diffuse through the parenchyma after release from nerve terminals, thereby providing for potential widespread concentration gradients of NE for interaction with adrenoceptors on target cells of the immune system.

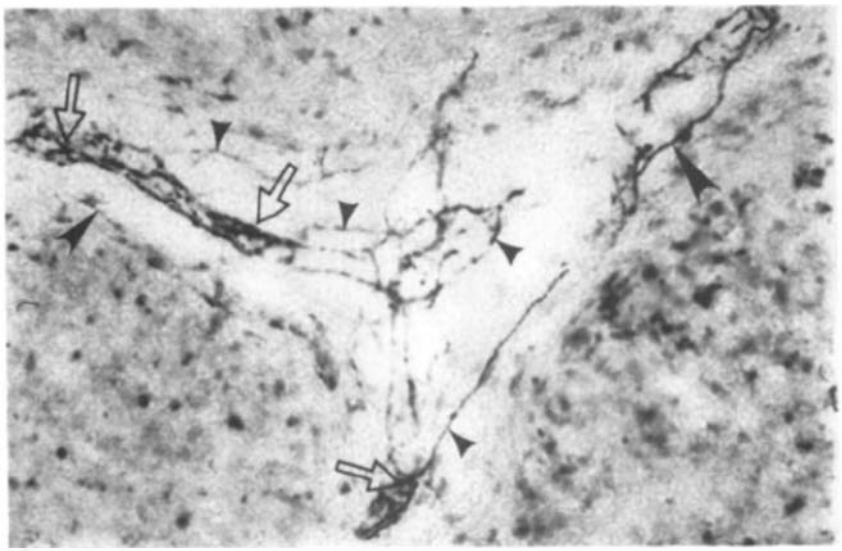

Figure 2 Tyrosine hydroxylase (TH)–positive postganglionic noradrenergic sympathetic nerve fibers in the white pulp of the spleen of an adult rat. These nerve fibers travel along the central artery of the white pulp (large open arrows), and also extend into the fields of T lymphocytes in the periarteriolar lymphatic sheath (PALS) (small arrowheads) and along the marginal sinus (large arrowheads). Electron microscopic observations have demonstrated that terminals from these noradrenergic nerve fibers form close neuro-effector junctions, as close as 6 nm, with T lymphocytes and macrophages in all compartments of the white pulp, even along the vasculature in the PALS. ×175

In lymph nodes, NA innervation is found within the subcapsular zone, paracortical and cortical regions, and medullary cords, but is absent from follicles, the B cell–containing areas (7, 13, 15). In spleen and lymph nodes, therefore, such processes as lymphocyte trafficking, antigen processing and presentation, and T lymphocyte function may be influenced directly by NE released from NA nerve terminals. B cells may encounter NE released from NA fibers that course along the edge of the follicles and in the marginal zone of the spleen; alternatively, B cell function may be affected indirectly through NE-induced alterations in cytokine production by T cells and macrophages. The compartmentation of NA nerves also suggests that the concentration of NE available for interaction with a specific target cell depends on its proximity to the NA nerve terminals.

NA innervation of primary lymphoid organs suggests that development of the immune system also may be influenced by the SNS. NA innervation supplies the thymus, where T cells mature, and the bone marrow, where B

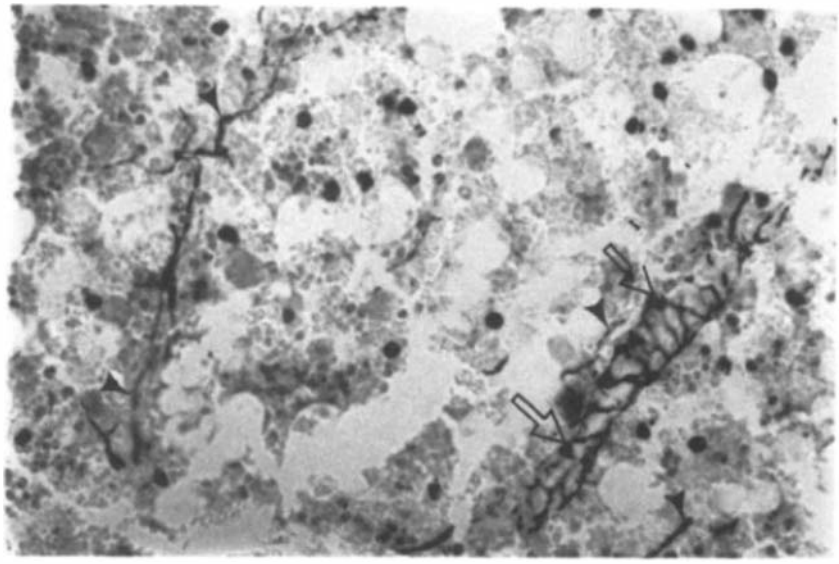

Figure 3 Tyrosine hydroxylase (TH)–positive sympathetic noradrenergic nerve fibers in the bone marrow of the femur of a young adult rat. Nerve fibers travel with the vasculature in the marrow (large open arrows) and also extend into the parenchyma and arborize among a variety of cell types (arrowheads). ×250

cells, macrophages, granulocytes, and other hematopoietic cell types develop. In the thymus, NA nerve fibers run in the subcapsular region with some branching into the cortex, which contains immature thymocytes (7, 16, 17). The site where mature thymocytes reside, the medullary cords, is sparsely innervated. The cortico-medullary boundary, an area important for immigration of mature thymocytes from the thymus, is densely innervated, particularly along the vasculature. In the bone marrow, NA nerve fibers enter the marrow with the arteries, travel with vascular plexuses deep in the marrow, and arborize in the substance of the marrow itself, where they may influence hematopoiesis and cell migration (7, 18, 19) (Figure 3).

Release of NE from NA nerve fibers, as well as its availability for interactions with cells of the immune system, also has been investigated. Splenic NE is primarily neurally derived; chemical destruction of NA nerve terminals with the neurotoxic drug 6-hydroxydopamine (6-OHDA) or with splenic nerve cuts or ganglionectomy resulted in loss of fluorescent nerve profiles and >90% reduction in NE levels, as measured by high-performance liquid chromatography (11, 20–23). One method of indirectly assessing NE release is to measure the rate of NE turnover. The rate of reduction of NE in the spleen is assessed

following inhibition of NE synthesis with α-methyl-para-tyrosine. NE turnover has been measured in rat spleen in adulthood and in development (24). Turnover is very low at birth, rises rapidly to approximately 50% of adult levels by day 14, and increases to adult levels (turnover time of 8–16 hours) between 56 and 90 days of age. This gradual increase in turnover parallels increasing NE concentrations in the spleen throughout development. Hori and colleagues have shown release of NE more directly in spleen following splenic nerve stimulation, using in vivo microdialysis with high-performance liquid chromatography (25). These results provide evidence that NE is released and is available to bind to lymphoid cells bearing the appropriate receptors throughout development and maturation of the immune system.

ADRENOCEPTOR EXPRESSION BY CELLS OF THE IMMUNE SYSTEM

NE and other neurotransmitters stimulate target cells through interactions with cell surface adrenergic receptors (adrenoceptors). The adrenoceptors are divided into two classes, α and β, each of which is divided into at least two subtypes, based on pharmacological, biochemical, and molecular criteria (reviewed in 26). Adrenoceptors have been defined classically by the potency exhibited following stimulation with NE, epinephrine (EPI), and isoproterenol (ISO). NE and EPI bind to α- and β-adrenoceptors with differing affinities, while ISO binds selectively to β-adrenoceptors. Intracellular signaling mechanisms also differ among adrenoceptors (reviewed in 27). β-Adrenoceptors are coupled intracellularly to the GTP-binding protein, G_s, of the adenylate cyclase complex, giving rise to increased intracellular cyclic AMP (cAMP) upon stimulation. α_1-Adrenoceptors stimulate phosphatidylinositol turnover and induce a rise in intracellular calcium. α_2-Adrenoceptors are linked to the G_i subunit of the adenylate cyclase complex. A third way of identifying and differentiating among adrenoceptors is through the use of selective radioligands. Using β-adrenoceptor-specific radioligands, lymphocytes have been shown to express high-affinity β-adrenoceptors, mainly of the β_2 subclass (28-32). α-Adrenoceptors have not been identified in rodent lymphocytes, although there are reports of human and guinea pig lymphocyte α-adrenoceptor expression (33–35). Activated rodent macrophages express α_2- and β-adrenoceptors (36, 37). As is discussed later in this review, rodent lymphocyte function can be altered by pharmacological agents targeted at α-adrenoceptors, suggesting that a small subset(s) of cells within unfractionated lymphoid populations may express α-adrenoceptors. Other hematopoietic cells involved in inflammation also express β-adrenoceptors, including neutrophils, basophils, and eosinophils (38, 39).

β-Adrenoceptor density varies among lymphocyte populations. In mice,

splenic B cells express twice the number of β-adrenoceptors expressed by T cells (40, 41). In humans, among the T cell subsets, suppressor T cells have the highest β-adrenoceptor density, cytotoxic T cells have an intermediate density, and T helper cells have the lowest density (42, 43). Intracellular cAMP levels generated by receptor-ligand interactions correlated with receptor density. The heterogeneity of receptor expression may be related to the concentrations of NE or EPI in the microenvironments of these cells. Desensitization or down-regulation of β-adrenoceptors occurs in the presence of agonist (44, 45; reviewed in 27); up-regulation may occur after chronic exposure to antagonists (46). The differences between T and B cell β-adrenoceptor expression might be related to the sparseness of innervation of B cell–dependent follicles in the spleen. Alternatively, the differences in β-adrenoceptor density may reflect heterogeneity within the T or B lymphocyte population. For example, T helper (Th) cell subsets have been identified and are characterized by the cytokines they secrete (47). T helper–1 (Th_1) cells secrete interleukin-2 (IL-2) and interferon-γ (IFN-γ), while Th_2 cells secrete IL-4, IL-5, IL-6, and IL-10. Th_1 cells participate in cell-mediated responses. Both Th cell subsets provide help for B cell production of immunoglobulin M (IgM) (48–51), albeit to different degrees, while their distinctive cytokines influence the production of particular antibody isotypes (47). Recently, resting Th_1 and Th_2 cells have been shown to differentially express the β-adrenoceptor (52). High-affinity β-adrenoceptors were detectable on resting Th_1 cells, but were undetectable on Th_2 cells. In addition, resting Th_1 cells accumulated cAMP intracellularly upon exposure to a β_2-adrenergic agonist, whereas resting Th_2 cells did not. Thus, differential expression of the β-adrenoceptor by Th subsets may provide a mechanistic basis for the modulation of Th cell subset function by NE, discussed below.

Lymphocyte β-adrenoceptor density also changes throughout development. β-Adrenoceptors are found on thymocytes as early as day 18 of gestation in the mouse (53), and the density increases with maturation. Mature (cortisone-resistant) thymocytes express twice the number of β-adrenoceptors per cell as immature (cortisone-sensitive) thymocytes (41, 54). In the rat spleen, β-adrenoceptors increased from approximately 500 sites per cell at birth to 1000 sites per cell (adult levels) by postnatal day 14 (24). The mechanism behind these maturational changes in lymphocyte β-adrenoceptor expression currently is unknown.

Lymphocyte activation with antigen or mitogen also may alter β-adrenoceptor density. Following intraperitoneal immunization with sheep red blood cells (SRBC), spleen cell β-adrenoceptor density was reduced (41). In contrast, following contact sensitization with picryl chloride, the number of β-adrenoceptors on draining lymph node cells was increased (55). Depending on the number of times the animal had been immunized previously, alloimmunization increased, decreased, or did not alter β-adrenoceptor density on B cells; ad-

renoceptor density on T cells was not altered (56). The alloimmunization-induced changes in receptor number were correlated with changes in receptor sensitivity, as measured by cAMP production. Availability of neurally released NE in the microenvironment following antigen challenge may alter β-adrenoceptor expression (57, 58), thereby regulating lymphocyte responsiveness to catecholamines at the level of the target cell. Treatment of lymphocytes in vitro with concanavalin A (Con A) has been reported to increase β-adrenoceptor density (59, 60). In contrast, when the signal transduction pathway induced by the T cell antigen receptor was activated with phorbol myristate acid (PMA) and a calcium ionophore, decreased T lymphocyte β-adrenoceptor density was demonstrated (60). This decrease was correlated with a reduction in ISO-stimulated cAMP. These conflicting results suggest that it is not activation per se but induction of specific signal transduction pathways that dictates how β-adrenoceptor number and sensitivity may be altered.

IN VITRO STUDIES OF CATECHOLAMINE INTERACTIONS WITH THE IMMUNE SYSTEM

An in vitro approach has been useful in identifying the potential range of direct effects of particular adrenergic agonists on immune reactivity. Based on early in vitro studies, a simple functional distinction was made between α- and β-adrenoceptor-mediated effects on cells of the immune system. β-adrenoceptor stimulation inhibited such activities as lymphocyte proliferation, antibody secretion, and production of pro-inflammatory factors (61–63), whereas α-adrenoceptor stimulation had the opposite effects (61). In this section, we review more recent studies assessing effects of adrenoceptor stimulation on immune reactivity in vitro. These studies indicate that regulation of immune responsiveness by catecholamines is more complex than initially proposed.

Reactivity of Catecholamines with Enriched Cell Populations of the Immune System

NEUTROPHILS, BASOPHILS, AND MAST CELLS Early studies reported that catecholamines and other agents that increase cAMP diminish both neutrophil phagocytosis and the release of lysosomal enzymes from neutrophils (64). More recently, Nielson (65) reported that low concentrations of ISO and PGE_2 inhibited the respiratory burst of neutrophils associated with degranulation. This effect was dependent on the mechanism of neutrophil activation, with inhibition observed following stimuli that increased intracellular calcium. Catecholamines may inhibit this rise in calcium, as suggested by the ISO-induced decrease in the concentration of neutrophil leukotriene B_4, a calcium-dependent metabolite of arachidonic acid (65). A closer examination of the respira-

tory burst reaction revealed that β-adrenoceptor stimulation and dibutyryl cAMP (dbcAMP) decreased the maximal rate of superoxide production and increased the rate of termination of superoxide production (66). Adrenergic stimulation also may alter neutrophil movement and chemotaxis. Incubation of rabbit peritoneal neutrophils with EPI and ISO inhibited spontaneous motility and chemotaxis (67). This effect was blocked by propranolol, indicating that the response was β-adrenoceptor-mediated. When human neutrophils were stimulated with two different chemoattractants, leukotriene B_4 and formyl-methionyl-leucyl-phenylalanine (FMLP), β-adrenoceptor stimulation also inhibited chemotaxis (68). Interestingly, forskolin, which induced a greater increase in cAMP than did ISO, had no effect on leukotriene B_4–induced chemotaxis, suggesting that increased cAMP may not be solely responsible for the change in responsiveness of neutrophils to various chemoattractants.

In one of the earliest reports of adrenergic effects on cells of the immune system, Lichtenstein & Margolis (69) demonstrated that ISO, EPI, and dbcAMP diminished antigen-induced release of histamine from previously sensitized basophils, in proportion to the ability of these agents to increase intracellular cAMP. This finding of a cAMP-induced reduction in IgE-mediated immediate–type hypersensitivity was extended to findings in a number of other cell types, including the release of histamine from peripheral blood leukocytes (63), the release of histamine and slow-reacting substance of anaphylaxis (SRS-A) from the lung (70, 71), and the release of SRS-A from rat mast cells. In each of these studies, the release of inflammatory mediators was inhibited by agents that increased cAMP, including prostaglandins, catecholamines, histamine, methylxanthenes, and dbcAMP. Bourne et al (63) further reported that the order of potency for the inhibitory action of adrenergic agents (ISO>EPI>NE>phenylephrine) and the ability of propranolol to block the inhibition suggested a β-adrenoceptor-mediated effect. The eosinophil, which also contributes to inflammatory processes, has been shown to express β_2-adrenoceptors that are coupled to adenylate cyclase (39). However, no effect of β-adrenoceptor stimulation on either superoxide anion generation or the release of eosinophil peroxidase was detected.

LYMPHOCYTES Complex interactions between adrenoceptor stimulation and lymphocyte activity have been demonstrated in studies of catecholamine-induced modulation of B lymphocyte proliferation and differentiation. The effect of β-adrenoceptor stimulation on B cell activity was dependent on the proliferative stimulus used. Lipopolysaccharide (LPS)-induced proliferation and differentiation by unfractionated spleen cells were enhanced in the presence of 5×10^{-6}–10^{-5} M NE; these effects were blocked by propranolol (72). The NE-induced enhancement was observed when LPS was added concurrently with the adrenergic agonist. In contrast, NE had no effect on B cell proliferation

when added 2 hours or more after incubation of the B cells with LPS (72). Removal of T cells and adherent cells did not alter this enhancement. With these highly purified B cells, the nonselective β-agonist ISO (5×10^{-6}–10^{-5} M) also enhanced proliferation; this effect was blocked by the β-blocker propranolol, but not by the α-blocker phentolamine. However, other agents that increase cAMP, such as forskolin and dbcAMP, inhibited LPS-induced proliferation. NE enhanced proliferation after stimulation with another polyclonal B cell mitogen, a membrane proteoglycan derived from *Klebsiella pneumoniae,* but NE or ISO inhibited proliferation induced by anti-IgM antibodies (73). Anti-IgM activates B cell proliferation via different intracellular mechanisms than those induced by LPS, suggesting that the intracellular pathway induced is an important factor in determining the nature of B cell–catecholamine interactions. Furthermore, these results suggest a divergent pathway in B cells subsequent to the increase in cAMP, because increased cAMP was not sufficient to predict the directional effect of catecholamine stimulation on B cell proliferation.

Likewise, recent data indicate that the signaling pathways induced in B cells by either LPS or crosslinking of surface Ig may be qualitatively different from the signaling pathway induced in B cells after an interaction with Th cells that recognize antigen expressed in conjunction with a class II major histocompatibility complex (MHC) determinant on the B cell (74, 75). Moreover, lower concentrations of cAMP are required to inhibit B cell activation induced by anti-Ig than by contact with Th cells (76). These results have suggested that the mode of B cell activation induces different signaling pathways intracellularly and that these pathways can be modulated differently by cAMP. The influences of β-adrenoceptor stimulation on Th cell–dependent B cell activation and antibody production are discussed later in this review.

Several groups have reported that T cell proliferation is inhibited by β-adrenoceptor stimulation and other cAMP inducers (61, 77, 78). ISO (10^{-5} M) and prostaglandin E_2 (PGE_2) inhibited anti-CD3-induced proliferation of T cells and enriched $CD4^+$, $CD8^+$, and CD45 RO^+ (memory) T lymphocyte subpopulations (79). ISO modestly reduced IL-2 production, but the decrease was not as great as the ISO-induced reduction in proliferation, suggesting that production of other lymphokines also may be reduced. The inhibition of proliferation correlated with increased intracellular cAMP, but equimolar concentrations of cAMP induced by ISO and prostaglandin E_2 did not induce similar levels of inhibition. Some factor other than peak cAMP levels may determine the level of inhibition, including the length of time cAMP is elevated or the isozyme of cAMP-dependent protein kinase stimulated. Also, as discussed previously, there may be differences in the level of proliferation because of a difference in the level of expression of β-adrenoceptor and PGE_2 receptors on different T lymphocyte subsets.

LYTIC ACTIVITY OF NATURAL KILLER (NK) CELLS AND CYTOTOXIC T LYMPHOCYTES (CTLs) Lytic activity, an effector function of NK cells and CTLs, also is altered by catecholamines. In one report, human NK cell activity was inhibited by ISO and other cAMP-inducers when added directly to target and effector cells in a ^{51}Cr-release assay (80). More complex effects also have been reported, in which EPI at 10^{-6} M inhibited, and at 10^{-8} M potentiated, NK cell lysis; propranolol prevented both of these changes (81). Pretreatment of effector cells with EPI, followed by extensive washing to remove the agonist, yielded similar results, demonstrating that the effects of EPI were mediated through NK cell β-adrenoceptors, and not through interactions of the catecholamines with the target cells. Finally, lysis of specific target cells by mature CTLs also was inhibited when ISO was added directly to the ^{51}Cr-release assay (82). This reduction correlates with recent reports using cloned CTLs, in which increased cAMP induced by forskolin and other cAMP-elevating agents inhibited exocytosis of granules via cAMP-dependent protein kinase (83). Together, these findings suggest that end-stage effector functions, such as cell lysis, are inhibited by catecholamines. However, as discussed below, catecholamines may exert enhancing effects on the initiation of CTL responses, in contrast to inhibition of effector cell function.

CYTOKINE PRODUCTION AND RESPONSIVENESS TO CYTOKINES LPS-induced production of tumor necrosis factor-β (TNF-β) was reduced when EPI or ISO was added to normal human peripheral blood monocytes or the monocytic cell line THP-1 (84). Production of THP-1 TNF-β was inhibited completely at 10^{-7} M EPI, and a β-blocker prevented the inhibitory effect of EPI. The reduction occurred when the agonist was added at the same time as the stimulator LPS. In contrast, when ISO was added to THP-1 cells 24 hours prior to LPS, TNF-β production was enhanced. These results suggest that the timing of β-adrenoceptor stimulation relative to the primary activating signal (LPS, in this case) may be critical in determining the direction of the effect of β-adrenoceptor stimulation.

Spengler et al (37) reported that NE and the α_2-agonist UK-14304 augmented LPS-stimulated TNF production by activated murine macrophages with an EC_{50} in the nanomolar range. β-Adrenoceptor stimulation with ISO reduced TNF production with an EC_{50} of 0.255 μM (85). These alterations in TNF production corresponded to changes in TNF mRNA production. Both the UK-14304-induced increase in TNF production and mRNA production were blocked by the α_2-blocker yohimbine; the ISO-induced reduction was blocked by propranolol. These results suggest that α- and β-adrenoceptor stimulation may influence immune responsiveness via adrenoceptor stimulation of cytokine production by accessory cells.

Responsiveness to cytokines also can be altered with adrenoceptor stimula-

tion. IFN-γ–induced activation of peritoneal macrophages was inhibited by NE (10^{-6} M) and EPI (10^{-6} M), as measured by reduced lysis of tumor cells or herpes simplex virus–infected cells (86, 87). This inhibition correlated with increased cAMP, and vasoactive intestinal polypeptide, which elevates cAMP, further augmented the NE-induced decrease in tumor cell lysis (87). IL-2 induction of lymphokine activated killer (LAK) cells was enhanced by the α-agonist phenylephrine (88). Finally, β-adrenoceptor stimulation acted in concert with IL-4 to synergistically increase expression of CD23, the low-affinity IgE receptor, on the human promonocytic cell line U937 (89). These results suggest that the nature of β-adrenoceptor stimulation of cells of the immune system may depend on simultaneous signaling from other cytokines in the microenvironment.

Reactivity of Catecholamines in Immune Responses Requiring Multicellular Interactions

GENERATION OF ANTIBODY PRODUCTION As mentioned previously, Melmon and coworkers (62) demonstrated that when ISO and other cAMP-elevating agents were added to spleen cells from mice immunized with SRBC 15 minutes prior to assay of plaque formation, fewer plaques were formed. Thus, it appears that the end-stage of B cell differentiation, antibody secretion, is reduced following β-adrenoceptor stimulation. More recent studies have demonstrated that β-adrenoceptor stimulation may have very different effects when a β-agonist is present during the activational phase required for generation of an antibody response.

Sanders & Munson pharmacologically assessed the effects of NE and adrenoceptor stimulation in the generation of anti-SRBC antibodies in vitro (90–92). Three types of cells participate in this response: antigen-presenting cells (macrophages, dendritic cells), T helper cells, and the antibody-forming B cells. NE (10^{-6}–10^{-5} M), added at the beginning of culture, enhanced the antibody response on the peak day of culture, day 5. This effect was blocked by the β-antagonist propranolol and mimicked with the β_2-agonist terbutaline. Addition of propranolol was required within 6 hours of initiation of culture to block the NE-induced enhancement of antibody production completely, suggesting that an early event was influenced by β-adrenergic stimulation.

α-Adrenoceptor-mediated effects also were demonstrated in this in vitro system (92). An α-adrenoceptor-mediated effect of NE was uncovered in the presence of propranolol. When NE and propranolol were added together, not only was the NE-induced enhancement on day 5 blocked, but the antibody response on day 4 was enhanced. The α_1-selective agonist methoxamine also enhanced the antibody response on day 4, and this effect was blocked with

the α-blocker phentolamine. Clonidine, an α_2-agonist, inhibited the antibody response on day 5 of culture; this effect was blocked by phentolamine.

These findings recently have been extended with trinitrophenyl (TNP)-specific B cells serving in the dual role of antigen-presenting cell and antibody-secreting cell (93). A keyhole limpet hemocyanin (KLH)–specific Th_2 cell line was used as the source of cytokines necessary for B cell differentiation to antibody-secreting cells. The primary advantage of using this model system is that it overcomes the problem of a low frequency of antigen-specific T and B lymphocytes in whole-splenocyte model systems, which limits the extent to which mechanistic studies can be conducted. In addition, the interpretation of results obtained using this model system is less difficult, since it eliminates the participation of $CD8^+$ T cells and macrophages. More importantly, this model system is physiologically relevant since, in vivo, the primary mechanism of both T cell and B cell activation involves the presentation of antigen by B cells to Th cells (94, 95).

Using this system, terbutaline increased the number of IgM anti-TNP-secreting cells as well as the amount of anti-TNP antibodies secreted by these cells. Limiting dilution analysis determined that β-adrenoceptor stimulation increased the number of anti-TNP B cell precursors that differentiated into TNP-secreting B cells, but did not increase the clonal proliferation of those precursors already induced to differentiate. No changes in B cell MHC class II expression or IgG_1 anti-TNP antibodies were observed, suggesting that lymphokine secretion by the Th_2 cell line was not affected by β-adrenoceptor stimulation. These results suggest that exposure of a Th_2-dependent B cell culture system to a β_2-adrenergic agonist induces a larger proportion of B cells to become capable of producing antibody.

GENERATION OF CYTOTOXIC T LYMPHOCYTES The generation of CTLs in vitro was examined in the presence of β-adrenoceptor stimulation (10, 96). Several cell types are necessary to induce mature CTLs, including antigen-presenting cells and T helper cells. The latter produce IL-2, which induces differentiation of precursor CTLs to mature CTLs. ISO (10^{-7} M), EPI (10^{-6} M), NE (10^{-4} M), and terbutaline (10^{-5} M), added at the initiation of culture, enhanced the generation of CTLs, measured as lytic activity at the end of the 5-day culture period (96). *l*-Propranolol, but not *d*-propranolol or phentolamine, blocked the enhancement. Phentolamine in the presence of NE increased lytic activity to a greater degree than that seen with NE alone. These results again show that β-adrenoceptor stimulation can lead to overall enhancement of a response, but when participation of several cell types is required, a balance between α- and β-adrenoceptor-mediated effects can be unmasked pharmacologically.

ANTIGEN-INDUCED PROLIFERATION Heilig et al (97) demonstrated that antigen-specific proliferation by in vivo–primed lymph node cells was reduced in

vitro in the presence of the α-agonist phenylephrine, but not the β-agonist ISO. This inhibition was greatest at suboptimal doses of antigen, and was completely blocked by the α-blocker phentolamine. Since the extent of α-adrenoceptor expression on mouse T lymphocytes is not known, it is possible that phenylephrine acted on receptors expressed by the antigen-presenting cells.

Intracellular Signaling of Lymphocytes by Adrenoceptor-Mediated Mechanisms

The in vitro studies described above demonstrate that the outcome of adrenergic stimulation is dependent on several factors, such as the adrenoceptor subtype stimulated, the cell type(s) present, the immune stimulus, and the timing of adrenoceptor stimulation. In order to predict how adrenergic stimulation will alter lymphocyte activity, the intracellular pathways involved in adrenergic signaling need to be elucidated. Numerous groups have studied the effects of increased cAMP on lymphocyte proliferation with potent cAMP inducers, such as PGE_2, in addition to β-adrenergic agonists. For example, recent studies show that IL-4 secretion by Th_2 cells is less sensitive to inhibition by a cAMP elevation induced by cAMP inducers than is secretion of IL-2 and IFN-γ by Th_1 cells (98–100). Despite intense investigation, the mechanism by which cAMP inhibits proliferation is not well understood. It is beyond the scope of this review to discuss fully the work that has been done so far in assessing the role of cAMP in modulating lymphocyte proliferation and other lymphocyte functions. However, recent evidence obtained with β-adrenoceptor agonists suggests that factors other than generation of cAMP must be considered when assessing β-adrenoceptor stimulation of lymphocytes.

Evidence is accumulating that measurement of cAMP at a single time point may not be sufficient to predict effects on lymphocyte activity. As discussed above, B cell proliferation and differentiation were differentially altered by a β-adrenoceptor-stimulated rise in intracellular cAMP, depending on the B cell stimulus (72, 73). In T cells, a direct comparison of equimolar cAMP levels induced by ISO or PGE_2 after T cell stimulation with anti-CD3 showed quantitative differences in inhibition of lymphocyte proliferation (79). This difference between ISO and PGE_2 may be related to the length of time cAMP is elevated, the compartmentation or sequestration of cAMP (101), or the isozyme of cAMP-dependent protein kinase that is activated following generation of cAMP (102). These differences suggest a divergence in the intracellular pathway following initial stimulation with a potent cAMP stimulator, such as PGE_2, compared to the relatively short-lived signal generated by ISO.

In addition, catecholamines may act to modify immune stimulators, suggesting that crosstalk between intracellular pathways follows, for example, activation of the T cell receptor and the β-adrenoceptor. Carlson et al (78) examined the cAMP response following stimulation of human lymphocytes

with two T cell activators, phytohemagglutinin (PHA) and anti-CD3 antibodies. T cell activation with either of these two agents alone did not increase cAMP levels; however, cAMP production was synergistically increased with ISO and PHA or ISO and anti-CD3, compared with ISO alone. This synergistic increase in cAMP was blocked completely by propranolol. To determine if T cell receptor activation was contributing to this increase, two substances were tested that mimic intracellularly the events that occur following T cell receptor stimulation: (*a*) activation and translocation of protein kinase C (PKC); and (*b*) an increase in intracellular calcium. PMA, which directly activates PKC, inhibited ISO-induced cAMP production, whereas ISO and ionomycin, a calcium ionophore, increased cAMP in a manner similar to ISO and PHA. These results provide evidence for crosstalk between the two major signal transduction pathways, phosphatidylinositol turnover and adenylate cyclase–cAMP–protein kinase. Such interactions between intracellular signal transduction pathways must be better elucidated to understand how the effects of adrenoceptor stimulation may be dependent on a specific immune activator. In addition, antigen-presenting B cells interact with, and activate, Th cells by stimulating T cell–associated CD28 with the B cell–associated molecule B7. Interestingly, stimulation of CD28 on T cells with an anti-CD28 antibody appears to overcome the inhibitory effect induced by stimulation of the β_2-adrenoceptor on T cell activation (103), suggesting that the interaction of B7 with CD28 during T cell–B cell interaction may have a similar effect. Thus, the "mechanism of action" of a catecholamine on the response of an individual cell in an immune response critically depends on the state of the other signal transduction pathways at the time of ligand-receptor interactions.

Summary of In Vitro Studies of Adrenoceptor Stimulation

In vitro studies to date have shown that the effect of adrenoceptor stimulation on lymphocyte activity cannot be categorized as a simple inhibition or enhancement. Rather, several factors must be taken into account, including cell type(s) involved, subtype of adrenoceptor stimulated, the immune stimulus, and when during the response the agonist is present. These in vitro studies indicate that if a β-adrenoceptor agonist is present during the activation phase of the response, then enhancement may occur, depending on the immune stimulus. If β-adrenoceptor stimulation occurs late in a response, then inhibition of effector functions, such as antibody secretion or lytic activity, is possible. It is also apparent that catecholamines are most effective when cells are activated by antigen, mitogen, or cytokines. This implies a synergistic, regulatory, or modulatory role for catecholamines; they do not initiate or completely suppress a response on their own at any single step. An in vitro approach is useful in carefully dissecting a response to adrenoceptor stimulation, particularly on the level of an individual cell. However, in vivo studies of catechol-

amine effects on immune responses are necessary to demonstrate both the physiological relevance of these in vitro phenomena and the full range of these interactions in an intact animal.

IN VIVO STUDIES OF CATECHOLAMINE INTERACTIONS WITH THE IMMUNE SYSTEM

The study of SNS–immune system interactions in vivo adds a level of complexity that is not present in vitro. SNS modulation of immune reactivity may occur directly via interactions of NE with adrenoceptors on cells of the immune system, or indirectly, through interactions with other cell types, such as reticular cells, endothelial cells, or smooth muscle cells associated with the vasculature. These interactions may alter a wide variety of functions, such as antigen presentation, lymphocyte proliferation, differentiation, expression of specific receptors, lymphokine production, or cell trafficking. Investigators have used several in vivo approaches to determine how the SNS may modulate the immune system.

Sympathetic Denervation

One approach to studying functional effects of NA innervation is to destroy NA nerve terminals in lymphoid organs, either chemically or surgically, followed by assessment of immune reactivity in vivo or in vitro. In the periphery, 6-OHDA is a NA-specific neurotoxin that rapidly depletes NE from target tissues by destruction of NA nerve terminals. In spleen and lymph nodes, NE levels and NA nerve terminal numbers are reduced by greater than 90% after treatment with 6-OHDA (21–23). When 6-OHDA is administered systemically to adults, sympathectomy is limited to the periphery because 6-OHDA does not cross the blood-brain barrier. In adults, the NA nerve terminals, but not the cell bodies, are destroyed by 6-OHDA, and NA nerve terminals can regenerate over time (21). If 6-OHDA is administered to neonates, cell bodies are destroyed, resulting in long-term depletion of NA nerve fibers in the periphery. Unfortunately, the blood-brain barrier is not completely formed in neonates, allowing 6-OHDA access to the CNS following which central NA and dopaminergic pathways also are destroyed, altering CNS outflow pathways that regulate both hormonal production in the anterior and posterior pituitary, and sympathetic outflow from the thoraco-lumbar spinal cord. Another ablative approach is the use of local injections of 6-OHDA in adult rodents into the fat pads and other tissue surrounding lymph nodes, producing selective denervation of that organ (104).

In chemically sympathectomized animals, antibody and cell-mediated responses are altered, demonstrating SNS modulation of immune reactivity in vivo. Antibody responses to T-dependent antigens were reduced in adult ro-

dents treated with 6-OHDA prior to immunization (29, 105–107). However, animals that were sympathectomized neonatally, and adult animals in which the splenic nerve was cut surgically, exhibited enhanced antibody production (20, 108). Miles et al (109), using adult sympathectomy, reported enhanced antibody responses to a T-independent antigen, but no effect on a T-dependent antibody response. The enhanced antibody responses to antigen following neonatal or surgical sympathectomy suggest that the timing of sympathectomy relative to immunization may influence the effect of sympathectomy on immune reactivity. Surgical sympathectomy may also remove a host of neuropeptide-producing nerve fibers from lymphoid organs (reviewed in 7, 8, 111).

Recent work in D Bellinger's laboratory (DL Bellinger, DL Felten, KS Madden & SY Felten, manuscript in preparation) demonstrated that sympathectomy modestly enhanced antibody production to KLH in young Fischer 344 (F344) rats but markedly enhanced antibody production in old rats, suggesting that age may play a role in the functional consequences of chemical sympathectomy. Other recent results from our laboratories (B Kruszewska, DL Felten, SY Felten & JA Moynihan, manuscript in preparation) have demonstrated an increase in antigen-induced IL-2 and IL-4 production in vitro following sympathectomy in KLH-immune mice. This increase in cytokine production in vitro was accompanied by an increase in serum antibody production in C57BL/6 mice, a TH_1-predominant strain, but not in BALB/c mice, a TH_2-predominant strain, suggesting that sympathectomy cannot further potentiate antibody production in a strain already shifted towards TH_2 cytokine production. These results suggest that T helper subtype predominance may determine the functional outcome of sympathectomy.

A cell-mediated immune response, delayed type hypersensitivity (DTH), was reduced in adult mice whether chemical sympathectomy occurred prior to or following the initial sensitization (55). In these animals, in vitro generation of CTLs specific for the contact sensitizing agent was reduced following chemical sympathectomy, most likely the result of reduced IL-2 production necessary for maturation of the CTL response. Chemical sympathectomy also reduced CTL responses to alloantigen in vitro and in vivo (110). The reduced cell-mediated responses following sympathectomy suggest that the SNS can potentiate immune responsiveness.

Non-immune animals also exhibit altered lymphocyte function following chemical sympathectomy. In sympathectomized adult mice, in vivo cellular proliferation was enhanced in bone marrow, spleen, and peripheral lymph nodes (inguinal and axillary) (23). Con A–induced T cell proliferation in both spleen and lymph nodes was reduced (112). This reduction corresponded to decreased Thy-1^+, $CD4^+$, and $CD8^+$ T cell numbers in lymph nodes, but not in the spleen. Reduced IL-2 production and IFN-γ production may have resulted in decreased Con A–induced proliferation of spleen cells. In lymph

nodes, LPS-induced B cell proliferation and sIgM$^+$ B cell number were enhanced following sympathectomy, whereas LPS-induced proliferation was reduced in spleen. Sympathectomy dramatically enhanced LPS-induced polyclonal IgG production in vitro by lymph node cells, whereas IgM production was reduced. IFN-γ production by lymph node cells also was increased following sympathectomy, and may be a contributor to the isotype switching in these non-immune mice. The differential effects of sympathectomy in spleen and lymph nodes suggest that sympathectomy may influence lymphocyte proliferation and differentiation by different mechanisms in spleen and lymph node, and further imply that NE may have regional or microenvironment-specific effects on the immune system.

One possible explanation for differences in responsiveness between spleen and lymph node cells following sympathectomy may be related to altered lymphocyte migratory patterns. We found evidence for sympathectomy-induced changes in lymphocyte trafficking using ^{51}Cr-labeled lymphocytes (23). Lymphocytes from control mice injected into sympathectomized animals exhibited increased migration to lymph nodes, but not to spleen. In contrast, lymphocytes from sympathectomized animals exhibited reduced migration to lymph nodes of animals with intact sympathetic nerves. These results suggest that sympathectomy induced changes in the migratory ability of the lymphocyte as well as changes in the microenvironment of the lymph node itself. Catecholamine effects on the expression of adhesion molecules on lymphoid cells and endothelial cells should be examined.

Infusion of Catecholamines

Another in vivo approach is peripheral infusion of the catecholamines EPI and NE in immunized and non-immunized animals. In mice, the timing of EPI administration relative to immunization determined the effect on the subsequent immune response (113). EPI [4 μg, interperitoneally (i.p.)] injected 6 hr before primary or secondary challenge with SRBC changed the kinetics of the IgM and IgG plaque-forming cells (PFC) response in spleen, shifting the peak response to a day earlier compared with control mice. However, EPI administered 2–4 days before immunization inhibited the primary response at all time points examined. Adoptive transfer studies demonstrated direct effects of EPI on the antibody response. Spleen cells treated with EPI in vitro (10^{-5} M for 1 hr) enhanced the PFC response in normal recipients, whereas EPI-treated recipients of non-exposed control cells showed no effect compared with control recipients, suggesting that EPI acted directly on lymphocytes to alter antibody responses.

Felsner et al (114) examined the effects of the continuous presence of EPI and NE using sustained release pellets in rats. Twenty hours after implantation of the pellets, plasma NE and EPI levels were increased about 10-fold com-

pared with sham controls. At this time point, peripheral blood lymphocytes from animals infused with NE exhibited a 50% reduction in Con A–induced proliferation in vitro, while EPI had no effect. Concomitant β-blockade by administration of propranolol with either EPI or NE reduced T cell proliferation in vitro by 80–90%. This large decrease in T cell proliferation occurred in the absence of changes in $CD5^+$ (pan) T cell number, suggesting that α-adrenoceptor stimulation markedly inhibited the ability of T cells to proliferate to Con A. Phentolamine administration blocked the NE-induced reduction in peripheral blood T cell proliferation, providing further evidence for an α-adrenoceptor-mediated event. No changes in Con A–induced proliferation by spleen cells were observed following infusion of either NE or EPI, suggesting that circulating catecholamines may have limited access to lymphocytes in the white pulp of the spleen.

Changes in lymphocyte migration also have been demonstrated following infusions of catecholamines. In humans, a single EPI injection induced transient increases in the number of circulating blood lymphocytes and monocytes, and decreased proliferative responsiveness to T cell mitogens (115, 116). These functional alterations were accompanied by reduced $T4^+$ cells ($CD4^+$, T helper cells) and increased HNK-1^+ cells (natural killer/killer cells), indicative of cellular redistribution (117). In guinea pig spleen, increased lymphocyte and granulocyte release was observed 5 minutes after intracardiac injection of either NE or the β-adrenergic agonist ISO (118). The enhancement induced by NE was blocked by pretreatment with phentolamine, and the effect of ISO was blocked by propranolol, suggesting both an α- and β-mediated component to this cellular migration. Blood flow was not altered by this treatment, suggesting that the primary effect was not on vascular smooth muscle. Ernström & Söder (119) also demonstrated that release of PFCs from guinea pig spleen after secondary immunization with SRBC was enhanced and sustained after intracardiac administration of EPI. Again, no change in splenic blood flow or smooth muscle contraction could be demonstrated, suggesting that a direct interaction of EPI with lymphocytes was necessary for enhanced release of antibody-producing cells. Lymph flow also is altered by sympathetic stimulation. Electrical stimulation of the lumbar sympathetic chain in sheep increased lymphocyte output from popliteal lymph nodes (120); increased lymph flow and lymphocyte counts accounted for this increased output. Thus, changes in immune reactivity following administration of catecholamines in vivo may reflect changes in lymphocyte redistribution and trafficking, as well as changes in lymphocyte responsiveness to various stimuli.

Catecholamines in Aging and in Pathological Conditions

AGING In numerous species, including humans, mice, and rats, aging is associated with significant decrements in cell-mediated immune responses, char-

acterized by reductions in such in vitro measures as Con A–induced proliferation, IL-2 production, and generation of CTL (121). These decrements presumably contribute to the increased incidence of certain tumors, infectious disease, and autoimmune diseases in an aged population (122). Changes in NA innervation may play a role in these age-associated deficits in immune function (123–125). The immune system also may contribute to changes in NA innervation of lymphoid organs associated with aging.

Fluorescent histochemistry and immunocytochemistry have demonstrated distinct age-associated changes in NA innervation of thymus, spleen, and lymph nodes of rats and mice (24, 124). In the thymus, the density of NA innervation increases after 9 months of age, and shows an inverse relationship with thymic involution (16). The same number of NA sympathetic nerve terminals persists, but in a markedly reduced volume compared with young animals prior to involution. This extraordinary increase in density of NA fibers and NE concentration (not total content) may contribute to the diminished capacity of the thymus to generate naive T lymphocytes.

In marked contrast, the NA sympathetic innervation of secondary lymphoid organs (spleen and lymph nodes) is greatly diminished by 18–27 months of age (123, 126). In Fischer 344 rats, the spleen has lost more than 80% of its NA nerve terminals by 27 months of age. NE levels also are reduced, although the magnitude (approximately 50%) is not as great as the reduction in NA nerve terminals, suggesting a compensatory increase in NE synthesis and release. β-Adrenoceptor expression on spleen cells is increased in aged animals, presumably in response to reduced NE levels. However, some reports suggest that these up-regulated β-adrenoceptors of aged lymphocytes are deficient in their transduction capacity to generate cAMP (reviewed in 24). A reduction in lymphocytes in the PALS and macrophages in the marginal zone begins around 9 months of age, and NA innervation remains intact as these compartments shrink. Preliminary studies suggest that the reduction in NA innervation is related to a retraction or destruction of nerve terminals, possibly by damage from free radicals generated by the oxidative metabolism of NE. In severe combined immunodeficiency (SCID) mice, a very high density of innervation was observed in the small splenic white pulps that were devoid of T and B lymphocytes (127); therefore, it is unlikely that NA nerves leave the spleen and lymph nodes because of changes in lymphocyte cellularity (128). Drug-induced diminution of lymphocytes in the spleen does not alter the NE content or NA innervation of this organ (128).

Sympathectomy-induced changes in immune reactivity in young adult animals mimic age-related changes in immune reactivity from secondary lymphoid organs showing age-related loss of NA nerves. In particular, denervated young animals and aged rodents with loss of NA fibers both show reduced cell-mediated immunity. Preliminary evidence from our laboratory indicates

that in young and old Fischer 344 rats, spleen cell Con A–induced proliferation and IL-2 production (already lower in old rats than in young rats) is reduced following sympathectomy (129). The sympathetic NA innervation gradually recovers spontaneously in both age groups, but only back towards its own age-related baseline. This neurochemical recovery is accompanied by a recovery of Con A–induced proliferation and IL-2 production, but only back to the age-related baselines. It is tempting, therefore, to hypothesize a causal relationship between reduced NA innervation and reduced cell-mediated immune responses in aging.

AUTOIMMUNE DISEASE In several animal models of autoimmune disease, experimental evidence is emerging that the SNS is an important regulator of immune reactivity to self-antigens. Sympathectomy with 6-OHDA enhanced the severity of autoimmune disease in two animal models, experimental allergic encephalomyelitis (EAE) and experimental rheumatoid arthritis, both induced in Lewis/N rats (104, 130). In the EAE model, sympathectomy was achieved with neonatal administration of 6-OHDA systemically. Induction of EAE with myelin basic protein and complete Freund's adjuvant in sympathectomized Lewis/N rats resulted in more severe pathology and inflammatory lesions in brain (130); treatment of Lewis/N rats with ISO, a β-agonist, protected them from EAE pathology and lesions (131). In the adjuvant-induced arthritis model, selective denervation with 6-OHDA of the reactive secondary lymphoid organs, the popliteal and inguinal lymph nodes, was achieved with local injection into the fat pads surrounding these lymph nodes (104). This denervation resulted in earlier onset and enhanced severity of inflammation and bone erosions compared with nondenervated rats, and suggests that the effects of ablation of NA nerves are exerted via influence on the reactive lymph nodes themselves; any effects of NE on the target organ (the joints) are additional and are exerted on the nerves and their receptors on target cells in the joint capsule. Levine et al (132) showed that systemic β-adrenoceptor blockade either before or after the onset of disease symptoms attenuated clinical symptoms in a rheumatoid arthritis animal model, suggesting that both the lymphoid organs and target tissue of autoimmune reactivity may be affected by catecholamines. It is likely that the major effects of β-adrenoceptor blockade, administered after the onset of rheumatoid arthritis, occurred at the level of NA innervation of the joints, and acted to reduce inflammation in the joints. Substance P nerves in the lymph nodes and joints also may exert important functional effects on the expression and severity of induced arthritis (for review, see 104, 123).

In the MRL lpr/lpr mouse, an animal model of systemic lupus erythematosus, a significant reduction in NA sympathetic innervation of secondary lymphoid organs occurs just prior to the onset of autoimmune symptoms (133).

This is consistent with findings of diminished NA innervation in other autoimmune mouse models, such as the NZB and the NZB×NZW F1 mouse (124). Together, the evidence points to a tonic inhibitory role for the SNS in certain autoimmune states; a more specific role for the SNS in induction of disease and reduction of autoimmune pathologies, once the disease has been established, remains to be elucidated.

Peripheral SNS-Immune Interactions Activated via the CNS

CNS ACTIVATION VIA INTRACEREBROVENTRICULAR INJECTIONS Pharmacological agents injected directly into the ventricles of the brain have been used to study the role of sympathetic outflow from the CNS in modulation of peripheral immune responses. For example, corticotropin-releasing factor (CRF) injected into rat brain lateral ventricles reduced splenic NK cell activity (134, 135). This administration of CRF activated both the hypothalamic-pituitary-adrenal axis and sympathetic pathways, since both plasma NE and corticosterone were elevated. Treatment with a peripheral ganglionic blocker, chlorisondamine, prior to intracerebroventricular administration of CRF, prevented both the increase in plasma NE and the reduction in natural killer cell activity; at the same time, adrenocorticotropin (ACTH) and corticosteroid levels remained elevated. Others have demonstrated that intracerebroventricular infusions of IL-1 suppressed macrophage IL-1 production, NK cell activity, and lymphocyte proliferation in the periphery (136, 137). These effects were completely or partially blocked by splenic nerve sectioning or administration of chlorisondamine, respectively.

CNS ACTIVATION VIA STRESSORS Several groups have reported that stress-induced changes in immune function were not correlated with changes in corticosterone, or occurred in adrenalectomized animals (138–140). Other pathways, including the autonomic nervous system, may be important in mediating stress-induced changes in immune function. For example, pretreatment with propranolol or nadolol, both nonselective β-blockers, prevented suppression of splenic T cell proliferation and IFN-γ production induced by foot-shock stress (141, 142). Nadolol does not cross the blood-brain barrier, demonstrating that peripheral β-adrenoceptors were involved in the response to the stressor. Restraint stress–induced suppression of immunity to herpes simplex virus was partially blocked by the glucocorticoid antagonist RU 486, but completely prevented by RU 486 and nadolol, demonstrating a role for both glucocorticoids and catecholamines in this system (143). Carr et al (144) showed that cold stress–induced enhancement of the secondary antibody response to SRBC was blocked by treatment with phentolamine, an α-blocker, and was potentiated by treatment with propranolol, a β-blocker. In addition to contrib-

uting to our understanding of SNS-immune system interactions, these results constitute further evidence for communication from the CNS to the immune system via the sympathetic nervous system.

IMMUNE SYSTEM COMMUNICATION WITH THE NERVOUS SYSTEM

There is considerable evidence suggesting that immune system signaling and activation is communicated to the CNS. Several groups have demonstrated changes in sympathetic activity following immunization or administration of immune cytokines. Besedovsky and colleagues observed that NE levels in spleen were inversely proportional to immunological activity, following initiation of a primary antibody response (57, 145), and in germ-free animals compared with animals raised in a specific pathogen-free environment (57). High antibody producers exhibited greater and more prolonged reductions in splenic NE levels, whereas NE levels of low responders were reduced transiently, and to a lesser degree (58). Fuchs and colleagues found an increase in the amount of the dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in the spleen after antigen exposure (29), which correlated with a decreased density of β-adrenoceptors on splenic lymphocytes (41). More recently, intravenous injection of recombinant IL-1 in rats was reported to increase firing rates of sympathetic nerves innervating the adrenal gland and the spleen (146). The NE turnover rate was reported to increase following i.p. injection of IL-1 in spleen and lung, but not in heart and other tissues in the rat (147). These results also suggest that the decreased concentration of splenic NE noted in the reports cited above resulted from increased turnover of NE, not reduced NA nerve activity.

Modulation of sympathetic activity by the immune system may be achieved by two routes: (*a*) through control of hypothalamic or other CNS centers regulating autonomic outflow, or (*b*) at a local level, through reciprocal interactions between the cells of the immune system and nerve terminals within lymphoid organs or their ganglionic cell bodies. Evidence for the first mode of regulation is found in the changes in firing rates and NE alterations (decreased levels, increased turnover) in discrete regions of the hypothalamus following antigenic challenge or peripheral administration of immune system–derived cytokines, such as IL-1 (148–150). An IL-1-induced increase in splenic NE release was shown to be related to activation of CRF-containing neurons in the CNS (25). The following indirect evidence exists for the local mode of regulation: (*a*) the close association between nerve terminals and lymphocytes, demonstrated by electron microscopy in the rat spleen (9); (*b*) the IL-1 enhancement of NE turnover in spleen (146, 147); and (*c*) the findings that two macrophage-derived cytokines influenced sympathetic nerve cells in vitro. In

one report, unstimulated and Con A–stimulated spleen cells were added to cultures of superior cervical ganglion (SCG) neurons (151). Unstimulated cells reduced SCG tyrosine hydroxylase (TH) mRNA and enhanced mRNA for neuropeptide Y (NPY), a neuropeptide colocalized with NE in spleen and lymph nodes (152), and preprotachykinin A mRNA (the mRNA for substance P). Con A–stimulated cells induced a further decrease in TH mRNA, but inhibited NPY mRNA. In another study, TNF reduced ^{3}H-NE release from superior cervical ganglia neurons depolarized with excess K^+, 6 min after the initial K^+-induced depolarization (153). In this system, neither TNF nor IL-1 altered baseline spontaneous release or initial ^{3}H-NE release, but exerted an effect at a later stage of ongoing release. These results suggest that secretory products of the immune system may influence the physiological activities of the sympathetic nervous system in vivo. Local regulation of sympathetic activity would allow rapid responsiveness to activation or perturbations of the immune system. In contrast, communication through the CNS would permit longer-term control and systemic coordination of the neuroendocrine, autonomic, and immune systems.

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