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Norepinephrine and β2-Adrenergic Receptor Stimulation Regulate CD4$^+$ T and B Lymphocyte Function in Vitro and in Vivo

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***Abstract*——Historically, norepinephrine and the sympathetic nervous system have been associated with the "fight or flight" response, and they also contribute to the regulation of autonomic activity within the body, such as cardiovascular function. In addition, evidence over the past 30 years suggests that norepinephrine may also regulate the function of immune cells that protect the body against pathogens. The presence of sympathetic nerve fibers and the release of norepinephrine within lymphoid organs represent a mechanism by which signals from the central nervous system may influence immune cell function. The T cell-dependent antibody response is essential to successful host defense against numerous environmental pathogens. It is during this response that CD4+ T and B lymphocytes are activated to produce cytokines and antibody, respectively, leading to immune competence and protection. The goal of this review is to discuss the evidence supporting the release of norepinephrine within lymphoid organs and the expression of the β2-adrenergic receptor by CD4+ T and B lymphocytes. We also discuss the mechanisms by which β2-adrenergic receptor stimulation affects the level of cytokine and antibody produced by these cells both in vitro and in vivo. In cases where conflicting findings have been reported, we discuss potential variables that may have contributed to these conflicting findings. To conclude, we discuss the disease- and health-specific implications of the basic research being done in the area of sympathetic nervous system regulation of T and B lymphocyte function.**

I. Background

A. Adaptive/Acquired Immunity

The basic function of the immune system is to clear "nonself" or "foreign" antigens such as bacteria and viruses from the body. The immune system is comprised of two general systems, the innate and the adaptive immune systems. Typically, the innate immune system is considered to be the "first line of defense," and its cells are the first to nonspecifically clear antigen from the body. Unlike the innate immune system, the adaptive immune system is characterized by two distinct features: specificity and memory. The specificity of adaptive immunity originates from the development of a diverse repertoire of T and B lymphocyte receptors that recognize a specific peptide sequence or "antigenic epitope". Therefore, since cells of the adaptive immune system possess the capacity to recognize and respond to minute amounts of antigen, it is essential that immune cell function be carefully regulated to prevent responses to "self" peptide antigens, while at the same time permitting the effective clearance of foreign antigens from the body.

The T cell-dependent antibody response is a critical component of adaptive immunity and serves as both a sentinel and a defender against bacterial and viral infections. In addition, the potential exists for the T cell-dependent antibody response to contribute to the development of autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus (reviewed in Boitard, 1992; Goodnow, 1997). In light of this potential for antibody production to both protect and damage the body, the immune system has developed a number of autoregulatory mechanisms to augment the antigen-specific response directed against a foreign antigen and, at the same time, to prevent responses directed against autoantigens. These regulatory mechanisms govern both B cell and T cell activation, as well as effector function during the T cell-dependent antibody response.

The two-signal hypothesis of B cell activation, as first described by Bretscher and Cohn (1970), represents one of these autoregulatory mechanisms (Fig. 1A). They proposed that B cell activation requires two signals, with signal 1 originating from stimulation of the antigen-specific B cell receptor (BCR) by a foreign antigen. Upon stimulation of the BCR, the B cell begins to prepare itself to produce antibody. However, without receiving another signal originating from the CD4+ T-helper (Th) cell, the B cell will not differentiate into either an antibody-secreting plasma cell or a memory B cell. This second signal from the Th cell was originally proposed to be in the form of cytokines. Thus, during a T cell-dependent antibody response, the B cell will differentiate into either an antibody-secreting plasma cell or a memory

[2] Abbreviations: BCR, B cell receptor; αAR, α-adrenergic receptor; Ab, antibody; Ag, antigen; ASC, antibody-secreting cells; βAR, beta-adrenergic receptor; βARK, β-adrenergic receptor kinase; BBB, blood-brain barrier; CNS, central nervous system; Con A, concanavalin A; CRF, corticotropin-releasing factor; CT, cholera toxin; db, dibutyryl; DNP, dinitrophenyl; DOPAC, 3,4-dihyroxyphenylacetic acid; Feno, fenoterol; GRK, G-protein receptor kinase; IBMX, 3-isobutyl-1-methylxanthine; IFN, interferon; Ig, immunoglobulin; IL, interleukin; Iso, isoproterenol; KLH, keyhole limpet hemocyanin; LPS, lipopolysaccharide; NE, norepinephrine; 6-OHDA, 6-hydroxydopamine; OVA, ovalbumin; PGE, prostaglandin E; PHA, phytohemagglutinin; PKA, protein kinase A; PKC, protein kinase C; PMA, phorbol ester; Salb, salbutamol; *scid*, severe combined immunodeficient; sRBC, sheep red blood cell; TCR, T cell receptor; Terb, terbutaline; Th, T-helper; TNF, tumor necrosis factor; TNP, trinitrophenyl.

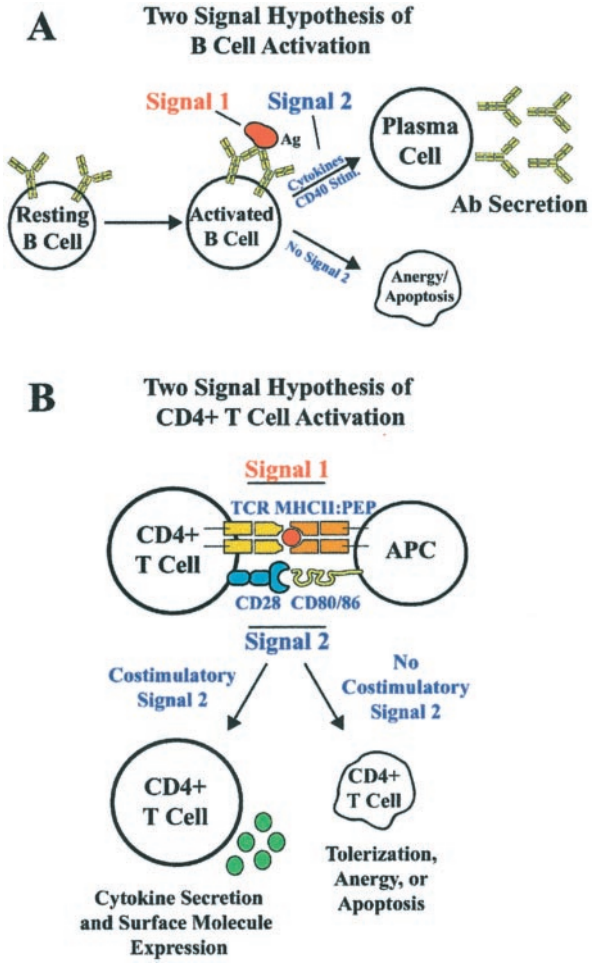

FIG. 1. Two-signal hypotheses of B and T cell activation. A, two-signal hypothesis of B cell activation (Bretscher and Cohn, 1970). Signal 1 to the B cell occurs upon stimulation of the B cell receptor (BCR) by antigen (Ag). Following signal 1, the B cell prepares to produce antibody (Ab) and awaits signal 2, which is delivered in the form of cytokine receptor stimulation. Only upon delivery of signal 2 will the B cell differentiate into either a plasma cell or a memory B cell. Without this costimulatory signal, the B cell will undergo apoptosis or anergy. B, two-signal hypothesis of $CD4^+$ T cell activation (Lafferty and Cunningham, 1975). A resting $CD4^+$ T cell receives the first activation signal following stimulation of the T cell receptor (TCR) by the MHC class II-antigen (Ag) peptide complex expressed by a professional antigen-presenting cell (APC). If the T cell does not receive signal 2, a costimulatory signal, the T cell is either anergized or induced to undergo apoptosis. However, if the T cell does receive a costimulatory signal, the T cell is activated to produce cytokines.

cell following both BCR stimulation and $CD4^+$ T cell cytokine production.

Similar to the process of B cell activation, an antigen-specific Th cell also requires two distinct signals to become activated to provide "help" to a B cell (Fig. 1B). As first proposed by Lafferty and Cunningham (1975), the first of these signals is generated by the recognition of the peptide antigen by the antigen-specific TCR expressed on the Th cell surface, which is now known to be presented by the B cell or another antigen-presenting cell in the context of MHC class II (the peptide-MHCII complex). In addition, if the Th cell receives costimulatory signals from the B cell, then the Th cell becomes fully activated to produce cytokines that provide the second signal, or "help", required by the B cell to differentiate into either an antibody-secreting plasma cell or a memory B cell. However, if the Th cell does not receive the additional costimulatory signals required for cell activation, such as a B7:CD28 interaction, the cell is either anergized or induced to undergo apoptosis (Schwartz, 1990). Thus, the antigen-specific physical interaction between the $CD4^+$ Th cell and the B cell represents a potent regulator of the Th cell-dependent antibody response (Sanders et al., 1986, 1988), which includes antibody secretion from plasma cells, affinity maturation of the BCR, antibody isotype switching, and memory B cell formation (Liu and Banchereau, 1997).

B. Bidirectional Communication Between the Nervous and Immune Systems

In addition to regulatory mechanisms that are provided by immune cells, it is now known that complex bidirectional interactions (Fig. 2) between the cells of the immune system and the nervous system contribute to additional regulatory mechanisms that influence the function of cellular activities associated with both systems (reviewed in Sanders and Munson, 1985a; Ader et al., 1990; Madden and Felten, 1995; Straub et al., 1998; Kohm and Sanders, 2000).

One mechanism by which signals from the immune system may regulate nervous system activity is via the stimulation of cytokine receptors expressed both on cells

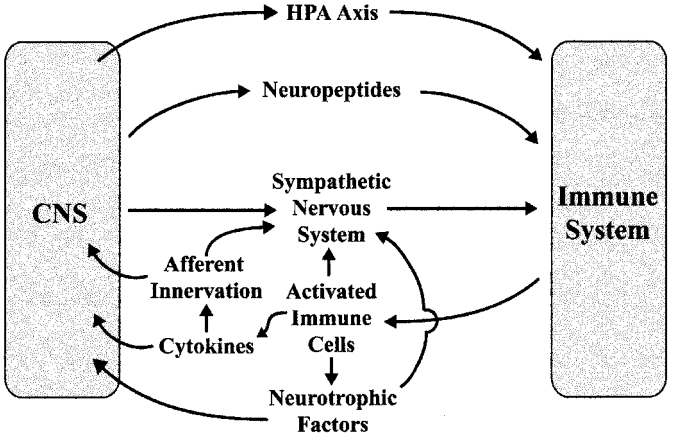

FIG. 2. Pathways of communication between the central nervous and immune systems. The presence of sympathetic nerve fibers in lymphoid organs and the release of norepinephrine from nerve terminals located in the direct vicinity of immune cells provide a mechanism by which norepinephrine might influence immune cell function. Upon release, norepinephrine binds to the β2-adrenergic receptor expressed on the surface of a variety of immune cells to influence their activity. The activity of sympathetic nerves originating in the central nervous system (CNS) may be influenced by products of activated immune cells because circulating cytokines and cells are actively transported into the CNS to influence their activity centrally and, also, by stimulation of cytokine receptors expressed on peripheral nerves to influence their activity peripherally. More importantly, a small number of lymphocytes actively patrol the normal CNS, but upon activation, increased numbers of lymphocytes enter the CNS and produce both cytokines and antibodies that can either protect against or contribute to a number of CNS pathologies. Finally, hormone production resulting from activation of the hypothalamic-pituitary-adrenal (HPA) axis may also influence a variety of systemic immune cell activities.

within the CNS (reviewed in Rothwell et al., 1996) and on peripheral sympathetic nerves and ganglia (Hart et al., 1993; Gadient and Otten, 1996; Marz et al., 1996; Cunningham et al., 1997). Cytokine receptor stimulation on peripheral nerves and ganglia alters the level of CNS activity possibly by afferent signal transduction. But more importantly, alterations in the level of CNS activity may alter the level of efferent nerve activity and neurotransmitter release in the periphery. Thus, peripheral cytokine production may influence efferent nerve activity and neurotransmitter release by binding to cytokine receptors expressed on peripheral nerves. In addition, cytokines produced in the periphery may cross the blood-brain barrier (BBB) via a number of specific cytokine transporter systems within the BBB to directly affect targets within the CNS (Banks and Kastin, 1987, 1991; Banks et al., 1991; Gutierrez et al., 1993; Plotkin et al., 1996). Additionally, activated immune cells may pass through the BBB to release cytokines directly into the CNS (reviewed in Weller et al., 1996), thus bypassing the need for afferent signaling pathways from the periphery. The integrity of the BBB can be disrupted under certain pathological conditions, such as viral infection of the CNS, allowing immune cells and other blood-borne mediators to enter the CNS (reviewed in Persidsky, 1999). Therefore, several mechanisms exist for the immune system to communicate with the CNS. However, in order for the CNS to influence an immune response, reciprocal pathways of communication from the CNS to the immune system must also exist.

The sympathetic nervous system is typically associated with the physiological "fight or flight" response, such that it is involved in the regulation of cardiovascular and respiratory function, especially during times of critical need. In addition, the sympathetic nervous system regulates gastrointestinal tract smooth muscle contraction/relaxation, gastric secretions, and other autonomic functions. Sympathetic neurotransmission from the CNS to the periphery occurs via projections extending from the paraventricular nucleus of the hypothalamus, rostral ventrolateral medulla, ventromedial medulla, and caudal raphe nucleus to preganglionic neurons of the spinal cord (Sawchenko and Swanson, 1982). The preganglionic cell bodies of sympathetic nerves reside in the intermediolateral cell column of the lateral horn of the spinal cord at T1-L2. These cell bodies send myelinated projections that exit the spinal cord via the ventral roots to synapse primarily on the superior mesenteric ganglia. From these ganglia, a second projection follows the vasculature to innervate target organs. Within the target organ, sympathetic nerves form terminals from which the sympathetic neurotransmitter norepinephrine (NE) is released to bind to adrenergic receptors expressed by various cell populations.

Most studies of sympathetic innervation of lymphoid organs incorporated immunohistological techniques in which the rate-limiting enzyme of norepinephrine synthesis, tyrosine hydroxylase, was detected. These studies demonstrated a rich sympathetic innervation of all primary (thymus and bone marrow) and secondary (spleen and lymph nodes) lymphoid organs (Calvo, 1968; Reilly et al., 1979; Williams and Felten, 1981; van Oosterhout and Nijkamp, 1984; Felten et al., 1988a,b). Additionally, these studies reported the presence of sympathetic innervation in both the splenic capsule and trabeculae, but more importantly, in the immune cell compartment of the spleen (the white pulp), especially the T cell-rich periarteriolar lymphoid sheath, the B cell-rich marginal zone, and marginal sinus areas (Felten et al., 1985, 1987a,b; Livnat et al., 1985; Ackerman et al., 1987; Felten and Olschowka, 1987). Whereas innervation is prominent in the white pulp, little innervation is present in the red pulp and represents less than 1% of the total splenic innervation. Electron microscopic studies of the white pulp reveal that sympathetic nerve terminals are in direct apposition to T cells and adjacent to both interdigitating dendritic cells and B cells (Felten et al., 1987a,b), with the neuro-immune junction being approximately 6 nm wide (Felten and Olschowka, 1987), in contrast to a typical CNS synapse that is approximately 20 nm wide. The close proximity of sympathetic nerve terminals to immune cells provides a mechanism not only for specific targeting of norepinephrine release to immune cells, but also for the containment of neurotransmitter release, possibly to permit differential modulation of only resident immune cells, depending on the specific immune response being evoked.

Finally, GAP-43 (a marker for an activated neuron)-positive sympathetic fibers enter the outer periarteriolar lymphoid sheath, marginal zone, and marginal sinus within the spleen following immunization, suggesting that not only can the immune response influence sympathetic outflow, but also that immune cell-derived neurotrophic factors may direct innervating fibers to the site of the response (Yang et al., 1998; Besser and Wank, 1999) to release norepinephrine to bind to β-adrenergic receptors (βARs) expressed on immune cell populations. Thus, a complete "circuit" appears to exist between the immune system and the CNS, such that the initiation of an immune response in the periphery signals the CNS, resulting in subsequent regulation of the immune response via activation of the sympathetic nervous system.

In summary, whereas behavioral conditioning studies provided the initial suggestion that an interaction between the CNS and immune system existed (Ader and Cohen, 1975; Rogers et al., 1976; Wayner et al., 1978; Cohen et al., 1979; Exton et al., 1998), research findings over the past 20 to 30 years have documented a number of complex bidirectional interactions between the nervous system and the immune system that appear to be necessary for the maintenance of homeostasis in both systems, as well as for the regulation of immune re-

sponses during the development and progression of immune-related disease states.

C. Norepinephrine and the β2-Adrenergic Receptor

The catecholamine norepinephrine is released from both postganglionic sympathetic nerve terminals found innervating all internal organs and from chromaffin cells residing in the adrenal medulla. Norepinephrine is the principal neurotransmitter of the sympathetic nervous system and is released into the periphery upon activation of the sympathetic nervous system. Norepinephrine is produced via multiple enzymatic alterations of tyrosine, of which the hydroxylation of tyrosine by tyrosine hydroxylase is the rate-limiting step (Zigmond et al., 1989). This enzymatic cascade is initiated upon the activation of sympathetic postganglionic nerve fibers. The final step in norepinephrine synthesis occurs within the nerve terminal storage vesicles and is mediated by the membrane-bound dopamine β-hydroxylase. Various fates await norepinephrine upon release from the nerve terminal, such as metabolization into normetanephrine by catechol-*O*-methyltransferase, reuptake back into the nerve terminal, diffusion, or receptor binding to influence target cell function.

The β-adrenergic family of receptors (βARs) binds norepinephrine and contains three subtypes: the β1AR, the β2AR, and the β3AR (reviewed in Bylund et al., 1994). The βAR is a seven-transmembrane receptor that classically leads to heterotrimeric guanine nucleotide-binding protein (G-protein) activation upon stimulation. Historically, the signaling capacity of the βAR has been attributed to the association of the cytoplasmic tail of the receptor with stimulatory G-proteins, in which stimulation of the receptor results in adenylyl cyclase activation, increased intracellular accumulation of adenosine 3′,5′-cyclic monophosphate (cAMP), and increased protein kinase A (PKA) activity (reviewed in Kobilka, 1992; Meinkoth et al., 1993). Upon activation, PKA regulates the activity of multiple targets via phosphorylation, including various transcription factors, such as NF-κB. Although stimulation of each of the three βAR-subtypes results in adenylyl cyclase activation, the β2AR appears to be more efficiently coupled to adenylate cyclase than is the β1AR or β3AR (reviewed in Strosberg, 1997).

However, over the last 5 to 10 years, a number of other signaling pathways have been reported to be activated following β2AR stimulation. One such pathway that is also relevant to lymphocyte function is the activation of protein kinase C (PKC). β2AR stimulation induces PKC activity (Kelleher et al., 1984), which may then mediate a number of intracellular events, including down-regulation of β2AR surface expression (Kelleher et al., 1984), positive or negative effects on adenylyl cyclase activity (reviewed in Houslay, 1991), and activation of Bruton's tyrosine kinase (reviewed in Mohamed et al., 1999) which ultimately activates the mitogen-activated protein kinase (MAPK) pathway. These same β2AR-induced signaling pathways are also involved in BCR signaling and, therefore, represent a mechanism by which β2AR stimulation may influence intracellular events in B cells following antigen recognition. Similarly, β2AR and BCR stimulation both result in Src kinase activation, which may induce down-regulation of β2AR expression (Daaka et al., 1997; Cornall et al., 1998; Lankar et al., 1998) and Ras activation (Daaka et al., 1997), as well as a number of additional intracellular events associated with BCR stimulation. Thus, as will be discussed later, due to the existence of overlapping intracellular signaling pathways associated with stimulation of the β2AR and the BCR, it is not surprising that stimulation of the β2AR by either an agonist or norepinephrine may influence B cell function.

II. Evidence and Mechanisms for the Release of Norepinephrine in Lymphoid Organs

As previously discussed, lymphoid organs are heavily innervated by sympathetic nerve fibers. However, in order for norepinephrine to influence immune cell function, it must be released at the immediate site of action, since it is either rapidly degraded by catechol-*O*-methyltransferase and monoamine oxidase, diffused into the circulation, or taken back up into the nerve terminal following release (reviewed in Glowinski and Baldessarini, 1966). Therefore, if norepinephrine is to influence immune cell function in response to antigen, it may be critical that mechanisms exist for enhancing the normally low basal level of norepinephrine released within the microenvironment in which immune cells reside (findings summarized in Table 1).

Splenic norepinephrine is derived from local sympathetic nerve terminals, as opposed to circulating catecholamine (Williams et al., 1981; Shimizu et al., 1994), and the electrical stimulation of the postganglionic splenic nerve results in norepinephrine release within the spleen (Lundberg et al., 1989). The rate of norepinephrine release from sympathetic nerve terminals is regulated by both positive and negative feedback mechanisms. For example, the release of norepinephrine from sympathetic nerve terminals is inhibited via stimulation of the α_2-adrenergic receptors (α2AR) expressed on the presynaptic nerve terminal itself but is enhanced by stimulation of the presynaptic β2AR (Elenkov and Vizi, 1991; Hasko et al., 1995; Vizi et al., 1995). In addition, although there are conflicting reports concerning the level of sympathetic nerve activity and the release of norepinephrine within the spleen during an immune response, there is increasing evidence that immune-derived factors may also influence the rate of norepinephrine release within lymphoid organs.

TABLE 1
The effects of immune cell activation and cytokines on sympathetic nerve activity and norepinephrine release within lymphoid organs

Stimulus	Effect	References
Endotoxin	↑[a] circulating NE	Heiffer et al., 1960; Rosenberg et al., 1961; Spink et al., 1966; Devereux et al., 1977; Feuerstein et al., 1981
	↓ lymphoid organ NE tissue content	Zetterstrom et al., 1964; Pohorecky et al., 1972; Pardini et al., 1982
LPS	↑ splenic NE turnover	Pardini et al., 1983; MacNeil et al., 1996, 1997
Pseudomonas aeruginosa infection	↑ bone marrow NE turnover	Tang et al., 1999
sRBC	↓ lymphoid organ NE tissue content	Besedovsky et al., 1979; Del Rey et al., 1981
	n.c. lymphoid organ NE tissue content	Fuchs et al., 1988; Delrue-Perollet et al., 1995
	↑ splenic DOPAC	Fuchs et al., 1988
Soluble protein antigen	↑ NE turnover in the spleen and bone marrow	Kohm et al., 2000
IL-1β	↑ NE release in the spleen	Akiyoshi et al., 1990; Ichijo et al., 1992; Shimizu et al., 1994
	↑ sympathetic nerve activity in the spleen	Niijima et al., 1991; Takahashi et al., 1992
	↓ sympathetic nerve activity in the spleen	Bognar et al., 1994
IL-6	[high] ↓ NE release [medium] n.c. NE release [low] ↑ NE release	Ruhl et al., 1994
IL-2	↓ splenic NE release	Bognar et al., 1994
TNF-α	↓ splenic NE release	Foucart and Abadie, 1996; Abadie et al., 1997

[a] Abbreviations: ↑, increase; ↓, decrease; [ ], concentration; DOPAC, 3,4-dihydroxyphenylacetic acid; IL, interleukin; LPS, lipopolysaccharide; n.c., no change; NE, norepinephrine; TNF, tumor necrosis factor.

A. Lipopolysaccharide- and Antigen-Induced Norepinephrine Release

1. Infection/Endotoxin. For many years, it has been known that systemic infection induces sympathetic nervous system activity via the endotoxin released from bacterial cell walls. Early studies reported alterations in the level of sympathetic nerve activity both in times of infection and shock by measuring circulating levels of norepinephrine and epinephrine as an indirect indicator of systemic sympathetic nerve activity (Heiffer et al., 1960; Rosenberg et al., 1961; Spink et al., 1966; Devereux et al., 1977; Feuerstein et al., 1981). In all of these studies, endotoxin exposure increased the levels of circulating norepinephrine, suggesting enhanced sympathetic nerve activity and norepinephrine release. In other studies, the total tissue content of norepinephrine was determined as a measure of sympathetic nerve activity following infection with *Escherichia coli* or injection of *E. coli*-derived endotoxin. Immunization of animals with endotoxin resulted in a significant decrease in the total tissue content of norepinephrine in the spleen (Zetterstrom et al., 1964; Pohorecky et al., 1972), possibly via altering norepinephrine reuptake mechanisms (Pardini et al., 1982). Such observations were interpreted in several ways, i.e., the decreased splenic norepinephrine levels may have been due to decreased norepinephrine production, increased norepinephrine release, increased norepinephrine diffusion/metabolism, and/or decreased reuptake of norepinephrine back into the nerve terminal.

Later studies addressed one of the aforementioned interpretations by performing experiments to determine whether the endotoxin-induced decrease in the level of splenic norepinephrine was due to alterations in the reuptake mechanisms for norepinephrine (Pardini et al., 1982). The uptake of [^{3}H]norepinephrine into splenic nerve terminals of endotoxin-injected animals was significantly lower than the rate of norepinephrine uptake in saline-injected control animals. Interestingly, endotoxin administration did not alter the activity of norepinephrine reuptake mechanisms in the heart, suggesting a lymphoid organ-specific effect of endotoxin on norepinephrine regulatory mechanisms. Therefore, while it is difficult to interpret data from studies measuring endotoxin-induced alterations in the total norepinephrine tissue content, one mechanism for infectious challenge to alter the level of splenic norepinephrine may involve a decrease in the efficiency of norepinephrine reuptake mechanisms, possibly mediated by stimulation of cytokine receptors on the local nerve terminal.

During normal homeostasis, the rate of norepinephrine release is balanced by the rate of norepinephrine synthesis, resulting in constant tissue levels of norepinephrine over a wide range of sympathetic nerve activity. In light of this, it is important to consider that alterations in total norepinephrine content may only be measured when this homeostatic mechanism is disrupted, i.e., when the rate of norepinephrine release is greater than the rate of norepinephrine synthesis. Therefore, previous studies reporting experimentally-induced alterations in the total norepinephrine tissue content may have disrupted these homeostatic mechanisms. Similarly, experimental conditions that do not induce detectable reductions in tissue norepinephrine concentrations provide little information about the level of norepinephrine release, except that the steady-state dynamics of the nerve terminal were not disturbed. Hence, reported changes in the tissue concentration of norepinephrine provide no information about the level of sympathetic nerve activity and resulting rate of norepinephrine release within the microenvironment in which the immune cells are responding to antigen challenge. To more accurately measure the specific rate of norepi-

nephrine release in immune organs, some studies have employed a pulse-chase technique that measures the rate of disappearance of tissue [^{3}H]norepinephrine over time, thus providing a more accurate measure of the rate of norepinephrine release. Therefore, norepinephrine turnover analysis provides an estimate of dynamic changes in sympathetic nerve activity that cannot be gained by the determination of tissue norepinephrine concentration alone (Neff et al., 1968; Brodie et al., 1978).

To further study the role of endotoxin in regulating the level of norepinephrine release, norepinephrine turnover analysis was used to determine the level of sympathetic nerve activity and norepinephrine release in the spleens of animals injected with lipopolysaccharide (LPS) (Pardini et al., 1983). LPS-induced activation of immune cell populations increased the rate of norepinephrine release in both the spleen and the heart during the first 12 h of exposure, suggesting that LPS exposure may have enhanced the level of systemic sympathetic nerve activity. In addition, since a previous study demonstrated a spleen-specific suppression of norepinephrine reuptake mechanisms following endotoxin exposure (Pardini et al., 1982), the effect of the norepinephrine reuptake inhibitor desmethylimipramine on the LPS-induced enhancement of norepinephrine release was also evaluated. In this same study, treatment of animals with desmethylimipramine did not alter the rate of norepinephrine release in either the spleen or the heart, suggesting that the LPS-induced increase in the rate of norepinephrine release was not due to alterations in norepinephrine reuptake mechanisms.

More recently, the effect of *Pseudomonas aeruginosa* infection on the rate of norepinephrine turnover in the bone marrow has been investigated (Tang et al., 1999). Using both isotopic and nonisotopic methods to measure the rate of norepinephrine turnover following *Pseudomonas aeruginosa* infection, it was shown that infection increased the rate of norepinephrine turnover in both the heart and bone marrow. Unfortunately, the exact mechanism by which infectious challenge enhanced the rate of norepinephrine release is currently unknown. However, additional studies have begun to investigate the mechanisms by which infectious challenge influences the level of sympathetic nerve activity in lymphoid organs (MacNeil et al., 1996, 1997). For example, systemic exposure to LPS (10–100 μg) via intravenous injection increased the level of splenic nerve activity within 15 to 25 min of injection (MacNeil et al., 1996). Importantly, indomethacin inhibited the LPS-induced enhancement of splenic nerve activity, suggesting a potential role for PGE_2 synthesis in mediating the effects of LPS exposure on nerve activity (MacNeil et al., 1997). Similarly, others have reported that infectious challenge results in a PGE_2-dependent increase in neuronal c-fos expression in brain regions known to control sympathetic outflow (Wan et al., 1993, 1994). Taken together, these studies support the hypothesis that infectious challenge induces PGE_2-dependent alterations in the level of both efferent sympathetic nerve activity and norepinephrine release in lymphoid organs.

Thus, immune cell activation following either infectious challenge or administration of bacterial products may increase the rate of norepinephrine turnover in lymphoid organs. In addition, these same stimuli may also increase the level of CNS nerve activity in brain regions known to control the level of efferent sympathetic nerve activity, suggesting that CNS-mediated regulatory mechanisms may respond to a peripheral endotoxin/bacterial insult to influence the rate of norepinephrine turnover in lymphoid organs following infectious challenge.

2. Particulate Antigens/Sheep Red Blood Cells. In addition to infectious challenge, other types of immune stimuli may also influence the rate of norepinephrine release within lymphoid organs. One of the earliest studies reporting a correlation between the splenic content of norepinephrine and an ongoing immune response to a particulate antigen was performed by Besedovsky et al. (1979). Immunization of animals with the particulate T cell-dependent antigen sheep red blood cells (sRBC) decreased the total norepinephrine content of the spleen in comparison with control animals. In later studies, this same group extended their findings to note that 3 days after immunization of rats with sRBC, the total norepinephrine content was lower in the spleen, lymph nodes, and thymus of immunized animals in comparison with nonimmunized control animals (Del Rey et al., 1981). Importantly, the effect of sRBC-induced immune cell activation on sympathetic nerve activity may be influenced by central regulatory mechanisms. For example, since central norepinephrine inhibits hypothalamic neuronal activity and efferent sympathetic nerve activity, the observation by Besedovsky and colleagues (1983) that sRBC-induced immune cell activation decreased the rate of norepinephrine release in the hypothalamus suggested less norepinephrine-mediated inhibition of CNS activity and increased sympathetic nerve activity in the periphery. Taken together, these findings support the hypothesis that sRBC-induced immune cell activation decreases the total lymphoid tissue content of norepinephrine by increasing the level of sympathetic nerve activity and norepinephrine release.

However, as discussed previously, since these studies detected sRBC-induced alterations in the total concentration of splenic norepinephrine, the homeostatic mechanisms that maintain a constant level of norepinephrine content over varying levels of sympathetic nerve activity may have been disrupted. In contrast, others did not observe an effect of sRBC immunization on splenic norepinephrine levels, suggesting that either immune cell activation did not influence the level of sympathetic nerve activity or that the rates of both norepinephrine release and synthesis were increased in these animals, thus main-

taining nerve terminal homeostasis while increasing the rate of norepinephrine release (Delrue-Perollet et al., 1995). Therefore, as with the early studies reporting effects of infectious challenge on the level of sympathetic nerve activity, it is difficult to determine the exact effect that sRBC-induced immune cell activation exerts on sympathetic nerve activity when measuring the total tissue content of norepinephrine alone. In addition, the rate of norepinephrine release was inferred from observations that sRBC administration resulted in lower tissue norepinephrine concentrations. This observation could be interpreted as the result of either an enhanced rate of norepinephrine release and metabolism, a suppressed level of norepinephrine production, or a suppressed level of norepinephrine reuptake by the nerve terminal.

Although they do not directly measure the rate of norepinephrine release, the levels of the dopamine metabolite 3,4-dihyroxyphenylacetic acid (DOPAC) were measured in the spleens of mice immunized with sRBCs (Fuchs et al., 1988b). Because the level of DOPAC correlates with the rate of norepinephrine synthesis, and because the rate of norepinephrine synthesis is equivalent to the rate of norepinephrine release during steady-state conditions, the concentration of DOPAC should correlate with the rate of norepinephrine release as long as nerve terminal homeostasis is maintained. Whereas the splenic norepinephrine concentration decreased following immunization with sRBC 48 h after immunization, there was no difference in the total norepinephrine content, suggesting that the decrease in norepinephrine concentration resulted from an increase in spleen size. Importantly, sRBC-induced immune cell activation increased the total level of splenic DOPAC within 48 h of immunization, suggesting an increase in the rate of norepinephrine synthesis and release. Thus, these findings suggested that sRBC exposure increased the rate of norepinephrine release in the spleen without disrupting the homeostatic mechanisms responsible for maintaining constant levels of norepinephrine.

Taken together, these studies suggest that sRBC-induced immune cell activation may influence sympathetic nerve activity to varying degrees, such that the steady-state nerve terminal dynamics may be disrupted in some model systems while being maintained in others. Regardless, sRBC-induced immune cell activation appears to increase the rate of norepinephrine synthesis and release in lymphoid organs. But thus far, few studies have investigated the effects of immune cell activation by a soluble protein antigen on sympathetic nerve activity and norepinephrine release in lymphoid organs.

3. Soluble Protein Antigen. In light of the previously discussed studies concerning the role of infectious challenge and particulate antigens on the level of sympathetic nerve activity, one study has used an antigen-specific model system to investigate the effects of a cognate soluble protein antigen on the rate of norepinephrine release in lymphoid organs by norepinephrine turnover analysis (Kohm et al., 2000). Severe combined immunodeficient (*scid*) mice were reconstituted with keyhole limpet hemocyanin (KLH)-specific Th2 cell clones and freshly isolated trinitrophenyl (TNP)-specific B cells prior to immunization with the cognate antigen TNP-KLH. Activation of Th2 cells and B cells increased the rate of norepinephrine release in the spleen and bone marrow 18 to 25 h, but not 1 to 8 h, following immunization. Since the rate of norepinephrine release was not measured between 8 and 18 h following immunization in these studies, it is possible that immune cell activation increased the rate of norepinephrine release at a time earlier than 18 h after immunization. Importantly, it was shown that immunization of *scid* mice reconstituted with antigen-specific cell populations with a noncognate antigen (fluorescein ovalbumin) that would not activate either the Th2 cells or B cells, but would activate resident macrophages, did not alter the rate of norepinephrine release in the spleen and bone marrow. Thus, these findings suggested that macrophage activation and inflammatory cytokine production are not responsible for the soluble protein antigen-induced increase in sympathetic nerve activity in this model system and that a cognate interaction between Th2 cells and B cells is necessary for soluble protein antigen-induced enhancement in norepinephrine release by a currently undetermined mechanism.

Finally, administration of the ganglionic blocker chlorisondamine completely blocked any effect of antigen administration on the rate of norepinephrine release in the heart, but only partially blocked the antigen-induced enhancement of norepinephrine release in the spleen and bone marrow (Kohm et al., 2000). These findings suggest a role for signals originating above or at the preganglionic cell body in regulating the level of antigen-induced nerve activity in lymphoid organs in this model system. One possible mechanism mediating the effects of antigen-induced lymphocyte activation on the rate of norepinephrine release may involve the production of immune cell-derived cytokines. The binding of cytokines to their specific receptors expressed on either the postganglionic nerve terminal or the postganglionic cell body may initiate afferent signals that must first be transmitted back to the CNS prior to the alteration in the rate of norepinephrine release from the local sympathetic nerve terminal. In this case, the blockade of ganglion signal transmission would block the ability of immune cell activation to increase the rate of norepinephrine release. However, because ganglionic blockade failed to completely block the antigen-induced enhancement of sympathetic nerve activity in the spleen and bone marrow, it is possible that local cytokine production may not only serve to initiate an afferent signal from the site of the immune response to the CNS, but may also modulate local nerve activity by binding to cytokine receptors on local nerve terminals or the postganglionic cell body. Thus, whereas signals emanating from the ganglion

may exert a significant regulatory influence on the level of sympathetic nerve activity in response to a specific cognate antigen, which is blocked by chlorisondamine, local cytokine receptor stimulation may also enhance norepinephrine release, which cannot be blocked by chlorisondamine. Thus, there may be multiple levels of cytokine-induced regulation of local sympathetic nerve activity and norepinephrine release within immune organs.

The following sections will discuss various mechanisms by which cytokine receptor stimulation may influence the level of sympathetic nerve activity and norepinephrine release in lymphoid organs, including the expression of cytokine receptors on peripheral nerves, the presence of afferent innervation in lymphoid organs, and the effects of cytokines on sympathetic nerve activity.

B. Cytokine Receptor Expression on Nerves

The hallmark experiments of Besedovsky et al. (1983) suggested that activated immune cells secrete "soluble factors" into the circulation that ultimately enter the CNS to stimulate neuronal activity in both the hypothalamus and brainstem. These studies were some of the first to show that soluble factors produced by cells of the immune system could alter noradrenergic nerve activity in the brain, as measured by changes in hypothalamic and brainstem norepinephrine content following α-methyl-*p*-tyrosine inhibition of norepinephrine synthesis. It is now known that these soluble factors were cytokines and that their transport into the CNS represents one possible mechanism of immune-to-brain communication. However, in order for cytokines to leave the blood and enter the CNS, a major obstacle must be overcome. The BBB, which is characterized by the astrocyte-mediated formation of tight junctions between endothelial cells composing the CNS vasculature, limits the entry of blood-borne proteins and cells into the CNS. The passage of molecules across the BBB is regulated on a variety of levels, including size, charge, lipophilicity, and adhesion molecule expression (Banks and Kastin, 1985a,b, 1987). Thus, the BBB serves as a biological filter for entry into the CNS. However, although several mechanisms exist for either the passage of, or signaling by, blood-borne cytokines into the CNS, this communication pathway is not considered a primary line of communication from the immune system to the CNS for a number of reasons, including: 1) the low concentration of cytokines present at the BBB, 2) the lack of specificity of cytokine signaling directly to the CNS, and 3) the observation that certain cytokine-related illnesses occur in the absence of detectable serum cytokine elevation (Kluger, 1991). Thus, although mechanisms exist for the transport of cytokines into the CNS, one alternative mechanism for immune cell-derived cytokines to signal the CNS is through the stimulation of cytokine receptors expressed on peripheral sensory nerves. By this mechanism, immune responses occurring near sites of sensory innervation could easily communicate signals to the CNS.

The interleukin-1 receptor (IL-1R) was the first cytokine receptor reported to be expressed on peripheral sensory nerves and, thus, became the focus of early studies concerning cytokine communication from the periphery to the CNS. IL-1 is a primary product of activated macrophages (Dinarello, 1998) and was a leading candidate for a mediator of the LPS-induced increase in sympathetic nerve activity and norepinephrine release. In addition, early studies suggested the presence of the IL-1R on peripheral nerves, because the peripheral administration of IL-1β increased CNS activity (Saphier and Ovadia, 1990; Dunn, 1992). However, these studies did not directly measure the expression of IL-1 receptors on peripheral nerves. A number of other studies have reported that peripheral administration of IL-1β resulted in increased vagus nerve activity, suggesting not only that IL-1 receptors are expressed on peripheral nerves, but that stimulation of these receptors by their specific cytokines may induce afferent nerve activity to the CNS (reviewed in Maier et al., 1998). In addition, some of these studies reported a CNS-localized effect of peripheral IL-1 administration as measured by cytokine-induced hyperalgesia, which can be blocked via administration of an IL-1R antagonist. Thus, not only did peripheral administration of IL-1 stimulate IL-1 receptors expressed in the periphery to induce vagal nerve activity, but in addition, it altered the CNS response to pain.

In later studies, the role of the vagus nerve in transmitting IL-1-induced signals to the CNS was further explored. For example, the injection of either LPS, a bacterial protein product that activates macrophages to secrete cytokines, including IL-1β, or the injection of IL-1β itself into the peritoneal cavity of mice and rats resulted in fever, hypothalamic norepinephrine depletion, and increased c-fos and acetylcholine expression in the brain (Fleshner et al., 1995; Gaykema et al., 1995; Sehic and Blatteis, 1996). Importantly, the effect of peripheral IL-1β on hypothalamic levels of norepinephrine were blocked by subdiaphragmatic vagotomy, suggesting a role for vagal afferents in mediating the effect of IL-1β on norepinephrine levels within the CNS (Fleshner et al., 1995). These findings were later supported by the observation that vagal paraganglia express IL-1 receptors, providing a direct mechanism by which IL-1β can directly activate vagal nerve afferent fibers (Goehler et al., 1997). Finally, others have shown that cultured sympathetic neurons express a functional IL-1R and that stimulation of this receptor results in the activation of NF-κB (Bai and Hart, 1998). Thus, it appears that the expression of functional IL-1 receptors on sympathetic nerves, such as the vagus nerve, provides one mechanism by which immune-derived cytokines can signal the CNS.

In addition to IL-1 receptors, the expression of other cytokine receptors on sympathetic nerves has been studied to a lessor extent. For example, sympathetic neurons appear to express too low a level of IL-6R to allow a functional effect of endogenous IL-6 on the neuron, but the exposure of sympathetic neurons to soluble IL-6R in vitro results in IL-6-induced neuron survival (Marz et al., 1998). This may be explained by the fact that the IL-6R ligand binding subunit does not possess tyrosine kinase activity, and therefore, IL-6-stimulated signaling relies on the dimerization of the ligand binding subunit of the IL-6R with the signaling subunit gp130 (reviewed in Dinarello, 1998). These studies suggest that although sympathetic neurons may express low levels of the ligand binding subunit of the IL-6R, they do express adequate levels of the signaling gp130 subunit. Thus, although sympathetic neurons may not constitutively express adequate levels of IL-6 binding subunits to respond to endogenous IL-6, either soluble IL-6R production or nerve injury may enhance the level of functional IL-6R expression on sympathetic nerves.

Finally, one study has detected the expression of IL-2 receptors on sympathetic neurons (Haugen and Letourneau, 1990). Using immunofluorescence staining, sympathetic neurons were shown to express detectable levels of IL-2R on their surface. In addition, treatment of cultured sympathetic neurons to IL-2 enhanced neurite outgrowth, suggesting that the IL-2R expressed by these cells is functional. Therefore, the presence of cytokine receptors on peripheral nerves provides a potential mechanism by which local immune cell-derived cytokines produced in the periphery may transmit signals to the CNS or to the peripheral nerve directly.

C. Afferent Splenic Innervation

As previously discussed, the effect of antigen-specific Th2 cell and B cell activation on the rate of norepinephrine release in lymphoid organs was significantly decreased by ganglionic blockade (Kohm et al., 2000). These studies suggested that the activation of antigen-specific cell populations induced the local release of norepinephrine via a mechanism that relied partially on ganglionic transmission. In light of these findings, it is reasonable to hypothesize that an immune cell-derived signal stimulated a neuronal reflex mechanism of norepinephrine release dependent upon structures at, or above, the sympathetic ganglia. Because the diffusion of locally produced immune-derived factors into the circulation would produce extremely low concentrations of circulating cytokine and, thus, would unlikely be able to induce CNS-regulated norepinephrine release in the spleen, it was more plausible to hypothesize that some local mechanism existed that was capable of responding to immune-derived signals to induce norepinephrine release from local sympathetic nerve terminals.

An early study noted the presence of afferent unmyelinated type C nerve fibers in the spleen (Herman et al., 1982), although others have observed that a small percentage of afferent fibers of the splenic nerve are myelinated (Utterback, 1944; Calaresu et al., 1984). Later studies suggested that approximately 5% of the splenic nerve is composed of afferent nerve fibers as determined by horseradish peroxidase retrograde tracing (Baron and Janig, 1988). These afferent splenic nerve fibers arose from the spinal cord at levels ranging from T4 to L2. However, the most significant origin of sympathetic afferent fibers (approximately 60%) appeared to be from levels T10 to T13. Importantly, the stimulation of these splenic afferent nerve fibers activated a reflex response via the splenic nerve increasing the level of cardiopulmonary sympathetic efferent nerve activity (Herman et al., 1982). Because activation of splenic afferents influenced cardiac efferent sympathetic nerve activity in these studies, such a mechanism may also play a role in the low level of cardiac norepinephrine release following activation of antigen-specific cell populations (Kohm et al., 2000). Interestingly, afferent signals from the spleen did not seem to originate from the capsule innervation but, instead, from vasculature-associated interior innervation, which is the location of cytokine-producing cells in the spleen (Herman et al., 1982). Finally, other studies reported that afferent fibers supplying the spleen may be activated by immune-derived stimuli (Niijima et al., 1991; Fleshner et al., 1995). Taken together, these findings support the participation of afferent innervation in transmitting the signals induced by locally produced immune-derived products to increase the rate of local norepinephrine release in lymphoid organs.

Interestingly, other studies in rats reported the absence of afferent innervation of the spleen using techniques similar to those previously discussed (Nance and Burns, 1989). The origin of these conflicting data is currently unclear. However, because these conflicting studies were performed in different animal species, the presence of afferent fibers in the splenic nerve may be a species-dependent observation. The existence of splenic afferent innervation is further supported by the report of afferent nerve fibers in another species, the guinea pig (Elfvin et al., 1992). Thus, the presence of afferent innervation in the spleen provides a specific mechanism by which locally produced cytokines or other immune cell-derived products may stimulate sympathetic nerve activity and norepinephrine release.

D. Cytokine-Induced Norepinephrine Release

Cytokines, which were once thought to only influence immune cell function, have now been shown to affect glial cell proliferation, neuron survival, neuronal proliferation and differentiation, and neurotransmitter expression (Giulian and Lachman, 1985; Yamamori et al., 1989; Jonakait and Schotland, 1990; Barbany et al., 1991; Freidin and Kessler, 1991; Hart et al., 1991; Schwartz et al., 1991; Brenneman et al., 1992). In addition, a number of studies have reported that a variety of

cytokines may influence peripheral sympathetic nerve activity and the rate of norepinephrine release.

As previously discussed, numerous studies have suggested that exposure of animals to infectious challenge or bacterial products, such as endotoxin, increases the rate of norepinephrine release in lymphoid organs. In light of the critical role of macrophage activation and IL-1β production in clearing infections and the role of norepinephrine in regulating macrophage activity (Miles et al., 1996), it is not surprising that a significant number of studies have investigated the role of IL-1β in regulating the level of norepinephrine release in vivo.

One indication that IL-1β may influence the level of sympathetic nerve activity is demonstrated by its ability to influence CNS activity. Because the hypothalamus is an area within the CNS that controls efferent sympathetic nerve activity, an IL-1β-induced increase in hypothalamic activity may enhance the level of efferent sympathetic nerve activity and the rate of norepinephrine release in the periphery. For example, peripheral injection of IL-1β enhanced both hypothalamic nerve activity and the level of CRF secretion from the hypothalamus (Sapolsky et al., 1987; Akiyoshi et al., 1990; Dunn, 1992; Fleshner et al., 1995). Also, peripheral IL-1β administration induced c-fos expression in CRF-producing cells in the paraventricular nucleus of the hypothalamus, suggesting that IL-1β increased hypothalamic neuronal activity (Ericsson et al., 1994). Because a number of studies have reported that peripheral IL-1β increases neuronal activity in the hypothalamus, it is reasonable to hypothesize that these IL-1β-induced alterations in hypothalamic activity may translate into alterations in efferent sympathetic nerve activity.

Using a nonisotopic technique employing either α-methyl-*p*-tyrosine to measure norepinephrine turnover in the spleen (Akiyoshi et al., 1990) or direct measurements of sympathetic nerve electrical activity (Niijima et al., 1991), it was shown that peripheral administration of IL-1β increased the rate of norepinephrine turnover in the spleen 1 to 6 h following exposure in a dose-dependent manner. Other studies measuring the level of sympathetic nerve electrical activity reported that peripheral IL-1β exposure increased the level of sympathetic nerve activity within 10 to 15 min of exposure in a dose-dependent manner (Takahashi et al., 1992) and that the rate of norepinephrine release in the spleen peaks within 40 min after peripheral IL-1β exposure (Ichijo et al., 1992; Shimizu et al., 1994). Finally, the effect of IL-1β on sympathetic nerve activity was specific for certain nerves, because it increased the rate of norepinephrine release in the spleen, but not in the heart (Akiyoshi et al., 1990). Because these studies administered IL-1β directly, it is not surprising that the rate of norepinephrine turnover increased much quicker than that in a study in which immune cells were activated via antigen exposure (Kohm et al., 2000). In contrast, others have reported an IL-1β-induced inhibition of splenic sympathetic nerve activity as measured by microdialysis or inhibition of [^{3}H]norepinephrine release from atria (Bognar et al., 1994; Abadie et al., 1997). Although the reason for these conflicting findings is currently unknown, the concentration of IL-1β used in these studies does not seem to be the source of these conflicting findings, inasmuch as studies reporting an IL-1β-mediated enhancement of splenic norepinephrine release have used varying concentrations of IL-1β.

Although the exact mechanism by which peripheral IL-1β increases the level of sympathetic nerve activity and the rate of norepinephrine release is currently unknown, prostaglandin synthesis may be a critical mediator of IL-1's effect on sympathetic nerve activity. For example, peripheral administration of cyclo-oxygenase inhibitors blocked the effect of IL-1β on sympathetic nerve activity in the spleen, suggesting a role for IL-1β-induced prostaglandin synthesis in regulating norepinephrine release (Niijima et al., 1991). In addition, the production of CRF within the CNS appears to be another critical mediator of IL-1β's effect on norepinephrine release, because central administration of a neutralizing antibody directed against CRF blocked the ability of peripherally administered IL-1β to increase the level of splenic norepinephrine release (Ichijo et al., 1992; Shimizu et al., 1994).

In summary, although there are conflicting reports concerning the level of splenic sympathetic nerve activity and norepinephrine release during an immune response, it appears that IL-1β may play an important role in mediating the level of sympathetic outflow in the spleen. However, IL-1β-induced regulation of norepinephrine release may only occur during immune responses involving macrophage activation, because these cells are the principal source of the cytokine. Therefore, a few studies have determined the role of other cytokine receptors in modulating norepinephrine release in lymphoid organs.

For example, whereas IL-6 does not affect the uptake of [^{3}H]norepinephrine into sympathetic nerve terminals, IL-6 does exert dose-dependent effects on sympathetic nerve activity. For example, 1 ng/ml IL-6 stimulated, 10 ng/ml IL-6 had no effect, and 100 ng/ml IL-6 inhibited [^{3}H]norepinephrine release from sympathetic nerve terminals in vitro within 2 h of cytokine exposure (Ruhl et al., 1994). Importantly, the combination of subthreshold concentrations of IL-6 (10 ng/ml) and IL-1β (0.1 ng/ml) significantly suppressed the level of sympathetic nerve activity and was blocked by an antagonist of either the IL-6 or the IL-1 receptor. Finally, others have shown that low concentrations of both IL-2 (Bognar et al., 1994) and TNF-α (Foucart and Abadie, 1996; Abadie et al., 1997) inhibited the rate of norepinephrine release in the spleen. Thus, IL-6, IL-2, and TNF-α may either enhance, inhibit, or have no effect on the rate of norepinephrine release, depending on both the cytokine concentration

and the presence of other cytokines in the microenvironment of the nerve terminal.

Taken together, these studies suggest that a physical mechanism for immune cell-derived cytokines to influence local sympathetic nerve activity is in place. Several studies have reported the presence of afferent innervation in the spleen, the presence of cytokine receptors on peripheral nerves, the ability of cytokine receptor stimulation to initiate afferent signals to the CNS resulting in alterations in hypothalamic neuronal activity, and finally, cytokine-induced alterations in sympathetic nerve activity and the rate of norepinephrine release in lymphoid organs. In light of these findings, immune cell-derived cytokine production may represent one mechanism by which an ongoing immune response may influence the rate of local norepinephrine release. Importantly, different types of antigen may lead to the activation of different populations of immune cells and affect which cytokines are produced during an immune response. The specific cytokines produced may, in turn, determine the mechanism that regulates the level of norepinephrine release within immune organs. Finally, it is possible that greater levels of infection involve the CNS-mediated regulatory mechanisms, whereas lower levels of infection may involve only local regulatory mechanisms of sympathetic nerve activity. However, in order for local norepinephrine release to influence immune cell function, lymphocytes must express receptors for the neurotransmitter.

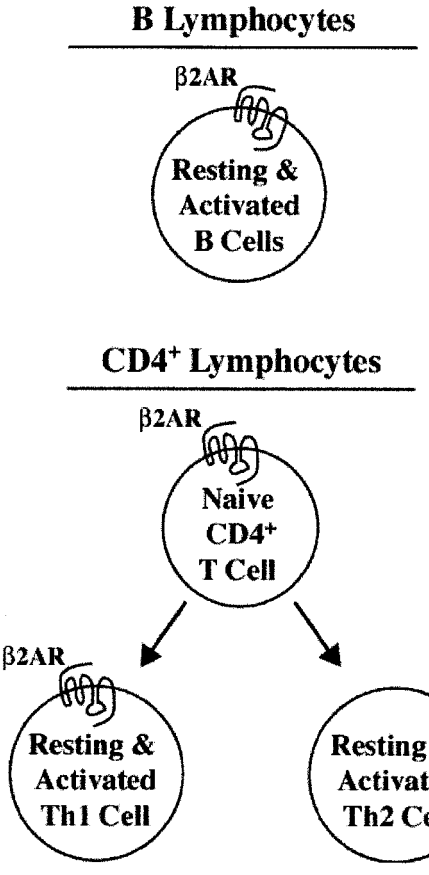

FIG. 3. β2-Adrenergic receptor expression on CD4+ cells and B cells. The predominant adrenergic receptor expressed on resting and activated B cells is the β2AR. Similarly, naive CD4+ T cells also predominantly express the β2AR. However, whereas β2AR expression is retained on clones and newly generated Th1 cells, β2AR expression is repressed on clones and newly generated Th2 cells.

III. β-Adrenergic Receptor Expression on CD4+ T and B Lymphocytes

A. CD4+ T Lymphocytes

1. Receptor Expression. Although few studies have reported the presence of αARs on T cells, early studies suggested the presence of a functional βAR on their surface. An important premise that made these studies possible was that stimulation of the βAR was found to increase the level of adenylyl cyclase activity and intracellular cAMP accumulation in other nonlymphoid cell types (reviewed in Wolfe et al., 1977). Therefore, using βAR agonists, early reports demonstrated that the exposure of lymphocytes to βAR agonists resulted in adenylyl cyclase activation and increased cAMP production (Bourne and Melmon, 1971; Makman, 1971; Bach, 1975). Thus, although βAR expression would not be measured directly on lymphocytes for another 6 years, early pharmacological and biochemical data suggested their functional presence. A recent review provides comprehensive discussion concerning the expression of adrenergic receptors on immune cells (Sanders et al., 2001). Figure 3 summarizes β2AR expression on both CD4+ T cells and B cells.

Williams et al. (1976) performed the original studies to measure the level of βAR expression on total human lymphocyte membranes directly via [3H]alprenolol saturation binding assays. These studies reported approximately 2000 βAR binding sites per lymphocyte. However, a number of subsequent binding studies reported a lower level of βAR expression on purified populations of T cells, as opposed to total lymphocytes (Pochet et al., 1979; Bishopric et al., 1980; Loveland et al., 1981; Krawietz et al., 1982; Bidart et al., 1983; Pochet and Delespesse, 1983; Khan et al., 1986; Westly and Kelley, 1987; Fuchs et al., 1988a; Van Tits et al., 1990; Radojcic et al., 1991). In general, the reported absolute number of βARs expressed on T cells varied, and this variance might be explained by the use of either different T cell isolation techniques, different types of radiolabel, i.e., ^{3}H versus ^{125}I, pharmacological ligands for which the receptor has differing affinities, and/or radioligand specific activity. Similarly, the cell population composition used in these studies may have also contributed to the varying number of βAR binding sites reported to be expressed by T cells, since it is now known that the different subsets of murine T cells (CD8+, naive CD4+, Th1, and Th2 cells) all express different levels of the βAR. Nevertheless, on average, most reports measured approximately 200 to 750 βAR binding sites per T cell.

Until the early 1980s, very little was known about the specific subtypes of βAR expressed on T cells. Subsequently, the primary βAR-subtype expressed on lymphocytes was found to be the β2AR, inasmuch as the β1AR-selective antagonist was unable to compete for the specific binding of [^{125}I]HYP, whereas L-propranolol, a nonselective βAR antagonist, competed for the specific binding (Loveland et al., 1981). This finding was supported by a number of other studies, suggesting that the primary subtype expressed on T cells was the β2AR (Bourne and Melmon, 1971; Williams et al., 1976; Conolly and Greenacre, 1977; Pochet et al., 1979; Loveland et al., 1981; Meurs et al., 1982; Ramer-Quinn et al., 1997; Sanders et al., 1997). Finally, functional studies indicated the lack of αARs on splenic and thymic T cells (Cook-Mills et al., 1995). Therefore, because no radioligand binding data showed the presence of a high affinity β1AR or β3AR on T cells, these findings suggest that previous studies measuring βAR expression on T cells were in fact measuring the level of β2AR expression.

Interestingly, some studies have reported that the number of βARs expressed on T cells varies during development. For example, immature T cells in the thymus may express a significantly lower number of βARs on their surface in comparison with circulating peripheral T cells (Pochet and Delespesse, 1983; van de Griend et al., 1983). These findings were supported by others who reported that thymocytes expressed a lower number of βARs than did peripheral T cells isolated from lymph nodes (Staehelin et al., 1985) or the spleen (Fuchs et al., 1988a), suggesting that β2AR expression may increase on the cell surface during T cell differentiation. The reason for such alterations in β2AR expression on developing T cells is unclear. However, it is possible that β2AR stimulation may impede T cell development. In this case, it would be beneficial for β2AR expression to be lower on developing T cells. However, future studies are needed to investigate this and other potential explanations.

It wasn't until the mid-1980s that the level of βAR expression was measured specifically on CD4$^+$ T cells. Approximately 750 βAR binding sites were reported to be expressed on Th cells (Khan et al., 1986), but these studies employed a nonselective βAR agonist (isoproterenol) and antagonists (propranolol and [^{125}I]CYP), leaving the subset of βAR expressed on the surface of the CD4$^+$ T cells unknown. However, later studies suggested that CD4$^+$ T cells expressed a β2AR with a "normal" affinity for isoproterenol (Dailey et al., 1988; Robberecht et al., 1989). Importantly, these studies used mixed populations of CD4$^+$ T cells, containing naive, Th1, and Th2 cell populations.

Recently, the expression of βAR subtypes has been measured on CD4$^+$ T cell subsets at both the protein and the mRNA level. In general, Th1 cells, but not Th2 cells, preferentially expressed the β2AR, and this was demonstrated by a number of techniques using both T cell clones and newly generated Th1 and Th2 cell populations. For example, resting Th1 cell clones, but not Th2 cell clones, showed a detectable level of β2AR protein expression using both radioligand binding with iodopindolol and immunofluorescence staining with a polyclonal anti-β2AR antibody directed against the cytoplasmic region of the β2AR (Sanders et al., 1997). This finding was later confirmed at the mRNA level (A. P. Kohm, M. A. Swanson, and V. M. Sanders, manuscript submitted for publication). Importantly, these studies were also performed using newly generated populations of Th1 and Th2 cells. Naive CD4$^+$ T cells receiving either antigen-presenting cells and antigen or anti-CD3 stimulation in the presence of IL-12 will preferentially differentiate into Th1 cells (Seder et al., 1993), whereas the same naive CD4$^+$ T cells stimulated in the presence of IL-4 will differentiate into Th2 effector cells (Hsieh et al., 1992; Seder et al., 1992). Thus, newly generated CD4$^+$ Th1 and Th2 cells provide another mechanism to study the phenotype and function of these two effector cell populations in vitro. Freshly isolated naive CD4$^+$ T cells expressed a functional β2AR, but not a β1AR or β3AR, as determined by mRNA analysis and functional studies (Swanson et al., 2001). Importantly, whereas β2AR mRNA expression was retained in newly generated Th1 cells, β2AR mRNA expression was repressed in newly generated Th2 cells (A. P. Kohm, M. A. Swanson, and V. M. Sanders, manuscript submitted for publication). Taken together, these findings suggested that the β2AR is differentially expressed on CD4$^+$ T cell subsets, with detectable receptor expression on naive CD4$^+$ T cells and Th1 cells, but not on Th2 cells.

The T cell activation status may also influence the level of βAR surface expression. For example, splenocyte activation by the T cell mitogen concanavalin (Con) A increased the level of βAR surface expression 24 h after cell activation, while exerting no effects on the affinity (K_d) of the receptor (Westly and Kelley, 1987). Others have also reported a similar effect of T cell activation on βAR expression both in vitro (Sanders and Munson, 1985b; Radojcic et al., 1991) and in vivo (Madden et al., 1989). Similarly, T cell activation may also influence βAR expression on subsets of CD4$^+$ T cells. As discussed previously, Th1 cell clones, but not Th2 cell clones, expressed detectable levels of β2AR surface protein (Sanders et al., 1997). Stimulation of the CD3 complex associated with the T cell receptor activates Th1 and Th2 cell clones, and more importantly, β2AR expression is upregulated on the surface of activated Th1 cell clones, but β2AR expression remains undetectable on the surface of activated Th2 cell clones (Ramer-Quinn et al., 1997). However, other studies have reported contrasting findings. For example, Con A-induced activation of lymph node T cells decreased the number of [^{125}I]CYP binding sites from 750 to 850 sites per cell to approximately 350 sites per cell within 3 days of culture, a time that also correlated with the peak in cell proliferation but induced

no change in βAR number within 24 h of activation (Cazaux et al., 1995). These findings are supported by others who reported that mitogen-induced activation of T cells results in a PKC-dependent increase in the expression of the β-adrenergic receptor kinase-1 (βARK1) and βARK2 mRNA within 48 h of stimulation, whereas no alterations were seen in G-protein receptor kinase-5 (GRK5) and GRK6, suggesting a selective regulation of the receptor-associated kinase subtypes (De Blasi et al., 1995). Because βARK is a serine-threonine kinase that regulates the level of βAR expression (reviewed in Inglese et al., 1993), βARK activation may contribute to the down-regulation of βAR expression at later times following T cell activation, e.g., at times longer than 24 h following T cell activation. Thus, whereas most studies report that T cell activation elevates the level of surface βAR expression, cellular activation may result in decreased levels of βAR expression at later times following T cell activation.

2. Mechanisms Regulating Differential Receptor Expression on CD4+ T Cell Subsets. Currently, the mechanisms regulating the differential expression of the β2AR on Th1 and Th2 cells is unknown. However, a few recent studies have begun to investigate possible mechanisms that may influence the level of β2AR expression on $CD4^+$ T cells.

As previously discussed, naive $CD4^+$ T cells express β2AR mRNA, but not β1AR or β3AR mRNA (Swanson et al., 2001). More importantly, Th1 cells newly generated from naive $CD4^+$ T cells retained the expression of the β2AR, whereas newly generated Th2 cells did not (A. P. Kohm, M. A. Swanson, and V. M. Sanders, manuscript submitted for publication). Because the cytokine microenvironment is the only difference between Th1- and Th2-promoting conditions in this model system, it seems reasonable that intracellular signals resulting from cytokine receptor stimulation during $CD4^+$ T cell differentiation may influence β2AR expression on subsequent generations of effector cells.

One mechanism by which cytokine receptor stimulation regulates gene expression is via alterations in both the level of histone acetylation (Ohno et al., 1997; Taplick et al., 1998; Cheung et al., 2000; Gray et al., 2000; Ito et al., 2000; Vanden Berghe et al., 2000) and DNA methylation (Hmadcha et al., 1999; Kang et al., 1999). To investigate whether either of these epigenetic mechanisms contributes to the regulation of β2AR expression in Th1 and Th2 cells, β2AR-negative Th2 cells were exposed to pharmacological agents that resulted in either histone hyperacetylation or DNA hypomethylation, both of which have been shown previously to regulate the level of βAR expression in other types of cells (Kassis et al., 1988; Buscail et al., 1990). Exposure of Th2 cells to the histone deacetylase inhibitor butyrate resulted in a dose- and time-dependent induction of β2AR mRNA expression in these cells (A. P. Kohm, M. A. Swanson, and V. M. Sanders, manuscript submitted for publication). Similarly, exposure of Th2 cells to the methyltransferase inhibitor 5-azacytidine also resulted in a dose- and time-dependent induction of β2AR mRNA expression, but with a longer time of onset. Not surprisingly, 5-azacytidine-induced DNA hypomethylation prior to butyrate-induced histone hyperacetylation resulted in a synergistic enhancement of β2AR mRNA expression in Th2 cells. Finally, pretreatment of Th2 cells with either the transcription inhibitor actinomycin D or the protein synthesis inhibitor cycloheximide revealed that the induction of β2AR mRNA expression following histone hyperacetylation and/or DNA hypomethylation was transcription-dependent, but translation-independent, suggesting that the basal levels of transcription factor expression may have been sufficient to induce β2AR gene transcription once the gene locus was accessible. Thus, epigenetic mechanisms such as histone acetylation and DNA methylation may play a critical role in regulating the differential expression of the β2AR in Th1 and Th2 cells. However, future studies are necessary to further investigate whether basal transcription factor expression plays a critical role on β2AR gene transcription in this model system.

In addition to epigenetic mechanisms, others have investigated the role of protein kinase activation in the regulation of βAR expression on T cells. One study has reported that Con A-induced T cell activation decreased the level of βAR surface expression in a PKC-dependent manner (Cazaux et al., 1995). In support of these findings, exogenous activation of PKC via a phorbol ester (PMA)/ionophore also decreased the level of βAR expression. Such a role for PKC-induced down-regulation of the β2AR has been reported previously in other cell types as part of the endogenous mechanisms inducing receptor desensitization (reviewed in Lefkowitz et al., 1998; Dzimiri, 1999). Finally, whereas treatment of cells with PMA alone did not down-regulate the level of βAR expression on T cells within 3 days, the addition of recombinant IL-2 (12.5 U/ml) significantly down-regulated βAR expression (Cazaux et al., 1995). It is interesting to note that IL-2R stimulation induces PKC activation (reviewed in Gomez et al., 1998), and thus, future studies may reveal a PKC-dependent component to the ability of IL-2R stimulation to influence β2AR expression on T cells. Thus, these studies suggest that PKC activation plays an important role in the regulation of β2AR expression in Th1 and Th2 cells.

Taken together, these studies suggest that $CD4^+$ T cells differentially express the β2AR, with detectable expression on $CD4^+$ naive T cells and Th1 cells, but not on Th2 cells. In addition, activation of $CD4^+$ T cells may result in an initial up-regulation of the level of β2AR surface expression, but then a later down-regulation of β2AR expression by PKC-dependent mechanisms possibly involving βARK activation. Although both the mechanism and the purpose of these biphasic effects of T cell activation on β2AR expression are currently unclear, such a mechanism may be critical to allow for the maintenance of $CD4^+$ T cell function while the cell participates in an ongoing immune response.

B. B Lymphocytes

An isoproterenol-induced accumulation of intracellular cAMP was the first finding to suggest the presence of a functional βAR on the B cell surface (Bach, 1975). In addition, early radioligand binding studies reported that peripheral lymphocytes, which contain between 30 and 50% B cells, expressed the βAR (Williams et al., 1976). However, it was not until a few years later that an enriched population of B cells was shown to express approximately 400 to 600 βAR binding sites per cell using a radioligand binding assay (Pochet et al., 1979). In addition, using three different βAR agonists, isoproterenol, epinephrine, and norepinephrine, an order of potency was observed that was consistent with the presence of a β2AR. Finally, T cells and B cells isolated from peripheral blood expressed a similar low level of βAR expression on their surface, and the affinity of the receptor was similar in both cell populations (Bishopric et al., 1980).

In contrast, other reports suggested that purified B cells expressed a higher level of the βAR on their surface in comparison with peripheral T cells (Pochet et al., 1979; Miles et al., 1981, 1984, 1985; Krawietz et al., 1982; Bidart et al., 1983; Paietta and Schwarzmeier, 1983; Pochet and Delespesse, 1983; Fuchs et al., 1988a; Griese et al., 1988; Korholz et al., 1988; Van Tits et al., 1990; Cremaschi et al., 1991). For example, some reported that B cells expressed approximately twice the number of surface βARs as T cells, but that the K_d values of the βAR were similar on both cell populations (Miles et al., 1984, 1985). Studies employing salbutamol displacement curves determined that the βAR expressed by both cell populations were of the β2AR subtype (Griese et al., 1988; Korholz et al., 1988), which is in agreement with the findings of others (Krawietz et al., 1982; Pochet and Delespesse, 1983; Fuchs et al., 1988a). These findings were supported by a recent study that investigated the expression of βAR-subtypes on antigen-specific B cells freshly isolated from the spleens of unimmunized mice (Kohm and Sanders, 1999). Radioligand binding analysis suggested that antigen-specific B cells isolated from the spleens of unimmunized mice expressed approximately 620 βAR binding sites per cell with an affinity of 0.1 nM. The βAR expressed on the B cell was shown to be of the β2AR-subtype, because B cells stained with an antibody directed against the cytoplasmic tail of the β2AR, but not with antibodies directed against the cytoplasmic tails of the β1AR. Thus, in agreement with previous studies investigating the expression of the βAR on purified B cell populations, these studies suggested that freshly isolated antigen-specific B cells preferentially expressed the β2AR.

IV. Effects on CD4$^+$ T Lymphocytes

A. β2-Adrenergic Receptor Signaling Components

Early studies measuring isoproterenol-induced intracellular cAMP accumulation in T cells supplied some of the initial suggestions that T cells expressed a functional βAR on their surface. For example, one early study observed that thymocyte exposure to isoproterenol elevated the level of cAMP accumulation 4 to 5 times that observed in similarly exposed peripheral T cells (Bach, 1975). This study not only suggested that both thymocytes and peripheral T cells expressed a functional βAR on their surface but, in addition, suggested that alterations in either the level or function of βAR expression may occur during T cell development. Other studies also reported that stimulation of either the βAR or β2AR results in increased levels of cAMP (Bishopric et al., 1980; Pochet and Delespesse, 1983; Staehelin et al., 1985; Khan et al., 1986; Dailey et al., 1988; Bartik et al., 1994; Cazaux et al., 1995; Sanders et al., 1997) or increased adenylyl cyclase activity (Bartik et al., 1994; Bauman et al., 1994) in T cells. In contrast, other studies reported that isoproterenol did not induce cAMP accumulation in human thymocytes, but did increase cAMP levels in both mouse thymocytes and peripheral human T cells (van de Griend et al., 1983). These findings correlated with binding studies showing that human thymocytes expressed a very low number of βAR binding sites compared with either mouse thymocytes or human peripheral T cells (van de Griend et al., 1983). Finally, the β2AR-selective agonist terbutaline induced an increase in the intracellular concentration of cAMP in clones of Th1 cells, but not in clones of Th2 cells (Sanders et al., 1997), a finding that is in agreement with the previously discussed data concerning the differential expression of the β2AR on Th1 and Th2 cell clones. Therefore, these studies suggest that stimulation of the β2AR expressed by mature T cells results in increased levels of intracellular cAMP accumulation.

Studies by Pochet and Delespesse (1983) reported that although maturing thymocytes possessed a lower number of βARs than did mature peripheral T cells, the few receptors that these cells did possess were more efficiently coupled to adenylate cyclase. They observed that even though thymocytes had 4.6 times fewer βARs per cell than splenocytes, as determined by radioligand binding studies, thymocyte stimulation by the βAR agonist isoproterenol resulted in 20 times higher levels of intracellular cAMP in comparison with stimulated splenocytes. Importantly, they also showed that the affinity of the βAR in both cell populations for isoproterenol was equivalent, and thus, differences in the efficiency of receptor stimulation could not be responsible for the observed differences in cAMP generation. In addition, immature thymocytes expressed a lower βAR density, but a greater cAMP response, to isoproterenol than more mature thymocytes. One interpretation of these findings may be that the efficiency of βAR coupling to adenylyl cyclase may be dependent upon the developmental status of the T cell. This proposal is supported by the observations that the different subtypes of βAR are coupled to adenylate cyclase with varying efficiencies

(Dixon et al., 1986; Frielle et al., 1987; Emorine et al., 1989). However, future studies are necessary to determine whether the same βAR subtype can be coupled to adenylyl with varying affinities and whether the developmental status of the T cell influences this coupling affinity. Thus, although the level of βAR surface expression may increase on T cells during maturation in the thymus, the level of isoproterenol-induced cAMP accumulation appears to decrease, suggesting that differentiation-dependent alterations in the efficiency of adenylyl cyclase coupling may exist.

In addition to comparing T cell populations at different developmental stages, other studies have compared the levels of βAR-induced cAMP accumulation in mature T and B cells. For example, even though purified B cells expressed a 2-fold higher number of βARs on their surface in comparison to peripheral T cells, both cell types responded equivalently to isoproterenol-induced cAMP accumulation (Pochet and Delespesse, 1983). Importantly, there were no differences in the K_d values of the βAR in either population of cells, and the βAR subtype expressed by both cell populations was determined to be of the β2-subtype by using salbutamol displacement curves. One explanation of these findings is that both cell types expressed varying β2AR affinities, with T cells expressing an increased number of the higher affinity β2AR in comparison with B cells. This difference may explain why the levels of isoproterenol-induced cAMP accumulation were equivalent in both cell populations, even though the T cells in these studies expressed a lower number of β2AR. Interestingly, the T cell cAMP response to isoproterenol was higher following 4 to 5 days of IL-2 induced proliferation in the absence of antigen (Dailey et al., 1988). Possible explanations include an increase in βAR expression, an increase in the catalytic subunit expression, or an increase in receptor coupling to adenylate cyclase. Thus, although T cells may express a lower level of the β2AR on their surface in comparison with B cells, the β2AR expressed on T cells may be more efficiently linked to adenylyl cyclase, and this efficiency of adenylyl cyclase coupling may be modulated by cytokine receptor stimulation.

Classically, stimulation of the β2AR initiates an intracellular signaling cascade leading to adenylyl cyclase activation, cAMP accumulation, and PKA activation. Exposure of T cells to either isoproterenol or PGE_2 induced PKA activity in a dose-dependent manner, but the PKA isoform activated in these studies was stimuli-dependent (Bauman et al., 1994). For example, PGE_2 exposure resulted in equal activation of two different isoforms of PKA, PKAI and PKAII, whereas isoproterenol exposure preferentially lead to the activation of PKAI. These data contradict previous studies suggesting that stimulation of either the PGE_2R or βAR induced PKA activation via identical pathways (Smith et al., 1971a; Goodwin et al., 1977; Baker et al., 1981; Johnson et al., 1981; Rappaport and Dodge, 1982; Makoul et al., 1985; Aussel et al., 1987; Hausdorff et al., 1990). One possible explanation of these findings may involve the cellular distribution of the different PKA isoforms, but there is still some controversy concerning the distribution of PKA in T cells (Chaplin et al., 1979; Hasler et al., 1992; Skalhegg et al., 1994). Regardless, stimulation of the T cell β2AR leads to PKA activation, and future studies are necessary to further investigate whether the PKA isoforms activated by receptor stimulation are cell type-, developmentally, and/or activation-dependent.

Other nonclassical signaling pathways have been described for βAR signaling in T cells. For example, one study used mutant T cell lines, which lack various classical components of the β2AR signaling cascade, to describe a PKA-independent component to β2AR-induced thymocyte apoptosis (Gu et al., 2000). Stimulation of the T cell β2AR resulted in the activation of Lck, a Src family tyrosine kinase, via physical interactions between the Gs subunit of the β2AR and Lck. However, future studies are required to determine the functional role of β2AR-induced Lck activation on T cell differentiation, proliferation, and function.

B. Proliferation, Differentiation, and Cell Trafficking

1. In Vitro Proliferation and Differentiation. Two of the major cellular activities of $CD4^+$ T cells are cell proliferation and cytokine production. Upon activation by recognition of an antigen peptide presented in the context of MHC class II by their antigen-specific T cell receptor, the small population of antigen-specific T cells must be expanded to magnify and successfully complete their effector functions, such as providing "help" to the B cell for antibody production. Therefore, cellular proliferation of $CD4^+$ T cells is a critical determinant of the magnitude of the ongoing immune response. Table 2 summarizes past findings concerning the effects of norepinephrine, β2AR stimulation, and cAMP-elevating agents on T cell proliferation in vitro and in vivo.

Early studies investigated the effects of isoproterenol-induced elevations in the intracellular level of cAMP on lymphocyte proliferation and found that isoproterenol exposure decreased the proliferation rate of phytohemagglutinin (PHA)-stimulated lymphocytes (Smith et al., 1971b). In addition, exogenous addition of db-cAMP (10^{-5} M) also inhibited PHA-stimulated lymphocyte proliferation, but only if present within the first hour of cell activation. Interestingly, low concentrations of db-cAMP (10^{-8}–10^{-9} M) had the opposite effect, to slightly increase the rate of lymphocyte proliferation. Therefore, these studies suggested that βAR stimulation may either inhibit or enhance the level of T cell proliferation, depending on the concentration of cAMP generated intracellularly.

Later studies supported the hypothesis that βAR stimulation decreased the level of mitogen- or anti-CD3 antibody-induced T cell proliferation. For example, isoproterenol exposure inhibited PHA-induced T cell prolif-

TABLE 2
The effects of norepinephrine, β2AR stimulation, and cAMP-elevating agents on T cell proliferation in vitro and in vivo

Effect	Stimulus	References
↑[a] Proliferation	In vitro cAMP-elevating agents	
	[lower db-cAMP] PHA-stimulated proliferation	Smith et al., 1971
	In vivo NE depletion	
	Ex vivo Con A-stimulated proliferation (DBA/2 mice)	Lyte et al., 1991
n.c. Proliferation	In vivo NE depletion	
	n.c. ex vivo Con A-stimulated proliferation (C57BL/6 mice)	Lyte et al., 1991
↓ Proliferation	In vitro βAR	
	PHA-stimulated proliferation	Smith et al., 1971; Feldman et al., 1987; Carlson et al., 1989
	anti-CD3 Ab-stimulated proliferation	Bartik et al., 1993; Bauman et al., 1994; Selliah et al., 1995
	Con A-stimulated proliferation	Johnson et al., 1981
	In vitro cAMP-elevating agents	
	[higher db-cAMP]: PHA-stimulated proliferation	Smith et al., 1971
	Theophylline: PHA-stimulated proliferation	Scordamaglia et al., 1988
	PGE_2-stimulated proliferation	Minakuchi et al., 1990
	In vivo NE depletion	
	Ex vivo anti-CD3 Ab-stimulated lymph node cell proliferation	Madden et al., 1994

[a] Abbreviations: ↑, increase; ↓, decrease; [ ], concentration; Ab, antibody; n.c., no change; Con A, concanavalin A; NE, norepinephrine; PGE_2, prostaglandin, PHA, phytohemagglutinin.

eration in a dose-dependent manner using concentrations of isoproterenol ranging from 10^{-6} M to 10^{-4} M (Feldman et al., 1987; Carlson et al., 1989). Importantly, isoproterenol-induced intracellular cAMP levels peaked within 10 to 20 min after exposure (Carlson et al., 1989). In the same manner, βAR stimulation by isoproterenol (10^{-9}–10^{-5} M) also decreased the rate of anti-CD3 antibody-induced T cell proliferation in a dose-dependent manner (Bartik et al., 1993), and isoproterenol-induced PKA activation correlated with an inhibition of T cell proliferation (Bauman et al., 1994). However, even though a number of reports suggested that either stimulation of the βAR, increased intracellular cAMP accumulation, or increased PKA activity inhibits T cell proliferation (Smith et al., 1971b; Johnson et al., 1981; Glibetic and Baumann, 1986; Feldman et al., 1987; Griese et al., 1988; Scordamaglia et al., 1988; Minakuchi et al., 1990; Bartik et al., 1993; Bauman et al., 1994), the exact mechanism by which these events may influence T cell proliferation was unknown.

Although early elevations in intracellular cAMP and PKA activation have been proposed as candidates for mediating the effect of β2AR stimulation on the rate of T cell proliferation, other mediators may also be involved. For example, one possible mechanism by which βAR stimulation inhibits T cell proliferation is by influencing the assembly of cytoskeletal elements. Under normal conditions, activation of T cells by anti-CD3 antibody resulted in the conversion of globular (G)-actin to filamentous (F)-actin to facilitate TCR activation, costimulation, and cell proliferation (Parsey and Lewis, 1993). However, stimulation of the βAR on anti-CD3-activated T cells inhibited F-actin assembly that occurred within the first hour of activation (Selliah et al., 1995). This effect of βAR stimulation on cytoskeletal elements was proposed to be cAMP-dependent, because similar results were observed in cells exposed to either forskolin or db-cAMP. Thus, one mechanism by which early elevations in intracellular cAMP may inhibit activation-induced T cell proliferation is via disruption of cytoskeletal events leading to cell division.

Therefore, studies conducted over the past 30 years suggest that stimulation of the β2AR decreases $CD4^+$ T cell proliferation via a mechanism that may involve elevations in the intracellular concentration of cAMP, increased PKA activation, and possible effects on cytoskeletal elements. Future studies are necessary to further determine whether the method of T cell activation or signals originating from other surface receptors may influence the effect of β2AR-induced elevations in cAMP on the rate of T cell proliferation. Finally, other mechanisms may also contribute to the effect of β2AR stimulation on T cell proliferation, such as β2AR-induced alterations in both cytokine production by T cells and cytokine receptor expression on T cells; however, this subject will be discussed in later sections.

In addition to proliferation, the process of cellular differentiation critically influences T cell function. Importantly, the cytokine microenvironment is one of the fundamental criteria that determine the fate of a differentiating $CD4^+$ T cell. For example, naive $CD4^+$ T cells receiving either antigen-presenting cells and antigen or anti-CD3 stimulation alone in the presence of IL-12 differentiate preferentially into Th1 cells (Seder et al., 1993), whereas the same naive $CD4^+$ T cells stimulated in the presence of IL-4 antibody will differentiate preferentially into Th2 effector cells (Hsieh et al., 1992; Seder et al., 1992). In light of the importance of the cytokine microenvironment in determining the path of $CD4^+$ T cell differentiation, norepinephrine-induced changes in the cytokine profile of antigen-presenting cells may influence whether a naive $CD4^+$ T cell differentiates into either a Th1 or a Th2 effector cell.

In support of this hypothesis, a number of studies suggested that elevated intracellular levels of cAMP may influence both the cytokine profile and the level of cytokine secreted by antigen-presenting cells. For example, individually, either the addition of PGE_2 exposure, or the direct addition of db-cAMP or norepinephrine (10^{-8}–10^{-6} M) to LPS-activated monocytes each decreased the level of IL-12 but increased the level of IL-10 produced by antigen-presenting cells (van der Pouw Kraan et al., 1995; Elenkov et al., 1996). In a similar manner, in vitro exposure of monocytes and dendritic cells to β2AR-selective agonists inhibited LPS- or anti-CD40-induced IL-12 production, respectively, but did not influence monocyte production of either IL-1α, IL-1β, IL-6, or IL-10 (Panina-Bordignon et al., 1997). Finally, one study reported no effect of norepinephrine on IL-12 production by antigen-presenting cells in culture (Swanson et al., 2001). Thus, although there are conflicting observations concerning the effects of β2AR stimulation and elevated levels of cAMP on the level of IL-10 production, these studies suggest that stimulation of the β2AR on a professional antigen-presenting cell may favor the development of Th2 cells by decreasing the level of IL-12 produced by antigen-presenting cells that is required for Th1 cell development.

In addition to effects on cytokine production by antigen-presenting cells, norepinephrine and β2AR stimulation may also influence $CD4^+$ T cell differentiation via direct effects on the naive $CD4^+$ T cell. Recently, Swanson et al. (2001) investigated the role of β2AR stimulation on the naive $CD4^+$ T cell during differentiation to a Th1 cell and on the function of progeny effector cells. Activation of naive $CD4^+$ T cells by anti-CD3/28 antibody and IL-12 in the presence of norepinephrine (10^{-6} M) generated effector Th1 cells that produced significantly higher levels of IFN-γ per cell upon restimulation in comparison with Th1 cells generated in the absence of norepinephrine. Importantly, the effects of norepinephrine on Th1 cell IFN-γ production were β2AR- and IL-12-dependent as demonstrated by the use of αAR and βAR antagonists, a β2AR-selective antagonist, and IL-12R-deficient mice. Therefore, these studies suggest that norepinephrine may influence Th1 cell function via stimulation of the β2AR expressed on naive $CD4^+$ T cell and via either augmentation or collaboration with the IL-12R signaling pathway.

2. In Vivo Proliferation and Cell Trafficking. When considering the effects of norepinephrine on in vivo T cell proliferation, one important factor is the model system being employed. Although very few studies have investigated the effects of norepinephrine on T cell proliferation in vivo, the findings of one study suggested a strain-specific effect of norepinephrine depletion on T cell proliferation. Whereas norepinephrine depletion significantly enhanced the level of in vitro Con A-induced T cell proliferation in spleen cells isolated from DBA/2 mice, no effect was observed on the level of proliferation of T cells isolated from C57BL/6 mice (Lyte et al., 1991). In addition, norepinephrine depletion seemed to also exert differential effects on T cell proliferation, depending on the T cell activation status. For example, when lymph node cells isolated from norepinephrine-depleted mice were restimulated in vitro with anti-CD3 antibody, the rate of T cell proliferation was significantly lower in comparison to lymph node cells isolated from norepinephrine-intact mice (Madden et al., 1994). In contrast, the basal rate of lymph node cell proliferation was significantly higher in norepinephrine-depleted mice in comparison to norepinephrine-intact mice as determined by injection of [^{125}I]deoxyuridine. In summary, findings from these few in vivo norepinephrine depletion studies suggest that norepinephrine release in lymph nodes may decrease the proliferation rate of unstimulated T cells but enhance the proliferation rate of activated T cells. Thus, whereas most studies suggest that norepinephrine and β2AR stimulation decreases the rate of T cell proliferation in vitro, regardless of the type of activation stimulus used, exposure of T cells to norepinephrine in vivo appears to induce both strain- and activation stimuli-dependent effects on cellular proliferation. However, these studies measured the rate of T cell proliferation by in vitro assays, which only provide an indication of how the T cell may have behaved in vivo. Thus, because none of these studies measured the effect of norepinephrine and/or β2AR stimulation on the rate of T cell proliferation in vivo, it is difficult to interpret the findings of these studies.

A greater number of past studies investigated the effects of norepinephrine and βAR stimulation on in vivo cell trafficking. Most of the early studies investigating the role of catecholamines in modulating cell homing were performed with epinephrine, suggesting a catecholamine-induced elevation in the number of circulating lymphocytes (reviewed in Benschop et al., 1996). Although a number of early studies dating back to the early 1900s were conducted to determine the effects of epinephrine on lymphocyte cell homing, the adrenergic receptor subtype responsible for the action of epinephrine was not determined until 1974. Using both αAR and βAR antagonists, βAR blockade alone was shown to inhibit the epinephrine-induced increase in circulating human lymphocytes, suggesting that earlier studies may have been describing the effects of βAR stimulation on lymphocyte homing (Gader, 1974). Supporting these findings, isoproterenol and salbutamol both increased the number of circulating lymphocytes in humans (Gader and Cash, 1975).

The source of the lymphocytes contributing to the catecholamine-induced increase in circulating cell numbers was also investigated (Ernstrom and Sandberg, 1973). Isoproterenol and norepinephrine both increased the number of lymphocytes leaving the spleen, without any apparent alterations in blood flow. These studies were the first to suggest that alterations in lymphocyte

adhesion molecule expression may mediate the norepinephrine-induced increase in the number of circulating lymphocytes, even though this was not tested directly. In contrast, others showed that the pretreatment of fluorescently labeled lymphocytes with isoproterenol (10^{-6} M) for 15 min prior to i.v. reconstitution increased the homing of lymphocytes to the spleen and peripheral lymph nodes in comparison to control cells (Carlson et al., 1997). More specifically, a significantly higher percentage of the cells homed to the white pulp in the spleen, particularly the T cell-containing periarterial lymphoid sheath.

In support of β2AR-mediated alterations in lymphocyte migration from the spleen, the βAR antagonist propranolol, but not the αAR antagonist phentolamine, decreased the number of lymphocytes leaving the spleen via both blood flow-dependent and -independent mechanisms (Rogausch et al., 1999). In contrast, others have reported that axotomy increased both the total number and percentage of Thy-1.2-positive T cells in the spleen, suggesting that norepinephrine depletion either increased the migration of non-T cells out of the spleen or the homing of T cells into the spleen (Miles et al., 1985). Thus, these studies support the findings of earlier studies that norepinephrine may alter the number of circulating lymphocytes and that β2AR stimulation may differentially influence the cell trafficking of specific cell populations. However, conflicting findings still exist concerning the exact role of norepinephrine and β2AR stimulation in regulating lymphocyte trafficking.

C. In Vitro and In Vivo Cell Surface Molecule Expression

In light of the apparent influence of β2AR stimulation on the rate of $CD4^+$ T cell proliferation, and because stimulation of the IL-2R plays a pivotal role in stimulating $CD4^+$ T cell proliferation, it was possible that β2AR stimulation influenced the level of IL-2 receptor expression on T cells.

Although a number of in vitro studies have reported that either isoproterenol or elevations in the intracellular level of cAMP down-regulate IL-2R expression on T cells at the protein level (Feldman et al., 1987; Johnson et al., 1988; Rincon et al., 1988; Krause and Deutsch, 1991; Anastassiou et al., 1992) and the mRNA level (Anastassiou et al., 1992), there are conflicting reports concerning which chains of the IL-2R are affected. For example, whereas one study reported that db-cAMP or forskolin decreased the number of only the high affinity IL-2R chain (p75 subunit) (Johnson et al., 1988), others have reported that similar concentrations of db-cAMP decrease the number of both the high and low affinity chains of the IL-2R on activated T cells in vitro (Rincon et al., 1988; Krause and Deutsch, 1991). To complicate matters, one other group has reported no effect of either similar concentrations of isoproterenol or db-cAMP on the level of IL-2R expressed by activated T cells in vitro (Chouaib et al., 1985). Although the source of these conflicting findings is currently unknown, the cell population being studied, the duration and type of T cell stimulus used, and the kinetics of IL-2R expression on the T cell should be considered. Finally, in addition to altering the level of IL-2R expression on T cells, elevations in intracellular cAMP may also influence signaling components associated with the IL-2R, such as JAK3, which is an essential mediator of IL-2R signaling (reviewed in Bacon et al., 1996). PGE_2 both inhibited the up-regulation of JAK3 in naive $CD4^+$ T cells and decreased JAK3 expression in activated $CD4^+$ T cells (Kolenko et al., 1999). The PGE_2-induced suppression of JAK3 was also mimicked by forskolin and db-cAMP, but exaggerated by IBMX, an inhibitor of cAMP phosphodiesterase. Thus, in addition to decreasing the level of IL-2R expression on naive and effector $CD4^+$ T cells in vitro, β2AR-induced elevations in cAMP may also down-regulate signaling components of the IL-2R in T cells.

In addition to cellular proliferation, surface molecule expression on $CD4^+$ T cells is critical for the execution of their effector functions. For example, CD40L is an essential molecule that is up-regulated on the surface of activated $CD4^+$ T cells, allowing them to provide the necessary "help" that B cells require to differentiate into antibody-secreting cells. Thus, alterations in CD40L expression on the $CD4^+$ T cell surface influences their ability to both initiate and modulate the level of B cell activation. Although exogenous addition of db-cAMP to T cells alone did not induce CD40L expression in vitro, exposure of PMA/ionomycin-activated T cells to db-cAMP synergistically increased both the level of CD40L mRNA and surface protein expression (Suarez et al., 1997). Another study reported that the effect of intracellular elevations of cAMP on the level of CD40L expression was dependent upon the mode of T cell activation. For example, whereas cAMP elevations inhibited TCR-induced levels of CD40L on T cells in vitro, cAMP enhanced Ca^{2+}-induced CD40L expression (Wingett et al., 1999). Thus, although no studies have specifically reported βAR-induced alterations in CD40L expression on T cells, it is likely that β2AR-induced elevations in the intracellular level of cAMP may regulate the level of T cell CD40L expression during the course of an immune response.

One important factor regulating T cell trafficking in vivo is adhesion molecule expression (reviewed in D'Ambrosio et al., 2000; Davenport et al., 2000). Multiple families of adhesion molecules have been described which regulate T cell homing, and the expression of these molecules on the T cell surface is regulated by a number of stimuli, such as cytokine receptor stimulation and cellular activation. Recent studies have investigated the effects of βAR stimulation on the level of other adhesion molecules expressed on T cells. For example, the incubation of T cells with isoproterenol for 2 h did not alter the level of LFA-1 or VLA-4 expression in vitro (Carlson et al., 1996), which are the counter-receptors

for ICAM-1 and VCAM-1 on T cells (Marlin and Springer, 1987; Dustin and Springer, 1988; Carlos et al., 1990). In agreement with these findings, others reported that isoproterenol infusion did not alter the level of LFA-1 expression in vivo, but significantly decreased the level of L-selectin on $CD4^+$ T cells (Mills et al., 2000). One possibility was that the effects of norepinephrine and βAR agonists on lymphocyte trafficking were mediated via the stimulation of adrenergic receptors expressed on endothelial cells, not on lymphocytes. However, βAR stimulation of endothelial cells did not alter their level of ICAM-1 and VLA-4 expression (Carlson et al., 1996). In light of these findings, the authors suggested that even though adhesion molecule expression may not be altered on either lymphocytes or endothelial cells, the affinity of the adhesion molecules may be influenced by βAR stimulation because this was shown to occur following interaction of lymphocytes with endothelial cells (Hourihan et al., 1993). Thus, further studies are necessary to gain a better understanding of the mechanisms by which norepinephrine and β2AR stimulation influence lymphocyte trafficking.

In addition to adhesion molecule expression on lymphocytes, chemokines also play an essential role in directing lymphocyte trafficking. T cells have been reported to express a number of different chemokine receptors, such as CXCR4, and stimulation of these receptors exerts a significant influence on the trafficking of these cells in vivo (reviewed in Syrbe et al., 1999). db-cAMP (10^{-4} M), forskolin (10^{-4} M), and norepinephrine (10^{-5} M) all enhanced the constitutive level of CXCR4 expression on $CD4^+$ T cells and $CD19^+$ B cells in comparison to unexposed cells (Cole et al., 1999). Interestingly, db-cAMP and norepinephrine both blocked the CD3/CD28 activation-induced decrease in CXCR4 expression, suggesting that intracellular elevations of cAMP not only elevated CXCR4 on resting $CD4^+$ T cells, but also maintained CXCR4 expression following activation of these cells. These findings are important because alterations in CXCR4 expression on lymphocytes influence their sensitivity to stromal cell-derived factor-1 (SDF-1) and may display altered trafficking patterns. Therefore, β2AR-mediated alterations in CXCR4 expression on $CD4^+$ T cells may influence their cell trafficking in vivo.

In summary, stimulation of the β2AR may differentially influence the trafficking of $CD4^+$ naive T cells, Th1 cells, and Th2 cells due to either differential β2AR expression or alterations in the level of adhesion molecules and chemokine receptor expression. However, future studies are necessary to further investigate the role of norepinephrine and β2AR stimulation in directing lymphocyte trafficking during an ongoing immune response, as well as in affecting chemokine production by both lymphocytes and nonlymphocytes.

D. T Cell Cytokine Production

1. In Vitro Th1-Like Cytokines. Table 3 summarizes past findings concerning the effects of norepinephrine, β2AR stimulation, and cAMP-elevating agents on T cell cytokine production in vitro and in vivo. A number of reports have suggested that the level of IL-2 production by activated T cells is affected either by agents that directly elevate the intracellular level of cAMP or β2AR-selective agonists. For example, exogenous addition of db-cAMP inhibited the level of IL-2 production by PHA-activated T cells (Chouaib et al., 1985; Van der Pouw-Kraan et al., 1992; Snijdewint et al., 1993). In addition, other cAMP-elevating agents such as PGE_2 (Minakuchi et al., 1990; Betz and Fox, 1991; Anastassiou et al., 1992), 8-bromo-cAMP (Anastassiou et al., 1992), cholera toxin (Anastassiou et al., 1992), or β2AR-selective agonists (Sekut et al., 1995; Ramer-Quinn et al., 1997; Holen and Elsayed, 1998) also decreased the level of IL-2 produced by either activated T cells or Th1 cell clones by decreasing the rate of IL-2 gene transcription (Anastassiou et al., 1992). Lastly, whereas most studies reported that either elevations in cAMP or β2AR stimulation decreased the level of IL-2 production by activated T cells, one study reported that the β2AR-selective salbutamol (albuterol) had no effect on IL-2 production (Sekut et al., 1995). However, most studies support the hypothesis that elevations in intracellular cAMP or stimulation of the β2AR decreases the level of IL-2 production regardless of the type of cAMP-elevating stimulus.

A few studies have investigated the mechanism by which elevations in the intracellular concentration of cAMP may inhibit IL-2 production by T cells. For example, PGE_2 exposure suppressed IL-2 production by decreasing the rate of calcineurin-dependent IL-2 gene transcription (Paliogianni et al., 1993). Similarly, restimulation of Con A-activated $CD4^+$ T cells 7 days later with either PMA and ionomycin, db-cAMP, cholera toxin, or PGE_2 resulted in a dose-dependent decrease in the level of IL-2 production (Lacour et al., 1994). Interestingly, the observed decrease in IL-2 production correlated with a decrease in the binding of nuclear factor of activated T cells (NF-AT) to the IL-2 promoter in these cells (Lacour et al., 1994). This finding was supported by others (Tsuruta et al., 1995) who also observed that elevations in the intracellular concentration of cAMP inhibited IL-2 gene transcription via both an inhibition of NF-AT binding and a decrease in NF-κB nuclear binding. Taken together, these studies suggest that β2AR-induced elevations in cAMP may decrease the level of IL-2 produced by $CD4^+$ T cells by decreasing the level of transcription factor binding to the IL-2 promoter site to decrease IL-2 gene transcription.

To gain a better understanding of the actions of norepinephrine on cytokine production by more physiologically relevant populations of Th1 and Th2 cells, recent studies investigated the role of β2AR stimulation in

TABLE 3
The effects of norepinephrine, β2AR stimulation, and cAMP-elevating agents on T cell cytokine production in vitro and in vivo

Effect	Stimulus	References
n.c.[a] IL-2	In vitro	
	β2AR	Sekut et al., 1995
	In vivo	
	NE depletion: lymph node cells	Madden et al., 1994
↑ IL-2	In vivo	
	NE-depletion: spleen cells	Kruszewska et al., 1995
↓ IL-2	In vitro	
	cAMP-elevating agents	Chouaib et al., 1985; Novak and Rothenberg, 1990; Munoz et al., 1990; Minakuchi et al., 1990; Betz and Fox, 1991; Anastassiou et al., 1992; Van der Pouw-Kraan et al., 1992; Paliogianni et al., 1993; Snijdewint et al., 1993; Lacour et al., 1994; Neumann et al., 1995; Tsuruta et al., 1995
	β2AR	Sekut et al., 1995; Ramer-Quinn et al., 1997, 2000; Holen and Elsayed, 1998; Swanson et al., 2001
	In vivo	
	NE depletion: spleen cells	Madden et al., 1994
↑ IFN-γ	In vivo	
	NE depletion: lymph node cells	Madden et al., 1994
↓ IFN-γ	In vitro	
	cAMP-elevating agents	Betz and Fox, 1991; Van der Pouw-Kraan et al., 1992; Snijdewint et al., 1993
	β2AR	Gajewski et al., 1990; Paul-Eugene et al., 1992; Ramer-Quinn et al., 1997; Sanders et al., 1997; Borger et al., 1998
	In vivo	
	NE depletion: spleen cells	Madden et al., 1994
↑ INF-γ	In vivo	
	NE depletion: lymph node cells	Madden et al., 1994
↓ IFN-γ	In vitro	
	cAMP-elevating agents	Betz and Fox, 1991; Van der Pouw-Kraan et al., 1992; Snijdewint et al., 1993
	β2AR	Gajewski et al., 1990; Paul-Eugene et al., 1992; Ramer-Quinn et al., 1997; Sanders et al., 1997; Borger et al., 1998
	In vivo	
	NE depletion: spleen cells	Madden et al., 1994
	NE-deficient: *L. monocytogenes* or *M. tuberculosis*	Alaniz et al., 1999
↑ IL-4	In vitro	
	cAMP-elevating agents	Betz and Fox, 1991; Lacour et al., 1994; Neumann et al., 1995; Teschendorf et al., 1996; Wirth et al., 1996
	In vivo	
	NE depletion: spleen cells	Kruszewska et al., 1995
n.c. IL-4	In vitro	
	cAMP-elevating agents	Munoz et al., 1990; Novak and Rothenberg, 1990; Betz and Fox, 1991; Van der Pouw-Kraan et al., 1992; Snijdewint et al., 1993
	β2AR	Paul-Eugene et al., 1992
↓ IL-4	In vitro	
	cAMP-elevating agents	Betz and Fox, 1991; Snijdewint et al., 1993; Borger et al., 1996
↑ IL-5	In vivo	
	cAMP-elevating agents	Lacour et al., 1994
n.c. IL-10	In vivo	
	cAMP-elevating agents	Teschendorf et al., 1996
↑ IL-10	In vivo	
	NE-deficient: *M. tuberculosis*	Alaniz et al., 1999
↓ IL-10	In vivo	
	NE-deficient: *L. monocytogenes*	Alaniz et al., 1999
↓ TNF-α	In vivo	
	NE-deficient: *L. monocytogenes* or *M. tuberculosis*	Alaniz et al., 1999

[a] Abbreviations: ↑, increase; ↓, decrease; β2AR, β_2-adrenergic receptor; IL, interleukin; n.c., no change; NE, norepinephrine; TNF, tumor necrosis factor.

modulating cytokine production by naive and newly generated $CD4^+$ T cells. Exposure of sort-purified naive $CD4^+$ T cells to norepinephrine (10^{-5} M) at the time of cell activation by anti-CD3 and anti-CD28 mAb stimulation decreased the level of IL-2 produced by naive $CD4^+$ T cells via stimulation of the β2AR (Ramer-Quinn et al., 2000). Implicating the role of the β2AR in these studies, terbutaline also decreased the level of IL-2 production by naive $CD4^+$ T cells in a concentration-dependent manner, and this effect was blocked by the βAR antagonist nadolol. Supporting these findings, another study used the β1AR-selective antagonist metoprolol and the β2AR-selective antagonist ICI 118,551 to determine that the norepinephrine-induced suppression of IL-2 production by naive $CD4^+$ T cells was mediated via stimulation of the β2AR expressed on these cells (Swanson et al., 2001). This was not surprising, since reverse transcriptase-polymerase chain reaction data also showed that naive $CD4^+$ T cells expressed β2AR mRNA, but not β1AR or β3AR mRNA. Thus, these studies sug-

gest a role for β2AR stimulation on the naive $CD4^+$ T cell in decreasing the level of IL-2 production by these cells.

In addition to IL-2 production by naive $CD4^+$ T cells, the role of βAR stimulation on the level of IFN-γ production by T cells has also been examined. PGE_2 exposure (10^{-6} M) inhibited the level of pigeon cytochrome *c*-induced IFN-γ production by a pigeon cytochrome *c*-specific Th1 cell clone (Betz and Fox, 1991). Other studies also reported that increased levels of intracellular cAMP decreased the level of IFN-γ production by T cells (Van der Pouw-Kraan et al., 1992; Snijdewint et al., 1993). Importantly, the β2AR-selective agonist salbutamol (10^{-5} M) decreased the level of IFN-γ production by PHA- and PMA-activated T cells (Paul-Eugene et al., 1992). In support of these findings, both the βAR-nonselective agonist isoproterenol and the β2AR-selective agonist fenoterol concentration-dependently inhibited Con A-induced IFN-γ and IL-3 mRNA expression by human T cells (Borger et al., 1998). Furthermore, the selectivity of the effect of βAR stimulation was demonstrated using a β2AR-selective antagonist ICI 118,551, the β1AR-selective antagonist atenolol, and the β3AR-selective agonist BRL 37344, showing that only stimulation of the β2AR expressed by these cells influenced the production of the cytokines examined. Finally, the β2AR-selective agonist terbutaline decreased the level of IFN-γ by resting (Sanders et al., 1997) Th1 cell clones in a concentration-dependent manner. Thus, the majority of past findings suggest that norepinephrine and β2AR stimulation may decrease the level of cytokine production by Th1 cells.

Finally, norepinephrine may exert different effects on Th1 cell cytokine production, depending on whether the naive or effector cell is exposed to norepinephrine. As previously discussed, naive $CD4^+$ T cells exposed to norepinephrine during the process of differentiation generated progeny Th1 cells that produced higher levels of IFN-γ upon restimulation with antigen in comparison to progeny Th1 cells generated in the absence of norepinephrine (Swanson et al., 2001). Thus, not only may norepinephrine exert activation stimulus-dependent effects on T cell cytokine production, but, in addition, norepinephrine may also exert differential effects on naive versus effector $CD4^+$ T cells.

2. In Vitro Th2-Like Cytokines. Although Th2 cells do not appear to express the β2AR (Sanders et al., 1997), some studies have investigated the effect of cAMP-elevating agents on the level of cytokine production by Th2 cells. Unfortunately, studies investigating the effect of elevated intracellular cAMP and PKA activation on IL-4 production have produced conflicting findings reporting either an increase, a decrease, or no change in the level of IL-4 production. For example, a number of studies have reported that cAMP-elevating agents increase the level of IL-4 production by $CD4^+$ T cells. Either db-cAMP, cholera toxin, or PGE_2 increased the level of IL-4 production by Con A-activated $CD4^+$ T cells restimulated 7 days later with PMA and ionomycin in a concentration-dependent manner (Lacour et al., 1994). Similarly, db-cAMP increased the level of IL-4 production by both mitogen-activated murine lymph node $CD4^+$ T cells and $CD4^+$ thymocytes in a concentration-dependent manner (10^{-6}–10^{-4} M) (Wirth et al., 1996). Finally, PKA activation by the cAMP phosphodiesterase inhibitor IBMX (100–750 μM) enhanced the level of IL-4 production by a mitogen-activated Th2 cell line in a concentration-dependent manner (Teschendorf et al., 1996). However, others have reported that high concentrations of db-cAMP (10^{-3} M) inhibited the level of IL-4 mRNA expression induced by the activation of human T cells by either Con A, anti-CD3 antibody, or anti-CD3/anti-CD28 antibody (Borger et al., 1996). Although the source of these conflicting findings is unknown, elevations in intracellular cAMP and PKA may increase the level of IL-4 production by T cells in a concentration-dependent manner until a threshold concentration is reached and these mediators begin to exert an inhibitory influence on IL-4 production. In addition, all studies reporting a cAMP- or PKA-induced increase in IL-4 measured cytokine production from mitogen-activated T cells, whereas the study reporting a cAMP-induced decrease in IL-4 production measured cytokine production by TCR-activated T cells. Thus, the method of cell activation may influence the role of cAMP and PKA in mediating IL-4 production by $CD4^+$ T cells.

In contrast, a number of studies have reported that both cAMP-elevating agents and the β2AR-selective agonist salbutamol failed to affect the level of IL-4 production by either mature T cells or Th2 cell clones (Novak and Rothenberg, 1990; Betz and Fox, 1991; Paul-Eugene et al., 1992; Van der Pouw-Kraan et al., 1992; Snijdewint et al., 1993). Because Th2 cells do not express the β2AR (Sanders et al., 1997), it is not surprising that salbutamol did not influence IL-4 production by Th2 cell clones. However, future studies are necessary to further investigate the importance of both the type of stimulus used to activate the T cells and the intracellular concentration of cAMP on the level of IL-4 production by Th2 cells.

In addition to IL-4, a few other studies have investigated the effects of cAMP-elevating agents and PKA activation on the level of IL-5 and IL-10 production by Th2 cells. For example, PGE_2 exposure (10^{-8}–10^{-6} M) enhanced IL-5 production in a concentration-dependent manner (Betz and Fox, 1991). Similarly, db-cAMP, cholera toxin, and PGE_2 all increased the level of IL-5 production by Con A-activated $CD4^+$ T cells restimulated 7 days later with PMA and ionomycin in a concentration-dependent manner (Lacour et al., 1994). Finally, PKA activation by the cAMP phosphodiesterase inhibitor IBMX did not affect the level of IL-10 production by a Th2 cell line (Teschendorf et al., 1996).

In summary, a number of studies have reported conflicting findings concerning the effects of cAMP-elevating agents on the level of IL-4 production by $CD4^+$ T cells, and fewer studies have investigated the effects of

these agents on other "Th2-like" cytokines. However, whereas a number of studies reported that elevations in intracellular cAMP do not affect the level of IL-4 production or IL-10 production by $CD4^+$ T cells, others have observed a concentration-dependent enhancement in the level of both IL-4 and IL-5 production. Importantly, if Th2 cells do not express the β2AR, then norepinephrine may only affect Th2 cell cytokine production indirectly via effects on other supporting cell populations, assuming that the αAR is not expressed during the resting or activated states of the Th2 cell.

3. In Vivo Cytokine Production. Unfortunately, there is a significant lack of literature concerning the effects of norepinephrine stimulation of the β2AR on cytokine production in vivo. Madden et al. (1994) reported an organ- and cytokine-specific effect of norepinephrine depletion on the level of cytokine production. For example, norepinephrine depletion increased the level of IFN-γ but did not affect IL-2 production by Con A-stimulated lymph node cells, in comparison with lymph node cells isolated from norepinephrine-intact mice. In contrast, norepinephrine depletion decreased the level of both IFN-γ and IL-2 production by Con A-stimulated spleen cells, in comparison to cells isolated from norepinephrine-intact mice. Thus, findings from these studies suggest that norepinephrine depletion may differentially affect the level of T cell cytokine production depending on both the specific cytokines measured and the target organ contributing the cells for study.

One study by Kruszewska et al. (1995) investigated the effects of norepinephrine depletion on the level of cytokine production in two strains of mice, C57BL/6J (Th1 cell-slanted strain) and BALB/c (Th2 cell-slanted strain) mice. Mice were injected once with 6-hydroxydopamine (6-OHDA), which significantly depleted norepinephrine in both strains of mice, and then immunized 2 days later with the T cell-dependent antigen KLH. Spleen cells isolated from norepinephrine-depleted C57BL/6J mice 3 or 6 days after antigen-induced activation in vivo produced significantly higher levels of IL-2 and IL-4 following restimulation in vitro, in comparison with cells isolated from norepinephrine-intact controls. In addition, similar findings were observed in experiments performed in Th2 cell-slanted BALB/c mice. Therefore, norepinephrine and β2AR stimulation may differentially affect $CD4^+$ T cell cytokine production, depending on the strain of mouse, the mode of T cell activation, and the specific cytokine measured. In addition, because the β2AR is differentially expressed by subpopulations of $CD4^+$ T cells, norepinephrine may also selectively influence naive and Th1 cell cytokine production in vivo.

4. Differential Effects on Th1 versus Th2 Cytokines. Studies by Gajewski et al. (1990) were some of the first to propose that alterations in intracellular cAMP may exert differential effects on Th1 and Th2 cell cytokine production. For example, cholera toxin and 8-bromo-cAMP more significantly inhibited the level of Th1 cell cytokine production in comparison with Th2 cell cytokine production. In support of these findings, cholera toxin inhibited TCR-induced IL-2 production and proliferation in Th1 cell clones, but not TCR-induced IL-4 production and proliferation in Th2 cell clones (Munoz et al., 1990). Since cholera toxin elevates the level of adenylyl cyclase activity by ribosylation of the G-stimulatory subunit of the G-protein, resulting in the accumulation of cAMP (Neer and Clapham, 1988), this differential effect of cholera toxin on Th1 and Th2 cytokine production may be directly related to the differential expression of the β2AR on these cell population, because β2AR-negative Th2 cells may express lower levels of G-protein to be activated by cholera toxin. However, because forskolin, a direct activator of adenylate cyclase, also failed to influence TCR-induced IL-4 production and proliferation in Th2 cells (Munoz et al., 1990), the lack of a cholera toxin-induced effect on Th2 cell IL-4 production is more likely to be the result of additional alternative mechanisms regulating the differential effect of cAMP-elevating agents on the level of cytokine production by Th1 and Th2 cells as opposed to a difference in G-protein expression. Finally, others suggested that the effect in intracellular cAMP may be gene-specific, not cell type-specific. Various cAMP-elevating agents decreased IL-2 mRNA expression by the murine thymoma EL4.E1 but had no effect on the level of IL-4 mRNA (Novak and Rothenberg, 1990). Therefore, intracellular cAMP may differentially affect Th1 and Th2 cell cytokine production via gene-specific mechanisms.

Surprisingly, both Th1 and Th2 cytokine responses were decreased in dopamine β-hydroxylase-deficient mice (Alaniz et al., 1999). Because dopamine β-hydroxylase enzymatically converts dopamine to norepinephrine (reviewed in Levi-Montalcini and Angeletti, 1966), mice deficient in this enzyme are deficient in norepinephrine. T cells isolated from the spleens of β-hydroxylase-deficient mice infected with *Listeria monocytogenes* produced lower levels of IFN-γ, TNF-α, and IL-10 in comparison with cells isolated from wild-type mice. However, T cells isolated from the spleens of β-hydroxylase-deficient mice infected with *Mycobacterium tuberculosis* produced lower levels of IFN-γ and TNF-α, but increased levels of IL-10, in comparison with cells isolated from wild-type mice. These studies suggest that norepinephrine may differentially influence both Th1- and Th2-like cytokine production, depending on the infection model system used. Because Th2 cells do not express βARs, the mechanism by which norepinephrine deficiency influences Th2 cell cytokine production is unknown. However, these findings may involve potential effects of norepinephrine deficiency on β2AR-positive naive $CD4^+$ T cells differentiating into Th2 cells or effects of norepinephrine deficiency on other cells, such as antigen-presenting cells, that influence the level of cytokine production by both Th1 and Th2 cells.

One study investigated the mechanism by which cAMP-dependent PKA activation inhibited the level of IL-2 production but enhanced the level of IL-4 production by T cells (Neumann et al., 1995). They reported a PKA-dependent increase in the level of expression, nuclear translocation, and DNA binding activity of a number of members of the Rel/NF-κB transcription factor family, such as c-Rel, p105/p50, and IκB. In addition, although there was no observed increase in the synthesis of the p65 subunit of NF-κB, increased PKA activity inhibited p65 nuclear translocation and DNA binding, possibly via stabilization of IκB-α. This finding was correlated with observations that forskolin inhibited mitogen-induced IL-2 promoter activity but enhanced the level of IL-4 promoter activity, due to the fact that the IL-2 promoter, but not the IL-4 promoter, contained an inhibitory κB binding site. Thus, the differential expression of various enhancing and inhibitory binding sites within the promoters of Th1 and Th2 cytokines may be another mechanism for the differential regulation of cytokine production in Th1 and Th2 cells.

V. Effects on B Lymphocytes

A. β2-Adrenergic Receptor Signaling Components

The earliest studies investigating the effects of βAR stimulation on immune cell function reported that stimulation of the receptor increased the level of intracellular cAMP accumulation and adenylyl cyclase activity in whole lymphocyte populations (Bourne and Melmon, 1971; Makman, 1971; Smith et al., 1971a,b; Williams et al., 1976; Astaldi et al., 1976; Conolly and Greenacre, 1977). Bach (1975) first reported that isoproterenol induced an accumulation of intracellular cAMP in murine splenic B cells. In contrast, this same group later reported that isoproterenol did not increase cAMP accumulation in human tonsillar B cells (Niaudet et al., 1976). However, because tonsillar B cells tend to display an activated phenotype in comparison to splenic B cells, which display a resting phenotype, these findings suggested that stimulation of the β2AR on resting, but not activated, B cells increased the level of intracellular cAMP. Subsequently, a number of studies reported that βAR stimulation induces adenylyl cyclase activity and increased intracellular cAMP accumulation in resting B cells (Galant et al., 1978; Bishopric et al., 1980; Pochet and Delespesse, 1983; Blomhoff et al., 1987; Holte et al., 1988; Kohm and Sanders, 1999).

As discussed previously, the number of βAR binding sites expressed on a B cell did not always correlate with the level of cAMP accumulation induced by βAR stimulation. A number of studies reported that whereas B cells expressed approximately twice the number of βARs as T cells, stimulation of the βAR on T cells generated higher levels of intracellular cAMP accumulation (Niaudet et al., 1976; Galant et al., 1978; Bishopric et al., 1980; Pochet and Delespesse, 1983). The mechanism responsible for the apparent inverse relationship between the level of βAR expression on T and B cells and the level of isoproterenol-induced cAMP accumulation in these cells is currently unknown. One possible explanation is that T and B cells have varying levels of membrane fluidity, such that if the cell membrane is more rigid, β2AR activation may less effectively activate membrane-bound adenylyl cyclase. Also, a number of additional mechanisms have been reported that influence the effectiveness of β2AR stimulation in initiating intracellular signaling cascades, such as the receptor phosphorylation state, and any one of these processes may be differentially active in T and B cells (reviewed in Hein and Kobilka, 1995; Bouvier and Rousseau, 1998; Lefkowitz et al., 1998).

B. B Cell Proliferation

Similar to CD4+ T cells, B cell proliferation is an essential component of an ongoing immune response. Only a small percentage of the body's B cells are capable of responding to any given protein antigen. Therefore, upon antigen-induced B cell activation, it is critical that the small antigen-specific cell population expand its numbers so that a suitable number of B cells are generated to differentiate into both antibody-secreting cells and memory B cell. Table 4 summarizes past findings

TABLE 4
The effects of β2AR stimulation and cAMP-elevating agents on B cell proliferation in vitro

Effect	Stimulus	References
↑[a] Proliferation	cAMP	
	IL-1 and anti-Ig-stimulated B cells	Hoffmann, 1988
	PMA/ionomycin-stimulated B cells	Cohen and Rothstein, 1989
	IL-4 and anti-Ig-stimulated B cells	Vazquez et al., 1991
	β2AR	
	LPS-stimulated B cells	Li et al., 1990
↓ Proliferation	cAMP	
	LPS-stimulated nude spleen cells	Diamantstein and Ulmer, 1975
	B cell precursor line	Blomhoff et al., 1987
	IL-4 and anti-Ig-stimulated B cells	Hoffman, 1988
	Anti-Ig-stimulated B cells	Cohen and Rothstein, 1989
	IL-2 and anti-Ig-stimulated B cells	Vazquez et al., 1991

[a] Abbreviations: ↑, increase; ↓, decrease; β2AR, β_2-adrenergic receptor; Ig, immunoglobulin; IL, interleukin; LPS, lipopolysaccharide; NE, norepinephrine; PMA, phorbol ester.

concerning the effects of β2AR stimulation and cAMP-elevating agents on B cell proliferation in vitro.

Studies by Diamantstein and Ulmer (1975) were the first to investigate the effects of intracellular cAMP levels on the rate of B cell proliferation. Using the B cell mitogen LPS, these findings suggested that the addition of exogenous cAMP (10^{-3} M) blocked the LPS-induced elevation in spleen cell proliferation in vitro. These studies were also performed using spleen cells isolated from nude mice, which lack T cells, to enrich for the percentage of B cells in the spleen. However, monocytes, which also expressed adrenergic receptors on their surface and are responsive to LPS stimulation, remained in these spleens and, thus, the exact role of norepinephrine specifically on B cells was still unknown.

In support of these early findings, forskolin decreased the proliferation of a B cell precursor cell line in vitro, a finding which correlated with the down-regulation of c-*myc* and c-Ha-*ras* expression, two known proto-oncogenes (Blomhoff et al., 1987). Importantly, the effect of cAMP elevation on the rate of B cell proliferation may be dependent upon certain activation signals. For example, db-cAMP (10^{-4} M) inhibited the effects of IL-4 (known then as BSF-1) but enhanced the effects of IL-1 on anti-IgM antibody-induced B cell proliferation in vitro (Hoffmann, 1988). Similarly, db-cAMP (10^{-4} M) and IBMX (10^{-4} M) both decreased the level of anti-IgM antibody-induced B cell proliferation in vitro, but increased the level of PMA/ionomycin-induced B cell proliferation (Cohen and Rothstein, 1989). Finally, one group reported that cAMP elevations influenced the rate of B cell proliferation in a cytokine-specific manner (Vazquez et al., 1991). For example, both forskolin and db-cAMP decreased the rate of anti-IgM antibody-induced proliferation in the presence of IL-2 but enhanced the level of B cell proliferation in the presence of IL-4. In addition to cAMP-elevating agents, the role of βAR agonists in modulating B cell proliferation has also been studied. Norepinephrine and isoproterenol both increased the rate of LPS-induced B cell proliferation in vitro, and this effect was blocked by the βAR antagonist propranolol, but not the αAR antagonist phentolamine (Li et al., 1990).

Thus, these studies emphasize the fact that the effects of cAMP on the level of B cell proliferation cannot be generalized, such that elevations in cAMP may exert differing effects on B cell proliferation in vitro, depending on the B cell activation stimulus used and the cytokines present within the microenvironment. Unfortunately, there is a lack of data concerning the effects of norepinephrine and β2AR stimulation on B cell proliferation in vivo.

C. B Cell Surface Molecule Expression and Function

1. In Vitro Surface Molecule Expression and Function. One molecule expressed on the surface of B cells that plays an important role in B cell function is B7–2 (CD86). B7–2 is a costimulatory molecule that is either induced or constitutively expressed on all types of antigen-presenting cells. B7–2 expression on the B cell serves two functions. First, B7–2-mediated stimulation of either CD28 or CTLA-4 expressed on the surface of T cells exerts a critical regulatory influence on T cell cytokine production and surface molecule expression, thus indirectly influencing B cell function by modulating the level of "help" that the cell receives from T cells. Second, stimulation of B7–2 sends a signal directly into the B cell to regulate the level of antibody production (Kasprowicz et al., 2000). Thus, alterations in the level of B7–2 expression on the B cell surface is one mechanism to both directly and indirectly regulate B cell function.

Presently, the exact regulatory mechanisms that govern B7–2 expression on a B cell are unknown. Whereas the B7–2 protein is expressed at very low levels on resting B cells (Lenschow et al., 1993), B7–2 mRNA expression and protein expression peak at approximately 12 and 24 h following BCR- or LPS-induced activation of B cells, respectively (Freeman et al., 1993; Lenschow et al., 1993, 1994). Therefore, cell activation appears to be one mechanism by which B7–2 protein expression is up-regulated on B cells. In addition to cell activation, a number of other stimuli are now known to enhance the level of B7–2 expression on B cells, such as cytokine receptor stimulation (reviewed in Lenschow et al., 1996). Interestingly, stimulation of the β2AR alone on resting B cells increased the level of B7–2 expression (Kasprowicz et al., 2000). In addition, concomitant stimulation of both the B cell receptor and the β2AR resulted in an additive increase in the level of B7–2 expression on B cells, suggesting the change in B7–2 expression may be one mechanism by which β2AR stimulation can influence the T-dependent immune response.

The mechanism by which in vitro stimulation of the B cell-associated β2AR regulates the level of B7–2 expression on the B cell has been investigated. One study reported two molecular mechanisms by which stimulation of the BCR and/or β2AR may cooperate to up-regulate the level of B7–2 surface protein and mRNA expression in B cells, i.e., increased mRNA stability and NF-κB-dependent gene transcription (A. P. Kohm and V. M. Sanders, manuscript submitted for publication). Importantly, the concurrent stimulation of both receptors resulted in an additive enhancement in the level of B7–2 expression on the B cell, and this cooperative effort between the BCR and β2AR may be one mechanism by which signals originating from the immune and nervous system synergize to regulate immune cell function. However, future studies are necessary to further dissect the mechanism by which stimulation of the BCR and β2AR cooperate to regulate the level of B7–2 expression on the B cell, as well as other B cell-associated molecules that may also be influenced by norepinephrine.

In addition to B7–2, stimulation of the β2AR may also alter the level of expression of various other surface

molecules on the B cell surface. Exposure to db-cAMP (10^{-4} M) slightly decreased the level of MHC class II expression and sIgD expression on resting B cells but did not alter the level of these molecules on PMA/ionomycin-activated B cells (Li et al., 1989). Similarly, norepinephrine and isoproterenol did not influence the level of MHC class II expression on LPS-activated B cells (Li et al., 1990). In agreement with these findings, antigen-induced activation or IL-4 exposure enhanced the level of MHC class II expression on antigen-specific B cells, whereas terbutaline alone did not alter the resting level of MHC class II expression (Sanders and Powell-Oliver, 1992).

In summary, in vitro findings suggest that norepinephrine and β2AR stimulation may cooperate with signals originating from the immune system to regulate surface molecule expression on the B cell surface, such as B7–2 and MHC class II. However, future studies are necessary to translate these findings into in vivo model systems, as well as to determine whether the expression of other surface molecules is affected by signals originating from the sympathetic nervous system.

2. In Vivo Surface Molecule Expression. Unfortunately, very few studies have investigated the effects of norepinephrine and/or β2AR stimulation on surface molecule expression in vivo. However, one important observation in respect to the previously discussed norepinephrine depletion studies is that norepinephrine depletion in vivo may alter the level of adrenergic receptor expression. For example, Miles et al. (1981, 1984, 1985) investigated the effect of 6-OHDA treatment on βAR expression on T and B cells. They showed that 1 week after norepinephrine depletion via 10 daily injections of 6-OHDA (100 mg/kg), there was a significant increase in the level of βAR expression on both splenic T and B lymphocytes in comparison to cells from saline-injected animals. In contrast, others reported no effects of 6-OHDA-mediated norepinephrine depletion on the lymphocyte surface density of βAR (Nahorski et al., 1979), even though the norepinephrine depletion protocol in these studies was milder than that used by Miles et al. (1984). Thus, norepinephrine depletion in vivo may influence the level of βAR expression on B cells, depending on the model system and treatment protocol used.

D. B Cell Differentiation and Antibody Production

1. In Vitro Direct Alterations Induced by Elevations in Intracellular cAMP. Resting B cells become activated following recognition of antigen by the surface immunoglobulin component of the BCR. Upon B cell activation, these cells must first differentiate into plasma cells prior to producing and secreting antibody. Therefore, the total amount of antibody produced in response to a specific antigen is dependent upon the number of B cells that differentiate into antibody-secreting cells, the level of antibody secreted per plasma cell, and the function of other accessory cells that are critical to the successful formation of a T cell-dependent antibody response. In light of this, it is important to determine whether norepinephrine and β2AR stimulation influence B cell function by either effects on B cell differentiation or plasma cell function. Tables 5 and 6 summarize past findings concerning the effects of cAMP-elevating agents on B cell differentiation into antibody-secreting cells in vitro and in vivo and the level of B cell antibody production in vitro, respectively.

Due to technological limitations at the time, early studies that examined the role of cAMP in modulating antibody production used whole spleen cell cultures. One early observation was that theophylline, a phosphodiesterase inhibitor that decreases cAMP degradation, and poly(A:U), a cAMP stimulator, both enhanced the number of antibody-secreting cells in response to the particulate antigen sRBC (Ishizuka et al., 1971; Robison and Sutherland, 1971). However, the concentration-dependent curves generated by these agents on B cell differentiation were bell-shaped, such that low (0.01–0.1

TABLE 5
The effects of cAMP-elevating agents on B cell differentiation into antibody-secreting cells in vitro and in vivo

Effect	Stimulus	References
↑[a] ASC	In vitro	
	[Low-med] sRBC-activated spleen cells	Ishizuka et al., 1971; Marchalonis and Smith, 1976
	[Low] sRBC + LPS-activated spleen cells	Watson et al., 1975
	[High] <24 h: spleen cells	Teh and Paetkau, 1974
	CT + LPS + IL-4-activated B cell IgG_1	Lyche et al., 1990; Coqueret et al., 1996
	LPS + IFN-γ-activated B cell IgG_{2a}	Stein and Phipps, 1991
	In vivo	
	sRBC immunization	Braun and Ishizuka, 1971
n.c. ASC	In vitro	
	[Low] sRBC-activated spleen cells	Marchalonis and Smith, 1976
↓ ASC	In vitro	
	[High] sRBC-activated spleen cells	Ishizuka et al., 1971; Marchalonis and Smith, 1976
	[High] sRBC + LPS-activated spleen cells	Watson et al., 1973; Watson et al., 1975
	[Med-High] sRBC-activated spleen cells	Melmon et al., 1974; Montgomery et al., 1975
	[High] >24 hours: spleen cells	Teh and Paetkau, 1974
	CT + LPS + IL-4-activated B cell IgM, IgA, and IgG_3	Lyche et al., 1990

[a] Abbreviations: ↑, increase; ↓, decrease; [ ], concentration; ASC, antibody-secreting cells; CT, cholera toxin; IFN, interferon; Ig, immunoglobulin; IL, interleukin; LPS, lipopolysaccharide; n.c., no change; sRBC, sheep red blood cell.

TABLE 6
The effects of cAMP-elevating agents on the level of B cell antibody production in vitro

Effect	Stimulus	References
↑[a] Antibody production	Exposure < 24 h: anti-Ig stimulation of primed spleen cell IgG	Kishimoto and Ishizaka, 1976
	Exposure < 24 h: anti-Ig stimulation of spleen cell IgM	Kishimoto et al., 1977; Cook et al., 1978; Stanford et al., 1979; Koh et al., 1995
	sRBC + IL-1-activated B cells	Gilbert and Hoffmann, 1985
	IL-4-activated B cell IgG_1 and IgE	Roper et al., 1999
	LPS + IL-4-activated B cell IgE	Coqueret et al., 1996
	CT + LPS + IL-4-activated B cell IgG_1	Lyche et al., 1990
↓ Antibody production	Exposure for > 24 h: anti-Ig stimulation of primed spleen cell IgG	Kishimoto and Ishizaka, 1976
	Exposure for > 24 h: anti-Ig stimulation spleen cell IgM	Kishimoto et al., 1977; Cook et al., 1978; Stanford et al., 1979; Koh et al., 1995
	IL-4-activated B cells IgM and IgG_3	Roper et al., 1999
	CT + LPS + IL-4-activated B cells IgM, IgA, and IgG_3	Lyche et al., 1990

[a] Abbreviations: ↑, increase; ↓, decrease; CT, cholera toxin; IFN, interferon; Ig, immunoglobulin; IL, interleukin; LPS, lipopolysaccharide; sRBC, sheep red blood cell.

ng/ml) and high (100 ng/ml) concentrations of theophylline had no effect, whereas medium concentrations (1–10 ng/ml) significantly enhanced the number of antibody-secreting cells. A similar pattern was observed with poly(A:U) treatment, except that high concentrations of poly(A:U) (100 ng/ml) suppressed the number of antibody-secreting cells. The role of cAMP in mediating the effects of poly(A:U) treatment on the number of antibody-secreting cells was further suggested by the potentiation of the poly(A:U)-induced increase in the number of antibody-secreting cells by theophylline (Braun and Ishizuka, 1971). In contrast, others reported that varying concentrations of either db-cAMP (10^{-5}–10^{-3} M) or theophylline (10^{-3} M) inhibited the number of antibody-secreting cells produced in response to sRBC-induced B cell activation (Watson et al., 1973; Melmon et al., 1974; Montgomery et al., 1975). Finally, whereas a high concentration of db-cAMP (10^{-3} M) inhibited the number of antibody-secreting cells in response to LPS and sRBC stimulation, a slightly lower concentration of db-cAMP (10^{-4} M) enhanced the number of antibody-secreting cells (Watson, 1975). Therefore, there appear to be conflicting findings concerning the effect of cAMP-elevating agents on the number of antibody-secreting cells.

Others have reported a biphasic effect by varying concentrations of cAMP on the formation of antibody-secreting cells following sRBC-induced B cell activation. For example, the addition of relatively low concentrations of db-cAMP (10^{-7}–10^{-6} M) to spleen cell cultures at the time of sRBC activation had no effect on the number of antibody-secreting cells, but moderate concentrations of db-cAMP (10^{-5}–10^{-4} M) increased and higher concentrations db-cAMP (10^{-3} M) decreased the number of antibody-secreting cells (Marchalonis and Smith, 1976). In addition, exposure of sRBC-activated spleen cell cultures to db-cAMP did not alter the kinetics of the ensuing antibody response, a finding that was mimicked when spleen cells isolated from nude mice, which do not possess peripheral T cells, were used. However, although these studies may have ruled out the possibility that cAMP-elevating agents were influencing B cell function indirectly via alterations in T cell function, other cell types may also have been affected in this model system, such as macrophages and dendritic cells, that might have contributed to the alterations in B cell differentiation.

Importantly, elevations in the intracellular concentration of cAMP did not always inhibit the formation of antibody-secreting cells. For example, a biphasic effect of cAMP on the formation of antibody-secreting cells was observed, depending on the length of exposure to the cAMP-elevating agent. For example, if the cAMP-elevating agents db-cAMP (10^{-3} M) or aminophylline (10^{-3} M) were present only during the first 24 h of B cell activation, but then washed out of the culture, the number of antibody-secreting cells was enhanced at days 4 and 5 of culture (Teh and Paetkau, 1974). However, if the cAMP-elevating agents were present at times later than 24 h, the formation of antibody-secreting cells was inhibited. Thus, these studies were some of the first to suggest that cAMP can exert time-dependent effects on B cell function, such that early elevations in the level of cAMP increase the number of antibody-secreting cells, whereas later elevations in cAMP inhibit the same response.

In addition to influencing the number of B cells differentiating into antibody-secreting cells, elevations in intracellular cAMP may also influence the level of antibody produced. DNP-primed spleen cells activated with either anti-Ig or DNP in the presence of db-cAMP (10^{-3}–10^{-4} M) produced more antigen-specific IgG and total IgG in comparison to B cells stimulated in the absence of db-cAMP (Kishimoto and Ishizaka, 1976). However, if db-cAMP was added after 24 h of activation, the level of IgG production was suppressed. In addition, similar results were obtained with the cAMP phosphodiesterase inhibitor aminophylline. Therefore, these studies and others suggested that cAMP may exert differential effects on B cell function, such that increases in intracellular cAMP concentrations during the early stages of T cell-dependent B cell activation enhanced the level of antibody production, whereas elevations in intracellular

cAMP during the later stages of cellular activation suppressed the level of antibody production (Kishimoto et al., 1977; Cook et al., 1978; Sanford et al., 1979; Koh et al., 1995).

Of importance, the previously discussed studies investigating the effects of cAMP-elevating agents on the level of antibody production used whole lymphocyte cultures. Because these cultures contained various cell populations that may be affected by elevations in cAMP and that may influence the level of antibody production by B cells, it was possible that elevations in cAMP were not exerting direct effects on B cells but, instead, were influencing the function of other accessory cells to modulate the level of antibody production by B cells.

Using purified populations of B cells, the influence of cAMP elevation specifically in B cells was investigated (Gilbert and Hoffmann, 1985). This study reported that B cells activated with either sRBCs alone or in combination with either IL-1 or cAMP (10^{-3} M) did not differentiate into antibody-secreting cells. However, when the sRBC-activated B cells were exposed to both IL-1 and db-cAMP concurrently, a significant number of antibody-secreting cells formed in comparison to B cells activated in the absence of db-cAMP. Importantly, these studies suggested that cAMP elevations were not sufficient to induce antibody formation, even following activation of B cells with sRBCs. Thus, it is possible that cAMP contributed to a signal generated by either IL-1R or BCR stimulation.

A later study further investigated the mechanism by which elevations in the intracellular concentration of cAMP decreased the level of antibody production during the later stages of cell activation. PGE_1-mediated increases in cAMP decreased the level of spontaneous IgM and PMA-enhanced IgM production by transformed B cells (Patke et al., 1991). These data support earlier findings concerning the relationship between the timing of cAMP exposure and the level of antibody produced. Since these studies utilized transformed B cells, which spontaneously produced antibody, the cells were already in an activated state. Thus, these studies supported previous findings suggesting that elevations in intracellular cAMP accumulation decreased the level of antibody production under conditions in which these elevations occurred either at a time later than 24 h following B cell activation or after the B cell had already differentiated into an antibody-secreting cell.

In contrast to previous studies that measured IgM production alone, elevations in cAMP have been reported to also influence the antibody isotype produced by B cells. For example, PGE_2 (10^{-8}–10^{-6} M) significantly enhanced the level of IgG_1 and IgE produced by LPS-activated B cells, but decreased the level of IgM and IgG_3 production, in the presence of varying concentrations of IL-4 (100–10,000 U/ml) (Roper et al., 1990). In addition, other cAMP-elevating agents such as cholera toxin (100 pg/ml) and db-cAMP (10^{-4} M) exerted similar effects on the level of antibody production. Thus, these findings suggested that intracellular elevations in cAMP contributed to the IL-4-dependent antibody response to increase the switching of antibody production to "Th2-like" isotypes, such as IgG_1 and IgE, or expanded a population of cells that had already switched to IgG_1 and IgE.

Because stimulation of the B cell IL-4R has been reported to increase the intracellular cAMP levels in B cells within 10–20 min of exposure (Finney et al., 1990; Rigley and Callard, 1991; McKay et al., 2000), it is possible that the level of intracellular cAMP influences the level of B cell IgG_1 and IgE production. In support of these findings, cholera toxin-induced elevations in intracellular cAMP synergistically enhanced the number of IgG_1-producing B cells and the level of germline γ1 transcript produced in B cells exposed to both LPS and IL-4, but decreased the number of IgM-, IgA-, and IgG_3- producing B cells (Lycke et al., 1990). These findings were extended to show that PGE_2 exposure of LPS-activated B cells resulted in a quicker generation of germline ϵ transcripts, as well as a higher level of gene transcription (Roper et al., 1995). It was also reported that cAMP-elevating agents increased the level of IgE production (Coqueret et al., 1996). Finally, IL-4 enhanced the effect of cholera toxin in a concentration-dependent manner to increase the number of IgG_1-producing B cells. Thus, these studies further supported the hypothesis that elevations in cAMP complement the IL-4-induced effects on B cells. However, other studies reported that elevations in the level of intracellular cAMP augmented the IFN-γ receptor signaling pathway. For example, exposure of IFN-γ-pulsed B cells to either db-cAMP, PGE_2, or cholera toxin enhanced both the number of IgG_{2a}-producing B cells and the total level of IgG_{2a} production following LPS-induced activation (Stein and Phipps, 1991). Thus, elevations in the intracellular concentration of cAMP may influence the effects of cytokines on the level and isotype of antibody produced by the B cell, perhaps by altering the signaling pathways associated with cytokine receptors.

In summary, the mechanism by which cAMP contributes to the intracellular signaling events that induce antibody production is unknown, but several possibilities exist. For example, it is known that the physical interaction between a T cell and a B cell results in cAMP accumulation within the B cell (Pollok et al., 1991). In addition, stimulation of the B cell receptor and cytokine receptors may result in the intracellular accumulation of cAMP, and elevations in cAMP may augment the signal transduction pathways initiated by stimulation of these receptors to increase either the number of antibody-secreting cells or the level of antibody produced by B cells.

2. In Vitro β2-Adrenergic Receptor Stimulation. Tables 7 and 8 summarize past findings concerning the effects of norepinephrine and β2AR stimulation on B cell

TABLE 7
The effects of norepinephrine and β2AR stimulation on B cell differentiation into antibody-secreting cells in vitro and in vivo

Effect	Stimulus	References
↑[a] ASC	In vitro	
	[Med NE/Terb] early: IgM and sRBC-activated spleen cells	Sanders and Munson, 1984a,b
	NE later: Ig and sRBC-activated spleen cells	Kouassi et al., 1988
	NE: IgM and TNP-KLH B/Th2 cell cultures	Sanders and Powell-Oliver, 1992
	In vivo NE depletion	
	Ig and sRBC immunization	Kasahara et al., 1977; Hall et al., 1982
n.c. ASC	In vitro	
	[Intermediate NE/Terb] late: IgM and Ig-sRBC-activated spleen cells	Sanders and Munson, 1984a,b; Kouassi et al., 1988
	NE: IgG and TNP-KLH B/Th2 cell cultures	Sanders and Powell-Oliver, 1992
	NE: IgM, IgG_1, and IgE-TNP-KLH + IL-4-exposed B cell IgG_1	Kasprowicz et al., 2000
	In vivo NE-depletion	
	Ig: T-dependent antigen	Miles et al., 1981
↓ ASC	In vitro	
	[Med-High NE/Iso] late: Ig-sRBC-activated spleen cells	Melmon et al., 1974
	In vivo NE depletion	
	Newborn depleted: Ig-sRBC immunization	Besedovsky et al., 1979
	Ig: T-independent antigen	Miles et al., 1981

[a] Abbreviations: ↑, increase; ↓, decrease; [ ], concentration; ASC, antibody-secreting cells; β2AR, β_2-adrenergic receptor; Ig, immunoglobulin; IL, interleukin; Iso, isoproterenol; KLH, keyhole limpet hemocyanin; LPS, lipopolysaccharide; n.c., no change; NE, norepinephrine; sRBC, sheep red blood cell; Terb, terbutaline; TNP, trinitrophenyl.

TABLE 8
The effects of norepinephrine and β2AR stimulation on the level of B cell antibody production in vitro and in vivo

Effect	Stimulus	References
↑[a] Antibody production	In vitro	
	NE early: TNP-KLH + IL-4-exposed B cells (IgG_1 and IgE)	Kasprowicz et al., 2000
	Terb early: Anti-Ig + anti-CD40 + IL-4-exposed B cells (IgG_1 and IgE)	Kasprowicz et al., 2000
	NE/Iso: LPS-activated B cells (IgM, IgG, and IgA)	Li et al., 1990
	Salb/Feno: LPS + IL-4-activated PBMC (IgE)	Paul-Eugene et al., 1992; Coqueret et al., 1996
	In vivo NE depletion	
	sRBC immunization	Kasahara et al., 1977; Williams et al., 1981; Hall et al., 1982
	NE depletion prior to 2° Ab response: effects measured on 2° Ab response-sRBC immunization	Kasahara et al., 1977
	TNP-KLH immunization	Alaniz et al., 1999
	KLH immunization BALB/c mice (IgG_1)	Kruszewska et al., 1995
	TNP-KLH immunization of scid mice reconstituted with B cells and Th2 cells (Ag-specific IgM and IgG_1)	Kohm and Sanders, 1999
n.c. Antibody production	In vivo NE depletion	
	NE depletion prior to 1° Ab response: effects measure on 2° Ab response	Kasahara et al., 1977
	KLH immunization BALB/c mice (IgM, IgG_1, and IgG_{2a})	Kruszewska et al., 1995
↓ Antibody production	In vivo Ne depletion	
	KLH immunization C57BL/6J mice (IgM, IgG_1, and IgG_{2a})	Kruszewska et al., 1995

[a] Abbreviations: ↑, increase; ↓, decrease; [ ], concentration; Ab, antibody; Ag, antigen; β2AR, β_2-adrenergic receptor; Feno, fenoterol; Ig, immunoglobulin; IL, interleukin; Iso, isoproterenol; KLH, keyhole limpet hemocyanin; LPS, lipopolysaccharide; n.c., no change; NE, norepinephrine; Salb, salbutamol; sRBC, sheep red blood cell; Terb, terbutaline; TNP, trinitrophenyl.

differentiation into antibody-secreting cells and the level of B cell antibody production in vitro, respectively. As with studies investigating the effects of elevated intracellular cAMP levels in B cells on antibody production, early studies investigating the effects of βAR agonists on the level of antibody production in vitro used whole splenic cell populations. Norepinephrine (10^{-6}–10^{-3} M) and isoproterenol (10^{-6}–10^{-3} M) both dose dependently inhibited the number of antibody-secreting cells in response to the particulate antigen sRBC when added at later times following B cell activation (Melmon et al., 1974). Importantly, the αAR agonist phenylephrine (10^{-6}–10^{-3} M) did not significantly affect the number of antibody-secreting cells, whereas later studies reported that α2AR stimulation decreases the level of antibody production (Sanders and Munson, 1985b). However, because these studies investigated the differentiation of B cells into antibody-secreting cells using unfractionated spleen cell populations, αAR-mediated effects may have been due to the expression of these receptors on non-B cells.

More specifically, concurrent addition of either norepinephrine (10^{-5} M) or terbutaline (10^{-5} M) and sRBCs to spleen cell cultures significantly enhanced the number of anti-sRBC antibody-secreting cells, whereas addition of norepinephrine to sRBC-activated B cells at later times following cell activation resulted in a loss of the norepinephrine-induced increase in the number of antibody-secreting cells (Sanders and Munson, 1984a). Thus, stimulation of the β2AR at earlier times during the B cell response to sRBC enhanced the number of antibody-secreting cells. These findings were supported

by later studies in which sRBC-activated spleen cells were exposed to either norepinephrine or terbutaline at the time of activation. At varying times following concurrent cell activation and β2AR stimulation, a βAR antagonist was added to the cultures and the number of antibody-secreting cells was determined on day 5 of culture (Sanders and Munson, 1984b). Addition of the βAR antagonist within 6 h of activation inhibited the maximal effect of β2AR stimulation to increase the number of antibody-secreting cells, whereas the addition of the βAR antagonist at either 6 h or later following cell activation did not alter the terbutaline-induced increase in the number of antibody-secreting cells. Thus, these studies suggested that stimulation of the B cell β2AR initiated intracellular signals during the first 6 h of cell activation that were critical to the β2AR-induced increase in the number of antibody-secreting cells. Others reported that norepinephrine enhanced the level of LPS-induced antibody production by whole splenic cell cultures depleted of T cells when norepinephrine was added to the cultures at the time of LPS exposure, but not when added 2 h following cell activation, an effect that was blocked by the βAR antagonist propranolol, but not by the αAR antagonist phentolamine (Kouassi et al., 1988). Taken together, these findings suggest that stimulation of the B cell β2AR at times early during cell activation may increase the number of antibody-secreting cells or the level of antibody produced by antibody-secreting cells, whereas B cell differentiation and function may be inhibited by β2AR stimulation at later times following cell activation.

However, because the frequency of antigen-specific B cells is relatively low in the spleen, and because other cell types are present in these cultures that also express adrenergic receptors, later studies elucidated the role of β2AR stimulation in modulating B cell function using enriched populations of antigen-specific B cells and T cell clones (Sanders and Powell-Oliver, 1992). TNP-specific B cells were cocultured with KLH-specific $CD4^+$ Th2 cell clones in the presence of TNP-KLH and terbutaline (10^{-6} M). Terbutaline increased the number of anti-TNP IgM-secreting cells 3 to 5 days following the initiation of culture. In addition, terbutaline increased both the number of anti-TNP IgM-secreting cells and the total level of TNP-specific antibody, in a concentration-dependent manner (10^{-9}–10^{-6} M). These effects of terbutaline were blocked by the βAR antagonists nadolol and propranolol, but not by the αAR antagonist phentolamine, ensuring the participation of β2AR stimulation in mediating the effects of terbutaline. This study also reported a nonsignificant effect of terbutaline on the number of IgG1-secreting cells; however, later studies reported that terbutaline increased the total amount of IgG1 secreted by B cells, not the number of secreting cells (Kasprowicz et al., 2000). In support of these findings, βAR stimulation by either isoproterenol or norepinephrine increased the level of IgM, IgG, and IgA produced by LPS-activated B cells (Li et al., 1990). Importantly, the effects of βAR-stimulating agents were blocked by propranolol, but not by phentolamine. Thus, these studies using B cells activated by either a soluble protein antigen (TNP-KLH) or a B cell mitogen (LPS) suggest that stimulation of the B cell-associated β2AR enhanced both the number of antibody-secreting cells and the total level of antibody produced by B cells.

As discussed earlier, previous studies using cAMP-elevating agents had implicated increases in the intracellular level of cAMP in augmenting IL-4R signaling. In addition, this hypothesis has been supported by studies that employed β2AR-selective agonists. Exposure of PBMC to either the β2AR-selective agonist salbutamol (10^{-10}–10^{-6} M) or fenoterol (10^{-10}–10^{-6} M) increased the level of IL-4-dependent IgE production which was blocked by the βAR antagonist propranolol (Paul-Eugene et al., 1992). These findings suggested that the effect of β2AR stimulation was mediated via the release of soluble CD23 receptors that modulated the level of B cell activation and IgE production (reviewed in Delespesse et al., 1989). Also, fenoterol enhanced both IL-4-induced IgE mRNA expression and protein secretion by human PBMC, and this effect correlated with the β2AR-induced increase in the level of intracellular cAMP (Coqueret et al., 1996). The role of cAMP in mediating the effect of β2AR stimulation on IgE production was further supported by the ability of PKA inhibitors, H8 (10^{-5} M) and Rp-AMP (10^{-5} M), which compete for the ATP-binding site of the PKA catalytic subunit, to block the effects of β2AR stimulation on IgE production. Pretreatment of PBMC with the cyclo-oxygenase inhibitor indomethacin significantly decreased the effects of fenoterol on the level of IgE production, suggesting that fenoterol may also stimulate the production of prostaglandins, which subsequently induce cAMP accumulation and PKA activation. Finally, fenoterol also enhanced the level of CD40-induced IgE production by purified B cells. Taken together, these studies suggest that β2AR-mediated increases in cAMP and PKA activity may contribute to the IL-4R signaling cascade, because IL-4R signaling results in both enhanced levels of intracellular cAMP (McKay et al., 2000) and PKA activity (Vazquez et al., 1991).

More recently, β2AR stimulation on the B cell alone was found to augment the level of IL-4-dependent IgG_1 and IgE produced by either the Th2 cell- or CD40L-activated B cell (Kasprowicz et al., 2000). Pretreatment of TNP-specific B cells with both antigen and terbutaline for 24 h prior to activation increased the responsiveness of B cells to IL-4 and the level of TNP-specific IgG_1 and IgE produced per B cell, but did not affect the number of antibody-secreting cells produced or the level of IL-4R expressed. Importantly, terbutaline increased the level of B cell IL-4 responsiveness in a concentration-dependent manner. This effect was blocked by the βAR antagonist nadolol and did not occur if B cells were isolated from the spleens of β2AR −/− mice. Therefore, these studies suggest that stimulation of the β2AR increases

the responsiveness of the B cell to IL-4 and the level of IgG_1 and IgE produced per cell.

In addition to influencing the level of IL-4 responsiveness, β2AR stimulation may also influence the level of BCR-dependent B7–2 signaling to the B cell. Previous studies suggested that stimulation of B7–2 on activated human tonsillar B cells increased the level of IgG_4 and IgE production (Jeannin et al., 1997). Importantly, exposure of murine B cells activated by CD40 and IL-4R stimulation produced more antigen-specific IgG_1 and IgE when exposed to anti-B7–2 antibody, anti-sIg antibody (or antigen), and terbutaline, in comparison with anti-sIg antibody and terbutaline only (Kasprowicz et al., 2000). These studies reported not only that B7–2 signaling to the murine B cell was dependent upon BCR stimulation to enhance the level of IgG_1 and IgE produced per B cell but, in addition, that stimulation of the β2AR further enhanced the level of B7–2 signaling in this model system.

Taken together, these studies support the hypothesis that stimulation of the B cell β2AR either prior to, or at the time of, cell activation may increase either the number of antibody-secreting cells or the amount of antibody produced per cell. In addition, a number of studies suggest that stimulation of the β2AR may generate intracellular signals that augment the IL-4R signaling pathway and/or the B7–2 signaling pathway to increase the level of IL-4-dependent IgG_1 and IgE produced per B cell.

3. In Vivo B Cell Differentiation and Antibody Production. Tables 7 and 8 summarize past findings concerning the effects of norepinephrine and β2AR stimulation on B cell differentiation into antibody-secreting cells and the level of B cell antibody production in vivo, respectively. An early study investigated the role of cAMP accumulation in regulating antibody-secreting cell formation in vivo (Braun and Ishizuka, 1971). Immunization of mice with poly(A:U) enhanced the number of antibody-secreting cells 48 h following sRBC immunization. A few years later, the effect of norepinephrine-depletion on the primary T-dependent antibody response in vivo was investigated (Kasahara et al., 1977b). Using a low dose of 6-OHDA, which selectively destroyed peripheral sympathetic nerve terminals (reviewed in Kostrzewa and Jacobwitz, 1974), both the hemagglutinin titer and the number of plaque-forming cells in response to immunization with sRBC were decreased. The suppressive effect of norepinephrine depletion on the hemagglutinin titer was measured 4 days following immunization by this group and others (Williams et al., 1981), but not at later time points after immunization. These findings were extended to determine the effect of norepinephrine depletion on the secondary (memory) response to a T-dependent antigen. Norepinephrine depletion at the time of primary immunization did not alter the secondary response to antigen administered 10 days following the primary antigen challenge (Kasahara et al., 1977a). However, 6-OHDA did inhibit the secondary antibody response in a dose-dependent manner when administered concurrently with the secondary exposure to antigen, suggesting that the level of norepinephrine at the time of antigen administration may be important. In contrast, others have reported that either surgical axotomy of the spleen or 6-OHDA-mediated norepinephrine depletion increased the number of antibody-secreting cells following immunization with sRBC (Besedovsky et al., 1979). However, it is important to note that norepinephrine depletion was performed on newborn animals in these studies, a procedure that not only results in permanent peripheral norepinephrine depletion but, in addition, alters central levels of norepinephrine as well. Thus, norepinephrine depletion may exert varying effects on the number of antibody-producing cells formed in response to sRBCs, depending on the mouse age, concentration, and timing of 6-OHDA administration in relation to the delivery of the antigen signal.

Using adult mice, norepinephrine depletion suppressed the level of the antibody titer to sRBC, as well as the number of antibody-secreting cells (Hall et al., 1982). More recently, the level of serum TNP-specific antibody produced by dopamine β-hydroxylase-deficient mice (norepinephrine-deficient) immunized with the soluble protein antigen TNP-KLH was significantly lower than the level of antibody produced by B cells in wild-type mice (Alaniz et al., 1999). Thus, these studies suggested that norepinephrine depletion suppressed the level of antibody produced in vivo following immunization with either a particulate (sRBC) or soluble protein (TNP-KLH) antigen. In contrast, peripheral norepinephrine depletion in mice increased the number of antibody-forming cells activated by T-independent antigens but did not alter the number of antibody-secreting cells activated by T-dependent antigens (Miles et al., 1981). In addition, it is difficult to determine the exact effect of norepinephrine depletion on these responses, because responses against two different antigens were initiated in each animal, and in some cases, T-dependent and T-independent responses were initiated in the same animal by immunizing mice with multiple antigens concurrently.

Using immunizations of cholera toxin, Lycke et al. (1990) reported that elevations in the intracellular level of cAMP slanted the in vivo T-independent antibody response toward a "Th2-like" profile, because cholera toxin-treated animals produced elevated levels of DNP-specific IgG_1, but not IgM or IgG_3. Zalcman et al. (1994) showed that exogenous IL-2 administration enhanced the antibody response against sRBC in vivo and that this effect was dependent upon intact splenic sympathetic innervation. It was possible that exogenous administration of IL-2 increased hypothalamic activity (Zalcman et al., 1994) to increase the level of peripheral norepinephrine released; and, because sympathetic

nerves express IL-2 receptors (Haugen and Letourneau, 1990), it was possible that IL-2 increased sympathetic nerve activity and norepinephrine release in the spleen. This hypothesis was supported by the finding that pretreatment of norepinephrine-intact animals with the βAR antagonist propranolol blocked the IL-2-induced enhancement in antibody production, whereas the αAR antagonist phentolamine had no effect. Finally, the timing of IL-2 administration was a critical factor that influenced this response because exogenous IL-2 had to be administered either the day before or the day of sRBC immunization to increase the number of antibody-secreting cells. Thus, these studies suggest that IL-2 administration may enhance the early in vivo antibody response via activation of the sympathetic nervous system to increase the level of norepinephrine release in lymphoid organs.

One study reported a strain-specific enhancement in antibody production in norepinephrine-depleted C57BL/6J (Th1-slanted strain) and BALB/c (Th2-slanted strain) mice (Kruszewska et al., 1995). For example, when C57BL/6J were depleted of norepinephrine using a single injection of 6-OHDA (100 mg/kg) and immunized with the T-dependent antigen KLH, serum levels of KLH-specific IgM, IgG, IgG_1, and IgG_{2a} were enhanced in norepinephrine-depleted animals 1 to 2 weeks post-immunization. In contrast, when similar studies were performed in BALB/c mice, serum levels of antigen-specific IgM, IgG, and IgG_{2a} were not significantly different in norepinephrine-depleted and norepinephrine-intact mice. However, norepinephrine depletion did slightly enhance the level of KLH-specific IgG_1. Interestingly, though, norepinephrine depletion seemed to create a trend, but not significant suppression of all isotypes in BALB/c mice during the first week. This finding suggested that a re-examination of the approach used to address these questions needed to be undertaken. When this was done, it became apparent that resident lymphocytes were exposed to a burst of norepinephrine when using 6-OHDA, because the mechanism of action of 6-OHDA is to displace norepinephrine before destruction of the sympathetic nerve terminal. Because adrenergic receptors are expressed by resident immune cells, it is probable that the displaced norepinephrine might affect these cells in a manner similar to norepinephrine released in response to antigen.

Because we now know that the spleen contains immune cells in all stages of differentiation, and because cells in these different stages may differentially express adrenergic receptors, a recent study addressed this deficiency in experimental design by using a reconstitution model system to specifically investigate the role of norepinephrine-induced β2AR stimulation on the B cell in regulating the Th2-dependent antibody response (Kohm and Sanders, 1999). KLH-specific Th2 cell clones (β2AR-negative) and TNP-specific B cells (β2AR-positive) were adoptively transferred into norepinephrine-depleted T cell- and B cell-deficient *scid* mice after the mice had been depleted of norepinephrine by 6-OHDA. A significantly lower serum level of TNP-specific IgM and IgG_1 was measured in response to the soluble cognate protein antigen TNP-KLH in norepinephrine-depleted animals. Importantly, the effects of norepinephrine depletion on the primary IgM response were reversed by the β2AR-selective agonists terbutaline and metaproterenol in a dose-dependent manner, suggesting that the effects of norepinephrine depletion on the in vivo antibody response were mediated via a lack of β2AR stimulation on the B cell. In addition, whereas the level of TNP-specific IgM returned to control levels following secondary immunization of animals that were depleted of norepinephrine prior to the primary immunization, serum levels of TNP-specific IgG_1 were still significantly lower. However, memory antibody levels were only measured for 3 weeks following secondary immunization; thus, the memory antibody response in norepinephrine-depleted animals may have been delayed, not inhibited. Finally, whereas norepinephrine depletion did not alter T and B cell trafficking to the spleen in this model system, spleen cell proliferation and germinal center formation were significantly lower in norepinephrine-depleted animals in comparison to norepinephrine-intact controls. Thus, these data suggest that stimulation of the B cell β2AR by endogenous norepinephrine released during the course of a T-dependent immune response (Kohm et al., 2000) is necessary to maintain an optimal level of antibody production in vivo.

Taken together, these studies demonstrate the potential for norepinephrine to exert varying effects on B cell function in vivo. For example, NE depletion may exert age-dependent effects on B cell function, because norepinephrine depletion of neonatal animals increases the number of antibody-secreting cells, whereas norepinephrine depletion in adults decreases both the number of antibody-secreting cells and the level of antibody production by B cells. In addition, the effects of norepinephrine depletion on the level of antibody production were dependent upon both the strain of mouse and the model system used. However, in addition to the these sources of conflicting findings in vivo, additional problems arise when comparing in vivo and in vitro effects of norepinephrine on B cell function. As previously discussed, most studies investigating the effects of norepinephrine on B cell function in vivo depleted normal mice of the neurotransmitter. Therefore, although in vitro studies could specifically study the effects of norepinephrine on B cell function, these in vivo studies were in fact studying the effects of NE depletion on all cell populations that both expressed adrenergic receptors and participated in the antibody response. Finally, in vivo studies may have also been studying the effects of norepinephrine on cells in various states of differentiation, because NE depletion mostly likely affected both naive and effector cells in these animals. Thus,

future studies are needed to further dissect the role of norepinephrine and β2AR stimulation in regulating B cell function both in vitro and in vivo. These studies may be assisted by the use of additional model systems, such as reconstituted *scid* mice, to investigate the role of norepinephrine in regulating the function of each cell type contributing to antibody production in vivo, or gene disruption of NE-synthesizing enzymes in specific cell populations in vivo.

VI. Disease- and Health-Specific Implications

In light of the ability of β2AR stimulation to influence the level of $CD4^+$ Th1 cell cytokine, $CD4^+$ T cell, and B cell proliferation; lymphocyte homing; B cell antibody production; and B cell costimulatory molecule expression and signaling, it is not surprising that norepinephrine or the stimulation of the lymphocyte β2AR has been reported to influence both the onset and progression of various diseases or age-related abnormalities, such as Down's syndrome (Morale et al., 1992), rheumatoid arthritis (Felten et al., 1992; Baerwald et al., 1997; Lombardi et al., 1999), multiple sclerosis (Zigmond et al., 1989), and aging (Callard and Basten, 1978; Doria et al., 1980; Kohno et al., 1986; Madden et al., 1989, 1995).

Decreases in the level of splenic innervation are present in aged subjects and individuals with certain pathological conditions. For example, an age-related withdrawal of sympathetic innervation was observed in both the spleen and lymph nodes of rats, but not in the thymus (Felten et al., 1987a,b, 1988a,b; Ackerman et al., 1991; Bellinger et al., 1992b). This observation may explain the declining T and B cell responses (Callard and Basten, 1978; Doria et al., 1980; Madden et al., 1989) and cellularity of the white pulp (Cheung and Verity, 1983; Bellinger et al., 1992a) that are associated with aging. Finally, the level of lymphoid organ innervation appears to be related to autoimmune disease expression, because sympathetic innervation was decreased in mice prone to the development of autoimmune disease prior to expression of the disease phenotype (Chelmicka-Schorr et al., 1988, 1992). Thus, alterations in the level of sympathetic innervation within lymphoid organs of individuals with certain disease states, or during the process of aging, may translate into alterations in the rate of norepinephrine release during the course of the immune response.

A number of studies have investigated the effects of aging on the level of β2AR expression and function on lymphocytes. For example, one study reported an age-dependent decrease in both the number of βARs expressed on the surface of spleen cells and the affinity (K_d) of the receptors (Kohno et al., 1986). In addition, others investigated the effects of age and norepinephrine depletion on the T-dependent antibody response in vivo (Madden et al., 1995). For example, norepinephrine depletion did not significantly influence the level of KLH-specific IgM or IgG production in young rats but significantly enhanced the level of KLH-specific IgM and IgG in aged animals. Similarly, norepinephrine depletion increased the level of KLH-, Con A-, and LPS/dextran sulfate-induced spleen cell proliferation in aged animals more significantly than in young animals. These findings are surprising in light of the possibility that the level of βAR affinity and expression on lymphocytes may be decreased in aged animals. However, it is possible that aged lymphocytes are more responsive to βAR-derived signals; thus, even though these cells express lower levels of βAR on their surface, stimulation of this receptor may still affect the function of aged cells more significantly than that of younger cells.

Others have investigated the effects of norepinephrine and β2AR stimulation on rheumatoid arthritis and reported that that βAR antagonists delayed both the onset and progression of rheumatoid arthritis. Importantly, lymphocytes isolated from rheumatoid arthritis patients do not expressed altered levels of β2AR expression or affinity; however, the activity and expression of GRKs were lower in patients in comparison with healthy controls (Lombardi et al., 1999). Because GRKs function to desensitize the β2AR, the lower activity and expression of GRKs in lymphocytes isolated from rheumatoid arthritis patients may account for both the higher levels of intracellular cAMP measured in these cells and the lower levels of TNF-α produced by diseased lymphocytes. In contrast, others have reported that the number of β2AR expressed on synovial fluid lymphocytes was significantly lower than the number of β2AR expressed on peripheral blood lymphocytes (Baerwald et al., 1997), thus suggesting a local mechanism for down-regulating the level of β2AR expression during arthritis. Such a decrease in the level of β2AR expression may remove the inhibitory influences of β2AR stimulation on T cell function, thus leading to enhanced inflammatory cytokine production. Finally, in support of the inhibitory role of β2AR stimulation on lymphocyte function during rheumatoid arthritis, depletion of peripheral norepinephrine via 6-OHDA resulted in both an earlier onset and enhanced severity of experimentally induced arthritis (Felten et al., 1992). Taken together, these studies suggest that norepinephrine stimulation of the β2AR expressed by lymphocytes may inhibit the progression of rheumatoid arthritis; however, future studies are necessary to determine the specific cell population(s) being influenced by norepinephrine and β2AR stimulation.

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