0031-6997/85/3703-0229$02.00/0
PHARMACOLOGICAL REVIEWS

Vol. 37, No. 3
Printed in U.S.A.

Norepinephrine and the Antibody Response

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I. Introduction

INVOLVEMENT of the sympathetic neurotransmitter norepinephrine in modulation of antibody responses has been a focus of considerable interest for the last two decades. Unfortunately, experimental findings have led to a considerable amount of confusion, mostly due to the nonspecific experimental systems used and the differing effects reported.

Recent findings indicate that lymphoid tissues are innervated with sympathetic nerve fibers and that immunocompetent cells residing therein possess adrenoceptors of high affinity and specificity. It therefore seems likely that the process of antibody formation, like other homeostatic mechanisms in the body, can be influenced by the sympathetic nervous system. Certain physiological and pathological conditions, including exposure to foreign antigens, could evoke neurotransmitter release in the vicinity of immunocompetent cells, and consequently the neurotransmitter could exert potent effects on various cellular metabolic and functional processes. It follows that release of norepinephrine during the development of the antibody response could ultimately modulate the magnitude and duration of an antibody response involving the complex processes of cellular communication, proliferation, and differentiation.

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† The research from this laboratory was supported in part by NIEHS Training Grant 1T32ESO7087 and Contract ES-1-5001.

Conflicting evidence exists in regard to changes in antibody responses following in vivo or in vitro exposure to norepinephrine or various pharmacological agents which mimic or modulate sympathetic neurotransmitter activity. The greatest progress in this area of research has occurred through the use of a complex in vitro system which involves heterogeneous cell populations maintained in culture. The in vitro primary immunization technique has been used to determine the effect of norepinephrine and adrenoceptor agonists on murine immunocompetent cells involved in IgM antibody production. Generation of the in vitro antibody response to a T cell-dependent antigen, e.g., to sheep erythrocytes, has been the assay of choice in most of these studies. This in vitro-generated response is qualitatively and quantitatively similar to that generated in vivo and depends on interactions among the macrophage, T cell, and B cell. However, this assay does have some inherent disadvantages associated with it, and these will be discussed throughout this review.

A few studies suggest that the antibody response can be modulated positively or negatively, depending on which adrenoceptor type (alpha or beta) or subtype (alpha-1, alpha-2, beta-1, or beta-2) is activated. The beta-2 adrenoceptor was recently identified pharmacologically to mediate the norepinephrine-induced enhancement of the antibody response during very early stages of the dynamic process of antibody formation (202, 203). Ad-

ditionally, a number of studies have utilized dibutyryl cyclic AMP, phosphodiesterase inhibitors, and bacterial toxins to confirm the involvement of the second messenger cyclic AMP in modulation of the antibody response. Use of the latter pharmacological agents has suggested that elevations of cyclic AMP *early* in the process of antibody formation produce an enhanced antibody response, while *late* elevations produce a suppressed response.

The role of alpha adrenoceptor activation in the process of antibody formation is less clear, although recent findings suggest that alpha-1 and alpha-2 adrenoceptors may also play a modulatory role in the antibody response (21, 205).

In this review,‡ we will attempt to summarize and critically evaluate previous as well as recent findings suggesting a role for the sympathetic nervous system in modulation of the antibody response. The involvement of other neurotransmitter and peptide modulators is addressed elsewhere (111, 228, 252). Initially, a selected review will be presented of the multiple adrenoceptor sites available for interaction with norepinephrine, as well as the mechanisms by which these adrenoceptors mediate their effects intracellularly. This section will be followed by a review of the complexities of the T cell-dependent antibody response and the assay most frequently used to evaluate its status. The role of the sympathetic nervous system and, in particular, the neurotransmitter norepinephrine in modulation of the antibody response will be the focus for a large part of this review. And, finally, in light of the important role of the antibody response to the maintenance of homeostasis, the relevance of these results to possible basic science and therapeutic applications will also be explored.

II. Adrenoceptor Sites and Mechanisms of Action

A. Beta Adrenoceptors

Norepinephrine is a catecholamine neurotransmitter released by nerve terminals of the sympathetic nervous system. Following release from nerve terminals, the neurotransmitter can interact with postsynaptic receptor sites. Ahlquist (4) initially proposed a subdivision of catecholamine receptors into alpha and beta receptors in order to distinguish different physiological responses elicited by various organ systems after exposure to catecholamines. The possibility that beta adrenoceptors were not a homogeneous population of receptors was first suggested by Moran (154), while the first experimental evidence in support of such a subclassification of beta adrenoceptors into beta-1 and beta-2 categories was first presented by Lands (130, 131). Lands showed that the rank order of potency of catecholamine agonists fell into two categories depending on the tissue response being examined. In tissues containing beta-1 adrenoceptors, the rank order of potency was isoproterenol > norepinephrine ≥ epinephrine, while in tissues containing beta-2 adrenoceptors, the rank order of potency was isoproterenol ≥ epinephrine > norepinephrine.

Three different lines of evidence support Lands' proposal for beta adrenoceptor subclassification: pharmacological; biochemical; and radioligand binding. Pharmacological evidence includes the relative orders of potency for a series of agonists and competitive antagonists in physiological responses in various tissues (58).

Biochemical evidence includes the measurement of adenylate cyclase activity in different tissues following exposure to a series of beta agonists and antagonists (36, 133). Sutherland and coworkers were the first to describe the stimulation of adenylate cyclase by epinephrine and other beta adrenoceptor agonists (159). Subsequently, a number of researchers have shown that adenylate cylcase stimulation is the mechanism by which many beta-adrenergic effects are mediated (189, 194). Interestingly, the resulting mediator from adenylate cyclase stimulation, cyclic adenosine 3′,5′-monophosphate (cAMP), had been discovered a few years earlier as a heat-stable mediator of epinephrine's effects on glycolysis (188). Recently, bifunctionality of the beta adrenoceptor protein has been demonstrated in a reconstituted system (45). The isolated adrenoceptor is shown to be a single polypeptide containing both the ligand binding site and the site responsible for stimulating adenylate cyclase via an interaction with the GTP-binding protein. Other biochemical evidence shows structural differences between the beta-1 and beta-2 adrenoceptors (225). Peptide maps from these studies suggest that alterations in the primary structure of adrenoceptors may be responsible for their pharmacological specificities.

Radioligand binding studies strongly support the existence of two beta adrenoceptor types (162, 226). Prior to such binding studies, it had been suggested that beta-1 and beta-2 adrenoceptors coexist in the same organ and may even mediate the same physiological response (44). This proposal was later supported by a number of studies using radioligand binding techniques (13, 150, 161). Since most tissues, e.g., lymphoid organs, are made up of a number of different cell types, it must be considered that two adrenoceptor subtypes existing within the same organ may actually be located on different cells.

A monoclonal antibody has been generated to the ligand binding site of the beta adrenoceptor (80). This antibody cross-reacts equally well with beta-1 and beta-2 adrenoceptors, suggesting that some homology exists between them. Two more recent tools for characterization of beta adrenoceptors have also been developed. Monoclonal anti-beta adrenoceptor ligand antibodies and antiidiotypic anti-beta adrenoceptor antibodies (46, 94) will allow for not only structural characterization of this receptor, but also characterization of the mechanisms underlying signal transduction.

‡ Several other reviews have addressed various aspects of this topic (22, 27, 98, 177, 245).

Beta adrenoceptor desensitization has been observed in a number of organ systems. Mechanisms to explain the desensitization phenomenon include (*a*) internalization of receptors as a consequence of agonist exposure (222), (*b*) decreased adenylate cyclase responsiveness due to factors distal to the receptor, e.g., a soluble inhibitor of adenylate cyclase which accumulates intracellularly (118, 181), (*c*) a change in coupling functions between receptor and adenylate cyclase (6), (*d*) activation of the inhibitory GTP-binding protein via beta adrenoceptor activation, an activation event which generally stimulates the stimulatory GTP-binding protein (8), (*e*) internalization and processing of binding sites (50, 51), (*f*) altered membrane phospholipid methylation (101), (*g*) long-term regulation of receptor synthesis and degradation by hormones, possibly via control of the transcription of genes coding for the beta adrenoceptor (25, 59, 226), and (*h*) phosphorylation of the beta adrenoceptor by cAMP or non-cAMP-mediated mechanisms (217, 218). The molecular aspects of agonist-induced desensitization prior to 1983 have been reviewed previously (100).

B. Alpha Adrenoceptors

Four types of evidence support subclassification of alpha adrenoceptors: anatomical; pharmacological; biochemical; and radioligand binding. The subclassification of alpha adrenoceptors based on anatomical location was first proposed by Langer (132). He proposed that classical postsynaptic alpha sites be referred to as alpha-1 and that presynaptic nerve terminal sites be referred to as alpha-2.

Pharmacological evidence includes the measurement of physiological responses in which alpha adrenoceptors are functionally differentiated by their ability to interact with a series of agonists and antagonists (17, 39, 144, 200, 223).

Biochemical evidence suggests that alpha-1 adrenoceptors are coupled to calcium gating or redistribution, possibly mediated via phosphoinositide turnover (77, 120, 147). Alpha-1 adrenoceptor activation in a number of tissues leads to a change in the steady-state concentration of free calcium in various intracellular compartments; thus calcium may be acting as the second messenger (250). A proposed mechanism for signal transduction following alpha-1 adrenoceptor activation includes the activation of a calcium-dependent protein kinase by diacylglycerol released from phosphatidylinositol metabolism (127). Another study suggests that alpha-1 adrenoceptors in isolated rat hepatocytes from mature rats may be coupled to separate signal transduction mechanisms (155). One mechanism is thought to involve calcium mobilization, while the other is thought to involve cAMP accumulation. The mechanisms involved in alpha adrenoceptor-induced phenomena have been reviewed recently by Exton (76).

In contrast, alpha-2 adrenoceptors are coupled to inhibition of adenylate cyclase activity (38, 84, 112, 254). Since alpha-2 adrenoceptor activation leads to a reduction in the level of cAMP accumulation in broken cell preparations, the decrease is assumed to be a consequence of a decrease in cAMP synthesis, as opposed to an increase in cAMP hydrolysis. Another proposed mechanism is that alpha-2 adrenoceptor-mediated effects are a consequence of adrenoceptor-induced effects at a step distal to cAMP generation (163). This was proposed from findings where the stimulatory effects produced by dibutyryl cAMP on insulin release could be inhibited by an alpha-2 adrenoceptor agonist.

Radioligand binding studies have also confirmed alpha adrenoceptor subclassification (251). Recent radioligand binding studies suggest that subtypes or species differences of the alpha-2 adrenoceptor may also exist (78).

In summary, the adrenoceptor system and the mechanisms by which these adrenoceptors mediate their effects are indeed complex. Those researchers attempting to understand adrenoceptor-induced modulation of the antibody response will need to include identification of not only the adrenoceptor subtype responsible for modulation of such responses, but also the cell population with which the adrenoceptor is associated. Only then will definitive studies be designed to determine adrenoceptor-induced changes in the functions of specific cells involved in this complex immune response.

III. The Primary Antibody Response

A. Cell populations and Interactions

The in vitro induction of a primary (IgM) antibody response in mouse spleen cells exposed to a T cell-dependent antigen, such as sheep erythrocytes, depends on the interactions among three cell populations. These cell populations include the B lymphocyte which produces the antibody, the T lymphocyte which regulates the B cell response, and the macrophage which processes and presents antigen (52, 157). Before attempting to present a review of the literature concerning sympathetic nervous system modulation of the antibody response, a brief review will be presented of the cells, soluble factors (cytokines), and regulatory mechanisms involved in the generation of this complex response. The reader is referred to fig. 1 for a diagram of the cells and soluble factors involved in the antibody response.

The macrophage performs a critical role in the in vitro T-dependent antibody response in that it functions to process and present antigen to the T lymphocyte (156) in a genetically restricted manner (197). Genetic restriction involves T cell recognition of antigen on the macrophage surface in conjunction with recognition of gene-encoded products on the macrophage surface, i.e., Ia antigens, which map to the I region of the murine major histocompatibility complex (MHC). Presentation of antigen by the macrophage initiates a series of cellular events involved in subsequent T and B cell interactions

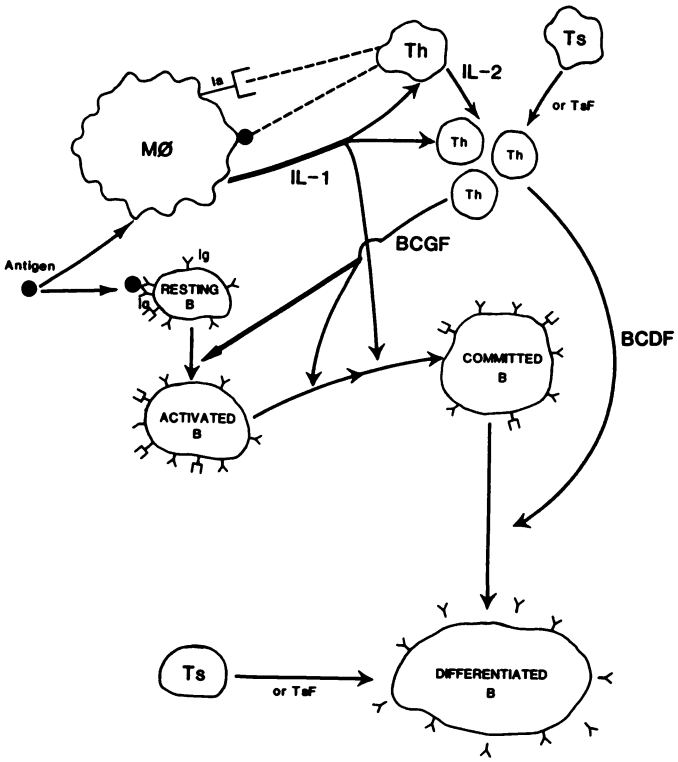

FIG. 1. Diagram of the cells and soluble factors involved in the antibody response.

(124). Interestingly, it has also been shown that resting B cells can present antigen to T cells in a similar genetically restricted manner (9, 49), since these cells also express high levels of Ia antigens.

The lymphocyte involved in up-regulation of the antibody response is called the T-helper cell. The macrophage is required for activation of the T-helper cell (73) as well as for the generation of soluble factors (lymphokines) released by this cell. The lymphokines released by the T-helper cell affect not only its own proliferation (238), but also B cell proliferation and differentiation (7, 105, 208, 209).

Antigen-specific T cell activation and proliferation require two separate signals from the macrophage (61). First, T cell *activation* requires the presentation of antigen to the T cell by macrophages in context with MHC antigens, and second, T cell *proliferation* requires the presence of IL-1, a cytokine released by the macrophage upon interaction with the T-helper cell. T-helper cells become responsive to IL-1 following activation. As a result of the macrophage-T-helper cell interaction, the T cells express receptors for, and begin secreting, interleukin-2 (IL-2) (153). Interleukin-1 (IL-1) appears to induce and amplify the production of IL-2 (89), possibly via an effect on the IL-2 producing cell itself. The subsequent increase in IL-2 concentration enhances the proliferation of T cells possessing receptors for IL-2 (153).

The B lymphocyte is critical for ultimate production of antigen-specific antibody. A number of theories (60, 106, 143) address the stages involved in development of the B cell response: (*a*) *activation* of the resting B cell to enter G_1 phase of the cell cycle; (*b*) *commitment* of this cell to proliferate and clonally expand; and (*c*) *differentiation* of this cell to the antibody-secreting stage.

Activation of the B cell is generally thought to involve the interaction of surface membrane immunoglobulin receptors with antigen in order to trigger the resting, i.e., G_0 phase, B cell into G_1 phase. This step has been successfully accomplished by interacting immunoglobulin receptors with low concentrations of anti-immunoglobulin antibody, thus creating a state in which the activation of B cells is limited to entry into G_1 phase (178). Initial *commitment* of the activated B cell is achieved by movement of the cell through G_1 phase of the cell cycle by the interaction of "receptors" on these cells with a soluble T cell product, B cell growth (stimulatory) factor (BCGFI or BSF-1).§ Ultimate commitment to S phase occurs by exposure of the activated B cell to the macrophage-soluble product, IL-1. It has also been reported that IL-2 receptors are expressed on activated B cells and that activation of these receptors by IL-2 allows for B cell proliferation (239, 256) and immunoglobulin synthesis (165).

Recently, Vitetta and coworkers (171) have proposed and presented experimental evidence that BSF-1 *prepares* resting B cells for subsequent antiimmunoglobulin-mediated entry into S phase of the cell cycle, and not vise versa as described by Howard and Paul (106). This finding suggests that "receptors" for BSF-1 are present on resting B cells and that their expression is not induced after interaction of immunoglobulin receptors with antiimmunoglobulin or antigen as previously suggested (106). This finding is most intriguing in light of a number of recent studies showing that BSF-1-containing supernatants (195) and purified BSF-1 (168) can increase the expression of Ia antigen on resting B cells. The latter observations become important, since B cell activation and subsequent antibody secretion in response to a T-cell-dependent antigen depend on linked recognition between the T and B cell (121), a phenomenon in which increased expression of Ia antigen on the B cell would allow for a more effective presentation of antigen to the T-helper cell in the context of MHC antigens.

Differentiation of the B cell to the antibody-secreting stage requires interaction of the committed B cell with other T cell-derived factors. Initially, the committed B cell acquires the ability to interact with T-helper cells or their soluble differentiation factors (128). This phase is also influenced by IL-1 which increases the number of factor-responsive B cells and/or enhances the expression of B cell surface markers (103). The B cells subsequently convert to antibody-secreting cells in response to T-helper cells and/or T-helper cell factors, referred to as B

§ For details on the new nomenclature for B cell factors, the reader is referred to ref. 107.

cell differentiation factors, which induce either IgM or IgG secretion (237).

The T-suppressor cell is involved in antigen-specific (1) and antigen-nonspecific (113, 123) suppression of the in vitro antibody response. These cells mediate suppression at various points along the pathway to antibody production by direct cell contact and/or by the release of lymphokines. T-suppressor cell induction in certain experimental models appears to require the generation of a suppressor-cell cascade (65). This cascade involves antigen-presenting cells, factor-presenting cells, and three different T-suppressor cells which release three different T-suppressor factors. Recently, it has been shown that antigen-nonspecific suppression can also be mediated by macrophages (5) and by B cells themselves (86, 87).

B. Soluble Factors

A number of cytokines are released by immune cells. Following interaction with antigen, these factors contribute to the overall function and regulation of the antibody response. This area of immunology is changing rapidly and is very controversial. Therefore, we have briefly summarized the major findings in this area in an attempt to emphasize the complexity of the system. Several reviews have addressed various aspects of this topic in detail (106, 128, 152, 153, 237).

The cytokines involved in the antibody response include IL-1, IL-2, BCGFI (BSF-1), BCGFII, BCDF, and suppressor factors. Interleukin-1 and IL-2 have been reviewed briefly in the preceding section. Two T cell-derived B cell growth factors have been functionally characterized in the murine system, BCGFI (BSF-1) (105, 168) and BCGF II (69, 230). As discussed previously, BSF-1 delivers a signal to resting B cells (171). B cell differentiation factor(s), also a T cell-derived soluble product(s), delivers a signal to the activated B cell to differentiate into an antibody-secreting cell (135, 187, 229). Two distinct differentiation factors have been characterized in the murine system (164). Differentiation factors have also been described which determine the antibody isotype, i.e., IgM or IgG, which will be secreted (237).

C. Regulation of B Cell Responses

A number of mechanisms responsible for regulating the magnitude of the antibody response in vitro and in vivo have been supported experimentally. Many of these mechanisms may prove to be targets for pharmacological modulation of this complex cellular system.

Humoral mechanisms of regulation include (*a*) B cell stimulatory factor 1 (BSF-1) (105, 106, 171), (*b*) B cell differentiation factor (135, 187, 229), (*c*) antiidiotype antibodies which occupy Ig receptors on resting B cells so they can no longer be activated by antigen (72, 115, 126), (*d*) antibody itself, possibly via Fc fragments, which inhibits or enhances the antibody response (30, 246), (*e*) antigen-antibody complexes which enhance or suppress the T-dependent antibody response (219, 235), (*f*) regulatory lymphokines which induce Ia expression on macrophages to enhance their functional activity of antigen presentation to T cells (15, 224), (*g*) macrophage regulatory factors (236), (*h*) complement proteins which enhance or suppress antibody production [e.g., C5a stimulates IL-1 production by macrophages allowing for direct stimulation of committed B cells, while C3 induces Ts cells (71)], and (*i*) prostaglandin-E2 which induces the generation of Ts cells to allow for feedback inhibition of the antibody response (91).

Cellular mechanisms of regulation include (*a*) a network of helper-suppressor activity by T lymphocytes (85), (*b*) isotype-specific T cells, activated by products from activated B cells, which regulate B cell Ig expression (196), (*c*) cytotoxic autoreactive T cells, activated by autologous I-A products, which enhance antibody responses via production of a nonspecific helper factor, or inhibit antibody responses via cytolysis of activated I-A-bearing macrophages or B cells (53), (*d*) suppressor-inducer T cells, activated by T-helper cells, which feedback to suppress the activated T-helper cell (70), (*e*) contrasuppressor-inducer cells which induce a cell to suppress the activity of the T-suppressor cell (92), (*f*)suppressor B cells which regulate B cell activity (86, 87, 129, 167), (*g*) B-helper cells (Ig^+, $Lyt\text{-}1^+$) which augment the antibody response in the absence of T-helper cells (214), (*h*) B cells which act as suppressor-inducer cells to induce the activation of T-suppressor cells (215, 216), and (*i*) natural killer cells which eliminate antigen-exposed accessory cells so that their antigen-presenting ability is eliminated (2).

Genetic mechanisms of regulation include the immune response genes linked to the MHC which determine the level of immune responsiveness to antigens (16).

D. The in Vitro Antibody Assay System

The technique most commonly used for inducing the primary antibody response in vitro is the Mishell-Dutton assay (151). In this system, dispersed spleen cells are cultured with an antigen, which is usually the T-dependent antigen, sheep erythrocytes. At the end of a certain number of days following sheep erythrocyte immunization, the number of IgM antibody-secreting cells are enumerated.

There are a number of special requirements for the generation of antibody-secreting cells using this system. Some, or all, of these requirements may be responsible for the differing results reported by different laboratories. First, fetal calf serum is essential in this assay, and consequently the batch of fetal calf serum (FCS) used can significantly alter the magnitude of the response. It has been suggested by Mishell and Dutton that the reason for this phenomenon is that the FCS may contain a level of antigens which cross-react with sheep erythrocytes (151). In addition, FCS also contains a number

of hormones, the level of which may influence the magnitude of the response. For example, FCS has been shown to contain the cytokine, IL-1, in contrast to adult bovine serum which contains no IL-1 (104).

Second, the system yields an optimal number of antibody-secreting cells when the sulfhydryl reducing agent, 2-mercaptoethanol (2-ME), is added to the cultures. The following are a number of theories proposed to account for the mechanism of 2-ME activity: (*a*) 2-ME functionally substitutes for macrophages (48, 54); (*b*) 2-ME enhances T cell mitogenic activity for T cells when present with FCS (134); (*c*) 2-ME activates a component of FCS which substitutes for macrophages (172, 173); (*d*) 2-ME stimulates the uptake of the essential amino acid, cystine, into murine spleen lymphocytes (169, 170); (*e*) 2-ME enhances the production of glutathione by spleen lymphocytes and protects against its loss, thereby allowing for adequate glutathione levels to be maintained for lymphocyte activation to proceed past the early stages of activation (255); and (*f*) 2-ME inhibits membrane lipid peroxidation via the antioxidant activity of reduced glutathione whose availability is enhanced by 2-ME (102).

Finally, the batch of sheep erythrocytes influences the magnitude of the response. This is most likely due to the number of antigenic determinants present on the surface of the sheep erythrocyte membrane.

The primary advantage of the in vitro system is that it closely parallels the in vivo response with respect to kinetics and magnitude (151, 156) and thus allows for dissection of the system in order to determine cellular and molecular mechanisms. But, the two systems are not exactly identical, and caution must be exercised in extrapolating results from one system to the other. For example, B cell responses to sheep erythrocytes appear to be driven primarily by T cell-derived antigen-nonspecific factors, whereas, B cell responses to protein-bound antigens are driven by antigen-specific factors (138). Also, participation of the hypothalamic-pituitary-adrenal axis is eliminated in the in vitro-generated antibody response. Various substances are released by this system during the immune response in vivo and have been shown to directly and indirectly impact on the magnitude of the antibody response (22, 56, 74, 75, 184, 211). Thus, the impact of this hormonal system in vivo must be considered for its overall effect on the antibody response, particularly since these conditions cannot be mimicked identically in vitro.

IV. Role of the Sympathetic Nervous System in Modulation of the Immune Response

A. Early Evidence

In 1962 the first anatomical evidence for nervous system involvement with an immunological organ was obtained. Electron micrographs of the white pulp of mouse spleen showed the presence of unmyelinated nerve fibers (83). The appearance of these fibers in the white pulp of the spleen was a significant finding since this area is where many immunocompetent cells reside.

It was not until 1984 that the first pharmacological evidence for involvement of the sympathetic nervous system in the immune response was obtained by using reserpine. Reserpine is a pharmacological tool which allows for the selective depletion of norepinephrine from sympathetic nerve terminals. This depletion is accomplished by the drug-induced inhibition of dopamine uptake into granules for conversion to norepinephrine by dopamine beta-hydroxylase (14). In reserpine-treated adult rats, a suppression of both cell-mediated and humoral immune status was observed, as indicated by a suppressed delayed-type hypersensitivity response to bovine serum albumin and tuberculin and depressed hemagglutination titers to sheep erythrocytes, respectively (67). These results were called into question in 1966 when the antibody suppression induced in reserpine-treated adult mice was shown to be a consequence of nutritional deficiencies (68). In these experiments, the antibody suppression induced by reserpine could be mimicked in starved mice and reversed by forced feeding.

At the same time, histochemical evidence showed the presence of monoamines and adrenergic nerve fibers in the rat thymus (81), an organ primarily responsible for T cell differentiation before exit to other immune organs, i.e., the spleen and lymph node. The nerve fibers containing catecholamines were associated with small arteries in the interlobular septa, while fibers containing 5-hydroxytryptamine were localized to mast cells within the thymus. The presence of adrenergic nerve fibers in the rat thymus raised the question once again as to whether immunocompetent cells within the thymus, as well as within other lymphoid organs, could be influenced by neurotransmitters released from such fibers. More recently, innervation of the thymus gland in the mouse has also been reported (33, 34).

In 1967, a technique developed by Mishell and Dutton allowed for the in vitro generation of a primary antibody response (151). Since adrenoceptor radiolabeling techniques were not available until the latter part of 1970, the in vitro exposure of spleen cells in the Mishell-Dutton assay allowed for an indirect determination, i.e., as opposed to radioligand binding, of the effect of neurotransmitters and various adrenoceptor agonists on antibody production. Any effect obtained in these assays was assumed to occur via a receptor-ligand interaction if it occurred in a dose-dependent manner and if it was blocked by appropriate antagonists.

Preliminary findings showed that norepinephrine and isoproterenol, at a concentration of 9×10^{-5} M, led to a suppression of the in vitro-generated murine antibody response to sheep erythrocytes (137). The suppression obtained following exposure to epinephrine was in a concentration-dependent manner between 10^{-6} and 10^{-4} M. However, no blockade of these responses by pharma-

cological antagonists was reported, and the medium from these cultures was changed every day following the initial exposure of these cells to agonists.

At the same time, human peripheral blood lymphocytes were being studied for their ability to take up tritiated thymidine following stimulation by the plant lectin, phytohemagglutinin, and simultaneous exposure to adrenergic agents (95). Norepinephrine, when added either early or late in the developing immune response, caused a concentration-dependent increase in the uptake of tritiated thymidine, epinephrine produced no change, and isoproterenol caused a suppression. The enhanced response to norepinephrine was shown to be alpha adrenoceptor mediated, since the enhancement could be blocked with the alpha antagonist, phentolamine. The alpha component to the enhanced response was further confirmed with epinephrine cultured in the presence of the beta antagonist, propranolol. In contrast, epinephrine cultured in the presence of phentolamine resulted in a suppression of the response similar to that obtained with isoproterenol, suggesting that a beta component was associated with suppression of this immune response. It should be noted that these results could only be obtained in cell cultures to which hydrocortisone had been added, a drug shown to enhance cell sensitivity to catecholamines (226). Nonetheless, in a letter to the editor of the *New England Journal of Medicine*, John Hadden suggested that sympathetic nervous system modulation of immune functions was working via adrenergic mechanisms (96).

Based on this early evidence, conflicting results accumulated over the next few years concerning the effect of norepinephrine and adrenoceptor agonists on the in vitro-generated primary antibody response. Before summarizing these data, evidence will be presented which confirms the presence of noradrenergic nerve fibers in the spleen and lymph nodes of rodents, as well as the presence of alpha and beta adrenoceptors on animal and human lymphocytes as determined by radiolabeled ligand binding studies.

B. Innervation of Lymphoid Organs

If the sympathetic nervous system is to have any modulatory role in the antibody response, the organs possessing cells responsible for generation of this response should contain an adrenergic nerve supply which releases norepinephrine in the immediate vicinity of these cells. Consequently, the presence of nerve endings in the immediate vicinity of these cells would allow for *immediate* homeostatic regulation of the functioning of these cells.

Early findings showed that nerve fibers were present in the white pulp of mouse spleen (83). This observation was confirmed and extended a number of years later by a combination of histofluorescence and electron microscopy (192, 193). These results showed that unmyelinated adrenergic fibers were adjacent to reticular cells and lymphocytes in the white pulp of the spleen close to the periarteriolar sheath, and that some of these fibers were actually in contact with lymphoid cells.

This observation was confirmed in 1981 when fluorescence techniques showed dense perivascular networks of noradrenergic varicose fibers mainly in the splenic white pulp of mice (248). To confirm the presence of these fibers, mice were injected at birth with 6-hydroxydopamine (6-OHDA) to produce a peripheral chemical sympathectomy. 6-Hydroxydopamine is a pharmacological tool used to produce a peripheral chemical sympathectomy. The mechanism by which this drug produces a sympathectomy is thought to be due to destruction of sympathetic nerve terminals, without effect on the adrenal medulla or cholinergic neurons (233). This drug has been used in a number of studies to confirm the relationship between the immune system and the sympathetic nervous system. When these 6-OHDA-exposed mice were examined using histofluorescence techniques, a decrease in the level of catecholamine fluorescence was seen in the spleen. These authors also included an examination of the thymus in these studies and found adrenergic innervation of the parenchyma of the thymus. In addition, it was shown that fluorescence intensity in the thymus was considerably less following treatment with 6-OHDA. A recent report by Felten et al. (79) summarizes a number of findings regarding the innervation of various lymphoid tissues by noradrenergic fibers.

Lymph nodes provide another site in which the cells for the production of antibody reside. In rats, fluorescence techniques confirmed the presence of adrenergic nerve fibers associated with the lymph node capsule and the medullary and internodular regions (90). No adrenergic fibers were found in association with blood vessels or in the cortical nodules. Fluorescent intensity and norepinephrine content of this organ were significantly decreased following superior cervical ganglionectomy, thus confirming adrenergic innervation of the lymph node.

When antibody responses were determined in vivo following 6-OHDA treatment, three different results were obtained: enhancement; suppression; or no change at all.

Enhancement of the antibody response to sheep erythrocytes was seen in female rats which had received either local denervation of the spleen or 6-OHDA treatment coupled with adrenalectomy at birth (21). These authors concluded that norepinephrine was imposing a suppressive effect upon the immune response, which was removed upon 6-OHDA-adrenalectomy treatment. This group further supported this hypothesis by showing that norepinephrine levels in the spleen, following sheep erythrocyte immunization, fell sharply just prior to the peak antibody response. The same group also reported that high responder animals had a longer persisting

decrease of splenic norepinephrine levels during the antibody response as compared to low responders. This observation suggested that the magnitude of the norepinephrine decrease was directly related to the magnitude of the response (62).

A few years later, it was reported that mice exposed to 6-OHDA at birth showed an enhanced antibody response without the need for adrenalectomy (248). These mice showed an even greater enhancement of the response when exposed to a combination of 6-OHDA and alpha-methyl tyrosine, a drug which was considered more preferable than adrenalectomy to inhibit possible adrenal effects on the antibody response. The authors suggest that removal of norepinephrine from the vicinity of the spleen and thymus created (*a*) an imbalance of cyclic nucleotide metabolism by the cells in these organs, resulting in a possible release from cyclic nucleotide-induced suppression, (*b*) a loss of regulation of histamine release from mast cells, resulting in a loss of histamine-mediated suppressive modulation of the antibody response, and/or (*c*) an alteration in T cell development within the thymus with consequent effects on the T cell-dependent antibody response.

Suppression of the antibody response following 6-OHDA treatment has also been reported (99, 122). The reasons for the discrepancies in these results with those discussed above may simply be due to the age or species of animals upon exposure to 6-OHDA. In contrast to the experiments presented by the Besedovsky (21) and Williams (248) groups where animals (rats and mice) received 6-OHDA at birth, the Kasahara (122) and Hall (99) groups exposed mice to 6-OHDA during adult life when full maturation of neuroendocrine pathways had occurred. In addition to the suppressed PFC response observed by Hall (99), spleen cells from 6-OHDA-treated mice also showed a suppressed proliferative response to the B cell mitogen, lipopolysaccharide, but an enhanced proliferative response to the T cell mitogen, concanavalin A (98).

The suppressed response measured by Kasahara (122) led him to postulate that 6-OHDA was allowing for a withdrawal of an alpha adrenoceptor-mediated component which suppressed cAMP levels in splenocytes. It was postulated that cAMP levels consequently accumulated in these cells, thus allowing for a suppression of the antibody response. Hall (99) suggested other possibilities for the suppressed antibody response after 6-OHDA treatment, which include (*a*) a release from a direct norepinephrine effect on the cells, (*b*) unopposed cholinergic input to these cells, (*c*) activation of glucocorticoid systems with resulting immunosuppression, and (*d*) denervation supersensitivity.

Another study utilizing adult mice exposed to 6-OHDA showed that no change in the antibody response occurred to a T-dependent antigen, whereas an enhanced antibody response was obtained to a T-independent antigen (148). It was pointed out in this report that the enhanced antibody response observed by Besedovsky and coworkers to a T-dependent antigen (21) was only seen when 6-OHDA treatment was coupled with adrenalectomy.

C. Adrenoceptor Identification on Lymphocytes

The presence of adrenoceptors on immunocompetent cells ultimately requires characterization using radiolabeled ligands selective for alpha or beta adrenoceptors, with subtype characterization requiring displacement of nonselective or selective radioligands by subtype-selective agonists and antagonists. Prior to the introduction of such radioligands, the presence of adrenoceptors on immunocompetent cells was surmised via indirect means such as (*a*) concentration-dependent changes in a response following exposure to pharmacological agents, (*b*) blockade of agonist-induced responses with an appropriate antagonist, (*c*) structure-activity relationships, and (*d*) biochemical changes occurring as a consequence of receptor activation.

Columns of Sepharose beads to which various agonists and antagonists had been conjugated were among the earliest attempts to verify the presence of adrenoceptors on immunocompetent cells (247). In this manner, human lymphocytes were shown to possess "receptors" for norepinephrine, since cells remained attached to Sepharose beads to which norepinephrine had been conjugated. Importantly, the norepinephrine-attached cells could only be displaced by epinephrine and propranolol, but not by norepinephrine.

A similar attempt to ascertain the presence of adrenoceptors on immunocompetent cells was made using in vivo-immunized mouse spleen cells (146). These cells were passed through drug-conjugated columns at various times following sheep erythrocyte immunization (days 6 to 13). The cells passing through the column were subsequently assayed for their antibody-forming ability. A suppressed antibody response was obtained using the fall-through cells when epinephrine or isoproterenol was insolubilized on the column, but no change from control was obtained when norepinephrine was used. This was an indication that beta adrenoceptors were present on already *differentiated* B cells. If cAMP levels were measured in the cells which adhered to the column, as opposed to those passing through the column, an accumulation of cAMP was obtained in these cells following isoproterenol stimulation. These data strongly suggested the presence of adrenoceptors on B cells which had *differentiated* to the antibody-secreting stage, but said nothing about the presence of adrenoceptors present on resting cells at the time of immunization.

Radioligand identification of adrenoceptors on murine immunocompetent cells provided a more definitive verification for the presence of these receptors. Most of the binding studies showed receptor characteristics which were saturable, reversible, and of a high affinity. Using the beta antagonist, [^{3}H]dihydroalprenolol, binding sites

were found on adult and fetal murine thymocytes (221). Equal numbers of sites were found on both adult and fetal thymocytes, but the fetal thymocytes possessed a higher affinity for the ligand.

Using spleen lymphocyte membranes from mice, two distinct populations of beta adrenoceptor sites were found using [^{3}H]dihydroalprenolol (116). One was a low-capacity, high-affinity site displaying no cooperative interactions, while the other was a high-capacity, low-affinity site more characteristic of a nonspecific binding site. In this study, no attempt was made to determine which cell population(s) (T, B, or macrophage) possessed the binding sites.

Another research group reported that no adrenoceptors could be found on rat splenic B cells or macrophages, but that a difference was found in receptor density between resting and activated T cells (227). At early stages of activation (induced by in vivo skin graft or in vitro mixed lymphocyte reaction), beta adrenoceptor number decreased to almost 50% of resting levels, whereas, during late stages of activation, beta adrenoceptor number increased 50 to 100% above resting levels.

Recently, changes in beta adrenoceptor density, without changes in affinity, were reported on spleen lymphocytes isolated from adult mice pretreated with 6-OHDA (149). B and T cells were found to have increased numbers of beta adrenoceptors accompanied by a shift in lymphocyte subset number to fewer numbers of B cells.

Alpha adrenoceptor binding sites have been characterized in spleen membrane preparations from guinea pigs (142), using [^{3}H]clonidine as the radioligand. High- and low-affinity sites were found, and the relative potency of agonists and antagonists indicated binding to alpha-2 adrenoceptors. Guinea pigs pretreated with 6-OHDA showed alpha-2 binding sites possessing an increased affinity with no change in receptor number.

Beta adrenoceptors have also been identified on human peripheral blood lymphocytes using various radioligands (26, 186, 249) with beta adrenoceptors being found on both T and B lymphocytes. Using both functional and radioligand binding techniques, human lymphocytes have been found to possess exclusively beta-2 adrenoceptors (31, 55, 82, 249).

D. Immune Responses to Norepinephrine and Adrenoceptor Agonists

Indirect verification for the presence of adrenoceptors on murine spleen cells involved in immune responses has been obtained from experiments in which a particular immune response was altered following exposure to neurotransmitter or adrenoceptor agonists. Some of these studies fulfilled the criteria necessary to classify the altered response as a receptor-mediated event, namely, changes occurring in a concentration-dependent manner and blocked by appropriate adrenoceptor antagonists.

Results obtained using the murine in vitro antibody assay have been conflicting, with either an enhancement or suppression of the response being seen. For clarity, the results from these experiments will be divided into those showing a suppression as opposed to those showing an enhancement of the response.

Suppression of the in vitro-generated antibody response was seen in a concentration-dependent manner when norepinephrine was added at the time of sheep erythrocyte immunization (21). This suppression was reported to have occurred in six of nine experiments, with the other three experiments showing an enhancement or no change at all. The norepinephrine-induced suppression was considered to support the 6-OHDA results obtained by this group, where 6-OHDA treatment allowed for an enhanced antibody response when coupled with adrenalectomy. The investigators concluded that norepinephrine normally exerted a direct suppressive effect in vivo which could be mimicked by a direct norepinephrine-induced suppression in vitro. It was also postulated that the suppression produced by norepinephrine was alpha adrenoceptor mediated, since a concentration-dependent suppression of the antibody response could also be obtained with the alpha-2 agonist, clonidine. No attempt was made to block the suppressive effect of clonidine with phentolamine.

Late phases of the antibody response were also found to be inhibited by addition of isoproterenol to in vivo-immunized mouse spleen cells 15 min prior to assay of the number of antibody-secreting cells (145). The inhibition produced by norepinephrine, however, was much less (145).

In vivo exposure of rats to beta-2 selective agonists caused a decrease in the total serum levels of IgE, with no change in IgM or IgA levels (179). When these rats were exposed to the beta adrenoceptor antagonist, propranolol, an increase in total serum IgE was obtained. Propranolol was also shown to enhance the in vivo anti-dinitrophenol response in rats (191) and the IgE response in mice (166).

In vivo exposure to isoproterenol produced an enhanced antibody response at low concentrations of isoproterenol, as opposed to a suppressed response seen at high concentrations (29). Both of these in vivo responses were blocked by administration of propranolol. An enhancement of the in vivo antibody response was also reported in response to epinephrine (63, 64). These authors postulated that the effect was beta adrenoceptor mediated and that the effect was due to inhibition of resting T-suppressor cells.

Enhancement of the in vitro-generated antibody response was obtained in a concentration-dependent manner when norepinephrine and/or isoproterenol was added to in vitro cultures at the time of immunization (29, 35, 180), but none of these studies attempted to block the induced response with propranolol. Burchiel and Melmon (35), via separation and reconstitution experiments, concluded that the antibody enhancement was due to an

effect of the agonist on B cells and/or macrophages, but not on T cells, although T cells were essential for a complete response to be obtained.

Recent findings have also shown a norepinephrine-induced enhancement of the in vitro-generated antibody response and, in addition, have determined pharmacologically the adrenoceptor type responsible for mediating the observed enhancement produced by norepinephrine (202). In this study, spleen cells were simultaneously exposed to norepinephrine and the alpha adrenoceptor antagonist phentolamine and/or the beta adrenoceptor antagonist propranolol. Norepinephrine alone or norepinephrine in the presence of phentolamine produced an enhanced antibody response when measured on days 5, 6, and 7 after immunization and drug exposure, whereas norepinephrine in the presence of propranolol produced an enhancement on day 4. These results show that beta adrenoceptor activation is responsible for the enhancement observed with norepinephrine alone on days 5, 6, and 7, while the day 4 enhancement is alpha adrenoceptor mediated and can only be unmasked in the presence of propranolol.

To determine which beta adrenoceptor subtype was responsible for mediating the enhanced response, relatively selective agonists for the beta-1 (dobutamine) and beta-2 (terbutaline) adrenoceptors were used, as well as the nonselective agonist, isoproterenol (202). Dobutamine is more selective for stimulating beta-1 adrenoceptors (234), and terbutaline is more selective for stimulating beta-2 adrenoceptors (182). While terbutaline possesses affinity for both beta-1 and beta-2 adrenoceptors, it possesses efficacy only at beta-2 adrenoceptors, suggesting different receptor-effector coupling mechanisms between the two adrenoceptor subtypes (150, 162, 201).

Beta-2 adrenoceptor activation with terbutaline enhanced the antibody response with a similar magnitude and kinetics to that produced by norepinephrine, and the enhancement could be blocked with propranolol (202, 203). This was one method used to conclude that beta-2 adrenoceptor activation was responsible for mediating the enhancing effect of norepinephrine. Relative activities for a series of agonists on the day 5 enhanced response were norepinephrine = terbutaline > isoproterenol > epinephrine $\gg$ dobutamine. It is of interest to note that the relative order of activity for the agonist-induced enhancement did not follow the classic order proposed by Lands (130, 131) for a tissue possessing beta-2 adrenoceptors. One reason for this discrepancy may be that the antibody response does not involve a system in which short-term responses are being measured. The antibody generating system requires at least 4 to 5 days for the processes of activation, proliferation, and differentiation to occur. We are dealing with a system where adrenoceptor agonists may not only mediate different effects at different times during this lengthy process of antibody formation, but may also mediate different effects on different cell populations. In future work, it will be critical to define both the *phase* of the response and the *cell population* which is being modulated by adrenoceptor activation.

Another approach to characterize the beta-2 adrenoceptor-mediated component to the norepinephrine-induced enhancement would be to antagonize the induced enhancement with *selective* antagonists for beta adrenoceptors. Preliminary studies suggest that the relative activities for antagonism of the day 5 norepinephrine-induced enhancement are ICI 118,551 (a beta-2 antagonist) > propranolol > metoprolol (a beta-1 antagonist) (43).

Adrenoceptor activation with dobutamine suggested that a later component of the enhanced response may be mediated by beta-1 activation (202). However, the prolongation produced after dobutamine exposure may be mediated by more of a beta-2 than a beta-1 mechanism, since recent studies have shown that the (+)-enantiomer of dobutamine can activate beta-2, as well as beta-1 adrenoceptors (198, 199). It is also possible that an alpha adrenoceptor-mediated component may be responsible for the prolonged effects produced by dobutamine, since the (−)-enantiomer activates the alpha adrenoceptor in myocardial tissue (125, 198). Dissection of these possibilities will require the use of each enantiomer separately, as well as the use of the racemic mixture in the presence of selective antagonists for each adrenoceptor subtype.

Addition of norepinephrine at times later than day 0 also produced an enhanced response, but of a lesser magnitude (202). It is possible that enhancement of the antibody response induced by norepinephrine at times of addition after day 0 may be mediated via another adrenoceptor type. The enhancement produced by activation of the beta-2 adrenoceptor by terbutaline appears to occur only within the first 24 h of agonist addition to cells immunized on day 0. Addition of terbutaline 24 h after immunization produces no enhancement of the antibody response (203). These studies are congruent with Strom and Carpenter's preliminary work (227), suggesting a decrease in beta adrenoceptor binding sites after immunization.

The role of drug metabolism appears to be minimal in the norepinephrine- or terbutaline-induced expression of a maximally enhanced antibody response when added at the time of immunization. Since catechol-O-methyl transferase (COMT) is present in spleen cells (10, 11), the rate of degradation of adrenergic agents must be considered. For example, terbutaline is not a catechol and is not acted on by COMT (183), and thus may remain in culture for a considerable length of time relative to norepinephrine. Antagonist pulse-chase experiments suggest that any metabolism of norepinephrine occurring early in the culture period is not sufficient to prevent the necessary receptor interactions from occurring to produce a similar enhancement to that seen with the more

stable terbutaline (203). In addition, the similarity between the time required for norepinephrine and terbutaline to attain a maximally enhanced PFC response (203) lends further support that norepinephrine mediates its enhancing effect via beta-2 adrenoceptor activation.

Antagonist pulse-chase experiments have also determined the minimal length of adrenoceptor exposure time required to produce a maximally enhanced antibody response measured a number of days after initial drug exposure (203). These results show that the enhancing effect measured 5 days after addition of norepinephrine or terbutaline was produced after at least 5 to 6 h of agonist exposure before addition of antagonists. The magnitude of the enhancement was found to increase over the first 5 to 6 h until a maximal enhancement was obtained.

Taken collectively, the time of addition and antagonist pulse-chase studies suggest that beta adrenoceptor-mediated events responsible for inducing the enhancement occur *early* in the antibody response. In addition, beta adrenoceptors may actually be down-regulating or becoming desensitized during the immune response itself, since addition of beta agonists at times from 24 to 72 h after immunization produced no change from the control level of response (203). This may be a homeostatic mechanism by which the immune response itself regulates its own magnitude via modulation of adrenoceptor number, affinity, or coupling mechanisms.

There are a number of possible explanations for the kinetic profile seen after agonist exposure. First, the spleen cell suspension used in these studies is a heterogeneous population of cells consisting of different cell types in different phases of the cell cycle. It is possible that adrenoceptor number and/or affinity may increase after antigen presentation or when the responding cell is in a certain phase of the cell cycle. Second, cell-cell interaction is necessary for initiation of a number of events leading to antibody formation (52, 157, 158). Such cell interactions may be required before the receptor-ligand interaction can mediate an effect. Since spleen cells are initially in suspension upon antigen and agonist exposure, they may require time to establish the necessary cell-cell contacts required to allow for maximal beta adrenoceptor-induced enhancement. For example, Ia expression on the macrophage is critical for recognition of antigen on the macrophage surface by the T-helper cell (197). Thus, it is possible that initial contact between the macrophage and the T-helper cell may allow for beta adrenoceptor-induced enhancement of Ia expression on the macrophage and thus allow for enhanced recognition of antigen by T-helper cells. Third, the receptor-ligand interaction may trigger the production and/or release of soluble factors necessary for enhancement of the activation, proliferation, and/or differentiation of cells producing antibody. The amount of soluble factor produced and/or released may be related to the length of time allowed for the receptor-ligand interaction and subsequently to the magnitude of the enhancement produced. For example, macrophage release of IL-1 and T cell release of IL-2 occur after interaction of the macrophage with the T-helper cell (152). This interaction may also allow for beta adrenoceptor expression on one, or both, of these cells. The resulting adrenoceptors may mediate, upon activation, an enhancement of IL-1 or IL-2 release.

Preliminary results suggest that beta adrenoceptor activation induces production of a supernatant possessing enhancing activity (140, 204). The enhancing activity is beta-2 adrenoceptor induced in a time-dependent manner, and its production is blocked by propranolol. Whether this enhancing supernatant contains a presently characterized soluble factor being produced by immunocompetent cells in an increased quantity, or a new as yet uncharacterized soluble product, is unknown at present. Biochemical characterization, coupled with the antibody assay to analyze functionality, will allow future characterization of the soluble product present in the supernatants from agonist-exposed spleen cells.

Adrenoceptor-induced responses involving other immune parameters, which relate to the final T-dependent antibody response, have also been reported. Since the bone marrow is the site from which all immunocompetent cells originate, and since this organ had been shown to be innervated (42, 243), it is important to know if the stem cells from this organ could be modulated by adrenergic agonists. Byron (40, 41) showed that bone marrow stem cells became sensitive to the cytocidal actions of [^{3}H]thymidine following exposure to isoproterenol, and that this effect could be blocked with propranolol. He suggested that this effect was beta-1 adrenoceptor mediated, since initiation of DNA synthesis in these stem cells required much higher concentrations of beta-2 selective agonists to produce effects equivalent to those obtained with isoproterenol. The major conclusion from these studies was that beta adrenoceptor activation was sufficient to trigger resting hematopoietic stem cells into cell cycle, i.e., G_0 to S phase.

Enhancement of the expression of fetal thymocyte cell surface antigens, such as Thy-1 and TL antigens, was shown to occur following exposure to isoproterenol (220). Blockade of the isoproterenol-induced expression of these markers was achieved with propranolol. This enhancement of Thy-1 marker expression was later confirmed in experiments using murine spleen cells and extended to show that isoproterenol enhanced B cell complement receptor number (an early B cell marker), while it suppressed B cell PC number (a late B cell marker) (207).

The latter findings are noteworthy since complement receptors may play a role in the cellular events leading to antibody production. An increase in B cell complement receptors following activation by isoproterenol, or by epinephrine in the presence of phentolamine, was also

seen by Ito and coworkers (110). In contrast to these results, no inhibition of complement receptor number was measured following exposure of spleen cells to norepinephrine or epinephrine in the presence of propranolol. These authors suggested that catecholamines influence complement receptor expression on B cells via beta adrenoceptor activation and thus exert a control on antibody production via modulation of complement receptors.

Lymphocyte responsiveness to the T cell mitogen, concanavalin A, and to the B cell mitogen, lipopolysaccharide, was found to be suppressed following exposure of mouse spleen cells to beta-2 selective agonists, norepinephrine, or epinephrine (117). Propranolol alone caused a suppression of these responses and was not used for blockade experiments. These authors suggested that their results indicated that the B cell response was more sensitive to the suppressive effects of norepinephrine and epinephrine than the T cell response. This suggested that the two cell types may possess different densities of these receptors or that beta adrenoceptors on these cells had different sensitivities for increasing cAMP levels.

The role of alpha adrenoceptor activation in modulation of the antibody response is less characterized. As described previously, Hadden (95) suggested that alpha adrenoceptor activation was responsible for the enhanced uptake of tritiated thymidine in phytohemagglutinin (PHA)-stimulated human lymphocytes exposed to norepinephrine. Besedovsky and coworkers suggested that norepinephrine exerted a suppressive effect on the antibody response via an alpha-2 adrenoceptor mechanism (21). Besedovsky also reported that the alpha-1 adrenoceptor agonist, methoxamine, mediated a suppression of the in vivo antibody response (23). Neither of the last two agonist-induced responses was reported to be blocked with an alpha selective antagonist.

Recent results provide evidence that enhancement of the antibody response measured 1 day prior to the peak control response in cultures exposed to norepinephrine in the presence of propranolol (202) is alpha-1 adrenoceptor mediated (205). Methoxamine is an agonist relatively selective for alpha-1 adrenoceptors (37). Exposure of immunized spleen cells to methoxamine produced an enhancement similar to that seen with norepinephrine in the presence of propranolol when assayed 1 day prior to the peak control response. The relative order of activity of agonists for this day 4 enhancement was methoxamine > epinephrine > norepinephrine = phenylephrine = clonidine. Alpha-1 adrenoceptor activation also produced a similar response to that produced by norepinephrine in the presence of propranolol on days 5, 6, and 7 where a return to control antibody response was measured (205). The nonselective alpha antagonist, phentolamine, was able to antagonize this day 4 enhancement induced by methoxamine. This is the first characterization of the alpha adrenoceptor subtype responsible for the enhancement occurring 1 day prior to the peak control response.

These results are in contrast to a reported decrease in the in vivo primary antibody response after exposure of rats to methoxamine just before sheep erythrocyte immunization (23). This apparent contradiction between in vivo and in vitro results in response to an adrenergic agonist must be considered. As described previously, the in vitro-generated response is devoid of a number of in vivo influences which can be precipitated by sympathomimetic amines. These influences may indirectly impact on the in vivo immune response itself and consequently modulate the magnitude of the antibody response.

The alpha-1 (205) and beta-2 (202, 203) adrenoceptor-induced enhancements of the antibody response, although occurring on different days, may possibly provide an interesting area of mechanistic investigation. There are examples of systems where alpha-1 and beta adrenoceptor activation produce similar physiological responses, albeit via different cellular mechanisms (190).

Clonidine is an agonist relatively selective for alpha-2 adrenoceptors (66) with some antagonist activity at alpha-1 adrenoceptors (141). It has been reported previously that clonidine produces a concentration-dependent suppression of the in vitro murine PFC response on the peak day of control response (21). Recent results (205) confirm the above observation and, in addition, show that the suppressed response can be blocked by phentolamine. The relative order of activity of agonists for the day 5-induced suppression was clonidine = phenylephrine > methoxamine > epinephrine > norepinephrine. Preliminary antagonist pulse-chase studies (206) indicate that clonidine may inhibit the antibody response within the first 6 h after antigen exposure, and that addition of clonidine between 24 and 72 h after antigen produces no change from a control antibody response. Again, this observation suggests down-modulation of receptor number and/or affinity after antigen and/or agonist exposure.

Since alpha-2 adrenoceptor activation induces a suppressed antibody response (205) on the same day that beta-2 adrenoceptor activation induces an enhanced response (202, 203), it is not unreasonable to postulate that the intracellular mechanisms through which these adrenoceptors act may explain the observed results. While beta-2 adrenoceptor activation causes an enhancement of adenylate cyclase activity, alpha-2 adrenoceptor activation causes an inhibition of adenylate cyclase activity in a variety of cell types. This information, coupled with evidence to be presented later in this review showing that changes in cAMP levels can modulate the in vitro antibody response, suggests that *preferential* activation of these different adrenoceptor types may alter the antibody response positively or negatively.

A number of endogenous and therapeutic substances are capable of elevating cAMP levels, e.g., catechol-

amines, prostaglandins, and theophylline, and may cause an undesirable enhancement of the antibody response if present at early times after immunization. It is possible that alpha-2 adrenoceptor activation may provide an endogenous, as well as a therapeutic, means of reversing the imposed increase in cAMP levels and subsequent antibody enhancement.

E. Immune Responses to Cyclic Nucleotides

Beta adrenoceptor activation is thought to mediate its physiological response via an enhancement of adenylate cyclase activity, with subsequent enhancement of intracellular levels of cAMP (189). This molecule is thought to represent the second messenger responsible for inducing a number of cellular responses. As data accumulated suggesting that beta adrenoceptor activation modulated immune responses, researchers began to investigate the effects produced by the cyclic nucleotide itself. Another area of investigation was to measure the levels of cAMP within immune cells at various times following adrenoceptor activation or cell surface modulation by mitogens or antigens. For clarity, the literature in this area will be limited to that involving the antibody response and will be presented in a chronological sequence.

Epinephrine was the first adrenergic neurotransmitter shown to cause an elevation of cAMP levels in murine immunocompetent cells (136). This elevation in cAMP induced by epinephrine occurred within 10 min, followed by active DNA synthesis within 30 min, with cells entering mitosis within 2 h. The conclusion was made that epinephrine alone acted as a mitogen, and that this action was mediated by cAMP.

Dibutyryl cAMP (dbcAMP) is often used to determine the effects of cAMP on cellular responses, since this form of the cyclic nucleotide more readily penetrates the lipid bilayer of cells. Following in vivo or in vitro exposure to dbcAMP, an enhancement of the antibody response to sheep erythrocytes was measured (108). The in vitro-induced enhancement suggested that dbcAMP was exerting a direct effect on immunocompetent cells involved in the antibody response, and not on other in vivo systems which indirectly affect antibody formation.

In an attempt to determine the cell population affected by cAMP, and thus the cell type responsible for mediating the enhanced antibody response, adherent (macrophage) and nonadherent cells (T and B cells) were exposed separately to dbcAMP and then reconstituted. In these experiments, poly A:U was used to stimulate cAMP accumulation (109). Whole cell preparations exposed to low doses of polyadenylic acid:polyuridylic acid for short periods of time prior to antigen exposure enhanced the antibody response, while high doses inhibited the response. Pretreatment of the nonadherent cells appeared to mimic this enhancement, while pretreatment of the adherent cells produced no change in the response from control values. This cAMP-mediated enhancement of the response also appeared to be a metabolism-dependent event, since pretreatment of the spleen cells at 5°C, as opposed to 37°C, did not allow for the enhancement.

A theory was proposed by Watson and colleagues (241) that the ratio of cAMP to cGMP (guanosine 3′,5′-monophosphate) was important for antibody induction, and that cAMP may be a mediator of immune paralysis or tolerance. Experimental data were collected using spleen cells exposed to dbcAMP for various lengths of time prior to wash-out. These data suggested that, when cAMP levels were elevated in the spleen cells up to 9 h following antigen, an enhancement of the antibody response was obtained. In contrast, if cAMP levels were elevated beyond this time, a suppression of the response was obtained. Watson proposed that two signals were required by the B cell for induction of antibody production. (*a*) Antigen coupled to the B cell produced a signal which led to accumulation of cAMP which over time became inhibitory to the cell, and (*b*) a T cell signal delivered to the antigen-B cell complex signalled an increase in the levels of cGMP which was able to overide the cAMP-induced inhibition (242). This latter cGMP signal was ascertained from experiments in which cGMP induced an enhanced antibody response in T cell-depleted spleen cultures, and in which addition of excess primed T cells overcame the inhibition produced in cultures exposed to cAMP.

In a review by Bourne and colleagues (27), a summary of the evidence suggesting that cAMP may regulate leukocyte function was presented. The authors outlined a hypothesis stating that regulation of immune response intensity was mediated by the action of cAMP which was inhibitory. There was a discussion of the possibility that amine receptors, i.e., catecholamine, histamine, dopamine, and 5-hydroxytryptamine receptors, may not develop on immunocompetent cells until after exposure to antigen; however, the experimental evidence for this statement is only found in a footnote accompanying this article. This evidence was in contrast to the work presented by Shearer *et al.* (210) in which spleen cells from unimmunized mice were passed through histamine-conjugated columns. The nonadherent cells were adoptively transferred in vivo and immunized with sheep erythrocytes, and the antibody response was subsequently measured. The result of an enhanced antibody response suggested that receptors for histamine were present on spleen cells prior to immunization and that these receptors must have been on T-suppressor cells. Subsequent injection of the eluate from these columns into irradiated mice reconstituted with bone marrow cells and immunized with sheep erythrocytes confirmed the presence of histamine receptors on T-suppressor cells by producing a suppression of the resulting antibody response.

When spleen cells were removed from immunized mice and treated with dbcAMP 15 min prior to assay for antibody-secreting cells, a suppression of the response occurred (145). Measurement of cAMP accumulation in

these immunized cells following exposure to isoproterenol showed a concentration-dependent accumulation of cAMP which correlated with the concentration-dependent suppression of the antibody response. These results suggested to the authors that cAMP acted as a second messenger to suppress the *late* antibody-secreting stage of the antibody response.

It now became evident that the timing of cAMP exposure in relation to generation of the antibody response was important to the final outcome of either enhancement or suppression of the response. A theory was soon proposed by Parker and colleagues that lymphocytes involved in the antibody response responded to cAMP in different ways (176, 177, 244, 245). First, the resting cell required some type of intracellular signal in order to be released from its resting G_0 phase to the G_1 phase of the cell cycle. This critical triggering signal was thought to be provided by an early increase in cAMP, since mitogens (212, 213, 240) as well as sheep erythrocytes (185, 253) had been shown to cause an early rise in cAMP levels of murine spleen cells. In an attempt to explain the discrepancies with data suggesting an inhibitory role for cAMP in the immune response, Parker and colleagues reported that different adenylate cyclases may be activated in different compartments within the cell by different agents. Thus, mitogens appeared to cause an accumulation of cAMP at the membrane, while isoproterenol caused an accumulation of cAMP in different areas of the cell (176). Second, the cell in G_1 phase of the cell cycle must be triggered to proceed through the G_1 phase into S phase. If cAMP levels are raised during this phase of the cell cycle, many cell types are inhibited from entering S phase (175). A soluble product produced by immunized immunocompetent cells, termed inhibitor of DNA synthesis, has been reported to raise cAMP levels in recipient stimulated lymphocytes during late G_1 phase of the cell cycle (114). Late G_1 phase was also shown to be the phase during which this soluble product had maximal effect on inhibition of subsequent DNA synthesis.

Further evidence for the timing of in vitro dbcAMP exposure in relation to subsequent antibody measurement continued to accumulate. Enhancement of the antibody response occurred when spleen cells were exposed to dbcAMP early in the culture period for up to 24 h following immunization (35, 119, 185, 231, 232, 241), whereas supression of the antibody response occurred when spleen cells were exposed to dbcAMP late in the culture period, i.e., if dbcAMP remained in the cultures from the time of immunization or were added or removed later than 24 h following immunization (35, 185, 231, 241).

Isoproterenol was shown to produce an optimal accumulation of cAMP in mouse spleen, thymus, and lymph node cells following only 10 min of exposure to the agonist (12). This accumulation was concentration dependent and could be blocked by the addition of propranolol. Similar cAMP accumulations were seen in splenic T and B cells, but lymph node B cells were found to accumulate cAMP to a greater magnitude than T cells. It is also interesting that thymocytes showed the greatest increase in cAMP content, and that the resting levels of cAMP in thymocytes were much lower than levels measured in spleen cells.

The role of cAMP in activation and proliferation of cells stimulated by sheep erythrocytes was investigated in vivo (185, 253). Within 2 min following sheep erythrocyte injection, cAMP levels in mouse spleen cells rose to a level dependent on the concentration of antigen injected. Cyclic AMP levels returned to basal levels by 20 min and fell below basal levels at 3 to 4 days. It should be noted that proliferation begins approximately 2 days after antigen exposure. These authors surmised that the early and late changes in cAMP levels following antigen exposure could explain the effects seen with drugs that modulated these levels. Any drug increasing cAMP levels given late in the active process of antibody formation would reverse the effect of antigen by itself, whereas the same drug given early in the response would enhance the antibody response further.

Yamamoto and Webb (253) reported that cAMP changes in spleen cells in response to antigen were dependent on the presence of T cells. The evidence for this was (*a*) spleen cells from nude mice deficient in T cells produced no change in cAMP levels following sheep erythrocyte exposure, and (*b*) corticosteroid pretreatment, which selectively enhances mature T cell number, allowed for an even greater enhancement in cAMP levels following sheep erythrocyte exposure. Sympathetic nervous system involvement in cAMP changes following sheep erythrocyte exposure was ruled out by pretreating mice with propranolol. This pretreatment still allowed for the increase in cAMP levels following antigen injection.

Attempts have been made to characterize the cell type(s) responsible for mediating the enhancing effect produced by early exposure to dbcAMP in vitro. Teh and Paetkau (232) postulated that dbcAMP had no direct effect on B cells or T-helper cells, but produced an effect on the antibody response by inhibiting macrophage and T-suppressor cell function. Johnson et al. (119) postulated that dbcAMP inhibited T-suppressor cell activity and interferon production, while enhancing T-helper cell activity. In contrast, Burchiel and Melmon (35) attribute the enhancing effect of early dbcAMP exposure to an effect on B cells and/or macrophages, although they report that T cells were important for generation of a complete antibody response. The latter researchers also showed that agents which elevated cAMP enhanced the antibody response in direct relation to their ability to maintain elevated levels of cAMP. Recently, forskolin activation of adenylate cyclase activity in enriched pop-

ulations of B cells has been found to interfere with the progression of activated B cells into S phase of the cell cycle (160). In another study, dbcAMP and IL-1 have been shown to stimulate antigen-specific and polyclonal antibody production from cultures containing purified B cells (88). In the same study, it was also shown that dbcAMP inhibited T-helper cell production of IL-2 as well as the production of an inhibitor of BCGF activity (88). Subtle differences exist between most of the assays used by the above researchers, but the point is clear that the latter studies have attempted to use purified cell populations to address the complex question involving the role of cAMP in functional modulation of the activity of the individual cells involved in the complex process of antibody formation.

If cAMP is involved in the activation process leading to an enhanced antibody resonse, phosphorylation of cellular proteins must be demonstrated (93). Attempts to isolate cAMP-dependent protein kinases and phosphorylated proteins have been made. Parker and colleagues (47) have shown cAMP-dependent protein kinase activity in both cytoplasmic and plasma membrane fractions of lymphocytes, and they have isolated phosphorylated proteins from these two cellular fractions. Ortez (174) has also measured cAMP-dependent protein kinase activity in resting murine spleen cells and determined that B cells had 6 to 10 times less activity than T cells.

The role of cGMP in the antibody response is very controversial and therefore will not be discussed at length in this review. The reader is referred to the reviews of Wedner (245), Parker (177), and Hadden (97) on the differing viewpoints regarding the involvement of this cyclic nucleotide in immune responses.

V. Evidence for an Interchange of Information between the Immune and Nervous Systems

If neurotransmitters affect the magnitude of immune responses, a communication between the activated immune process and the central nervous system must be ascertained. Recent findings suggest that such a communication exists, and that the end result of this communication may result in modulation of neurotransmitter levels in the vicinity of an ongoing immune process in order to modulate the magnitude and duration of the response.

Preceding references in this review have strongly suggested that sympathetic neurotransmitters can affect the magnitude of the antibody response both in vivo and in vitro. The afferent limb of this response, i.e., communication between the immune response and the central nervous system, is currently being investigated. Besedovsky and his colleagues have been in the forefront of this research. This research group injected rats with sheep erythrocytes and measured an increase in serum corticosterone levels during the peak of the antibody response and a drop in thyroxine levels just prior to and during the peak antibody response (18, 20). This suggested that the immune response itself affected hormonal levels measured in the blood. Stronger evidence suggesting an afferent pathway between the activated immune system and the central nervous system was found when sheep erythrocyte-immunized rats showed an increase in the firing rate of neurons in the hypothalamus as compared to saline-injected rats (19). An afferent pathway was further substantiated by injection of supernatants from concanavalin A-treated rat or human spleen cells into rats, with a subsequent increase in corticosterone levels within 30 min (22). In 1983, it was reported that the injection of sheep erythrocytes or supernatants from concanavalin A-treated spleen cells into rats produced a decrease in the norepinephrine content of the hypothalamus and brainstem, as measured by a decrease in the turnover rate of this neurotransmitter (24).

Additional evidence comes from animal studies in which lesions of selected brain nuclei cause a decrease in spleen and thymus cell number, as well as a decrease in the responsiveness of spleen cells from these animals to concanavalin A (32). In addition, lesions of the anterior hypothalamus have been shown to suppress the cytotoxic activity of natural killer cells (57), and lesions of selected areas of the brainstem have altered various immune responses (139). Also, a number of studies have utilized behaviorally conditioned modulation of immune responses to demonstrate a link between the central nervous and immune systems (3, 28).

VI. Conclusions

The immune process has generally been considered to be a self-regulating process controlled by a number of the mechanisms described earlier in the immunology section of this review. However, the pharmacological studies outlined in this review provide evidence that an additional mechanism for immune regulation exists, namely modulation by the sympathetic nervous system. The research in this area has only begun to answer the many questions which must be addressed for a complete understanding of this mechanism of antibody modulation. Concurrently, the second messengers mediating the effects of a number of the soluble immune factors are being researched. It is most likely that adrenoceptor activation at specific times during the immune response will modulate the magnitude of such factor-induced effects via modulation of the level of intensity of such second messengers as cAMP, cGMP, and calcium. Future research will need to identify not only the number and affinity of the subtypes of adrenoceptors on each individual cell involved in this complex response, but also the cellular functions induced or suppressed upon selective adrenoceptor activation. This latter area of research will become increasingly important, since these adrenoceptors are most likely being up- and down-modulated during the many different stages of activation of these cells. These types of research will better be accomplished in the future by the use of individual cell types, such as

macrophages and antigen-specific T and B cells, by the development of more selective adrenoceptor agonists and antagonists, and by the use of purified, as well as recombinant, cytokines.

Acknowledgments. Acknowledgments. We thank Ms. Brenda Rexrode for her excellent preparation of each draft of this manuscript. The critical reading of the immunology section of this review by Drs. Ellen Vitetta and Kathie Brooks is greatly appreciated.

REFERENCES

1. Abbas, A. K., Takaoki, M., and Greene, M. I.: T lymphocyte-mediated suppression of myeloma function in vitro. IV. Generation of effector suppressor cells specific for myeloma idiotypes. J. Exp. Med. **155:** 1216–1221, 1982.
2. Abruzzo, L. V., and Rowley, D. A.: Homeostasis of the antibody response: immunoregulation by NK cells. Science (Wash. DC) **222:** 581–585, 1983.
3. Ader, R., and Cohen, N.: Conditioned immunopharmacologic effects. *In* Psychoneuroimmunology, ed. by R. Ader, pp. 545–574, Academic Press, New York, 1981.
4. Ahlquist, R. P.: A study of the adrenotropic receptors. Am. J. Physiol. **153:** 586–600, 1948.
5. Allison, A. C.: Mechanisms by which activated macrophages inhibit lymphocyte responses. Immunol. Rev. **40:** 3–27, 1978.
6. Anderson, W. B., and Jaworski, C. J.: Isoproterenol-induced desensitization of adenylate cyclase responsiveness in a cell-free system. J. Biol. Chem. **254:** 4596–4601, 1979.
7. Andersson, J., Schreier, M. H., and Melchers, F.: T-cell-dependent B-cell stimulation is H-2 restricted and antigen dependent only at the resting B-cell level. Proc. Natl. Acad. Sci. USA **77:** 1612–1616, 1980.
8. Asano, T., Katada, T., Gilman, A. G., and Ross, E. M.: Activation of the inhibitory GTP-binding protein of adenylate cyclase, Gi, by beta adrenergic receptors in reconstituted phospholipid vesicles. J. Biol. Chem. **259:** 9351–9354, 1984.
9. Ashwell, J. D., DeFranco, A. L., Paul, W. E., and Schwartz, R. H.: Antigen presentation by resting B cells. Radiosensitivity of the antigen-presentation function and two distinct pathways of cell activation. J. Exp. Med. **159:** 881–905, 1984.
10. Axelrod, J., and Tomchick, R.: Enzymatic O-methylation of epinephrine and other catechols. J. Biol. Chem. **233:** 702–705, 1958.
11. Axelrod, J., Inscoe, J. K., and Daly, J.: Enzymatic formation of O-methylated dihydroxy derivatives from phenolic amines and indoles. J. Pharmacol. Exp. Ther. **149:** 16–22, 1965.
12. Bach, M.: Differences in cyclic AMP changes after stimulation by prostaglandins and isoproterenol in lymphocyte subpopulations. J. Clin. Immunol. **55:** 1074–1081, 1975.
13. Barnett, D. B., Rugg, E., and Nahorski, S. R.: Direct evidence of two types of beta adrenoceptor binding site in lung tissues. Nature (Lond.) **273:** 166–168, 1978.
14. Bein, H. J.: The pharmacology of Rauwolfia. Pharmacol. Rev. **8:** 435–483, 1956.
15. Beller, D. I., and Ho, K.: Regulation of macrophage populations. V. Evaluation of the control of macrophage Ia expression in vitro. J. Immunol. **129:** 971–976, 1982.
16. Benacerraf, B., Katz, D. H., Kapp, J. A., and Pierce, C. W.: Regulatory mechanisms in the immune response. *In* Cellular Selection and Regulation in the Immune Response, ed. by G. M. Edelman, pp. 265–281, Raven Press, New York, 1974.
17. Berthelsen, S., and Pettinger, W. A.: A functional basis for classification of alpha adrenergic receptors. Life Sci. **21:** 595–606, 1977.
18. Besedovsky, H., and Sorkin, E.: Changes in blood hormone levels during the immune response. Proc. Soc. Exp. Biol. Med. **150:** 466–470, 1975.
19. Besedovsky, H., Sorkin, E., Felix, D., and Haas, H.: Hypothalamic changes during the immune response. Eur. J. Immunol. **7:** 323–325, 1977.
20. Besedovsky, H., and Sorkin, E.: Network of immune-neuroendocrine interactions. Clin. Exp. Immunol. **27:** 1–12, 1977.
21. Besedovsky, H. O., DelRey, A., Sorkin, E., DePrada, M., and Keller, H. H.: Immunoregulation mediated by the sympathetic nervous system. Cell. Immunol. **48:** 346–355, 1979.
22. Besedovsky, H. O., and Sorkin, E.: Immunologic-neuroendocrine circuits: physiological approaches. *In* Psychoneuroimmunology, ed. by R. Ader, pp. 545–574, Academic Press, New York, 1981.
23. Besedovsky, H. O., DePrada, M., DelRey, A., and Sorkin, E.: Immunoregulation by sympathetic nervous system. Trends Pharmacol. Sci. **2:** 236–238, 1981.
24. Besedovsky, H., DelRey, A., Sorkin, E., DaPrada, M., Burri, R., and Honegger, C.: The immune response evokes changes in brain noradrenergic neurons. Science (Wash. DC) **221:** 564–566, 1983.
25. Bilezikian, J. P., and Loeb, J. N.: The influence of hyperthyroidism and hypothyroidism on alpha- and beta-adrenergic receptor systems and adrenergic responsiveness. Endocrine Rev. **4:** 378–388, 1983.
26. Bisphoric, N. H., Cohen, H. J., and Lefkowitz, R. J.: Beta adrenergic receptors in lymphocyte subpopulations. J. Allergy Clin. Immunol. **65:** 29–33, 1980.
27. Bourne, H. R., Lichtenstein, L. M., Melmon, K. L., Henney, C. S., Weinstein, Y., and Shearer, G. M.: Modulation of inflammation and immunity by cyclic AMP. Science (Wash. DC), **184:** 19–28, 1974.
28. Bovbjerg, D., Ader, R., and Cohen, N.: Acquisition and extinction of conditioned suppression of a graft-vs-host response in the rat. J. Immunol. **132:** 111–113, 1984.
29. Braun, W., and Rega, M. J.: Adenyl cyclase-stimulating catecholamines as modifiers of antibody formation. Immunol. Commun. **1:** 523–532, 1972.
30. Britton, S., and Moller, G.: Regulation of antibody synthesis against *Escherichia coli* endotoxin. I. Suppressive effect of endogenously produced and passively transferred antibodies. J. Immunol. **100:** 1326–1334, 1968.
31. Brodde, O-E., Engel, G., Hoyer, D., Bock, K. D., and Weber, F.: The beta-adrenergic receptor in human lymphocytes: subclassification by the use of a new radioligand, (+)-^{125}I iodocyanopindolol. Life Sci. **29:** 2189–2198, 1981.
32. Brooks, W. H., Cross, R. J., Rozman, T. L., and Markesbery, W. R.: Neuroimmunomodulation: neural anatomical basis for impairment and facilitation. Ann. Neurol. **12:** 56–61, 1982.
33. Bulloch, K., and Moore, R. Y.: Innervation of the thymus gland by brain stem and spinal cord in mouse and rat. Am. J. Anat. **162:** 157–166, 1981.
34. Bulloch, K., and Pomerantz, W.: Autonomic nervous system innervation of thymic-related lymphoid tissue in wild type and nude mice. J. Comp. Neurol. **228:** 57–68, 1984.
35. Burchiel, S. W., and Melmon, K. L.: Augmentation of the in vitro humoral immune response by pharmacologic agents. I: An explanation for the differential enhancement of humoral immunity via agents that elevate cAMP. Immunopharmacology **1:** 137–150, 1979.
36. Burges, R. A., and Blackburn, K. J.: Adenyl cyclase and the differentiation of beta-receptors. Nature (Lond.) **235:** 249–250, 1972.
37. Burn, P., Crooks, P. A., Ratcliff, B. L., and Rees, J. M. H.: Sympathomimetic effects of *exp-* and *endo-*isomers of 2-aminobenzonorbornene in vitro and in vivo. J. Pharm. Pharmacol. **32:** 87–91, 1980.
38. Burns, T. W., Langley, P. E., Terry, B. E., Bylund, D. B., Hoffman, B. B., Tharp, M. D., Lefkowtiz, R. J., Garcia-Sainz, J. A., and Fain, J. N.: Pharmacological characterizations of adrenergic receptors in human adipocytes. J. Clin. Invest. **67:** 467–475, 1981.
39. Bylund, D. B., and U'Prichard, D. C.: Characterization of alpha-1 and alpha-2 adrenergic receptors. Int. Rev. Neurobiol. **24:** 343–422, 1983.
40. Byron, J. W.: Evidence for a beta-adrenergic receptor initiating DNA synthesis in haemopoietic stem cells. Exp. Cell. Res. **71:** 228–232, 1972.
41. Byron, J. W.: Manipulation of the cell cycle of the hemopoietic stem cell. Exp. Hematol. **3:** 44–53, 1975.
42. Calvo, W.: The innervation of the bone marrow in laboratory animals. Am. J. Anat. **123:** 315–328, 1968.
43. Campbell, K. S., Sanders, V. M., and Munson, A. E.: Further characterization of the beta-2 adrenoceptor mediated enhancement of the murine primary antibody response in vitro (abstract). Fed. Proc. **44:** 1488, 1985.
44. Carlsson, E., Ablad, B., Brandstrom, A., and Carlsson, B.: Differential blockade of the chrontropic effects of various adrenergic stimuli in the cat heart. Life Sci. **11:** 953–958, 1972.
45. Cerione, R. A., Strulovici, B., Benovic, J. L., Lefkowitz, R. J., and Caron, M. G.: Pure beta-adrenergic receptor: the aingle polypeptide confers catecholamine responsiveness to adenylate cyclase. Nature (Lond.) **306:** 562–566, 1983.
46. Chamat, S., Hoebeke, J., and Strosberg, A. D.: Monoclonal antibodies specific for beta-adrenergic ligands. J. Immunol. **133:** 1547–1552, 1984.
47. Chaplin, D. D., Wedner, H. J., and Parker, C. W.: Protein phosphorylation in human peripheral blood lymphocytes. II. Phosphorylation of endogenous plasma membrane and cytoplasmic proteins. Biochem. J. **182:** 537–546, 1979.
48. Chen, C., and Hirsch, J. G.: The effect of mercaptoethanol and of peritoneal macrophages on the antibody-forming capacity of nonadherent mouse spleen cells in vitro. J. Exp. Med. **136:** 604–612, 1972.
49. Chestnut, R. W., and Grey, H. M.: Studies on the capacity of B cells to serve as antigen-presenting cells. J. Immunol. **126:** 1075–1079, 1981.
50. Chuang, D. M., Kinnier, W. J., Farber, L., and Costa, E.: The biochemical study of receptor internalization during beta-adrenergic receptor desensitization in frog erythrocytes. Mol. Pharmacol. **18:** 348–355, 1980.
51. Chuang, D. M.: Beta-adrenergic receptor internalization and processing: role of transglutaminase and lysosomes. Mol. Cell. Biochem. **58:** 79–89, 1984.
52. Claman, H. N., and Mosler, D. E.: Cell-cell interactions in antibody production. Prog. Allergy **16:** 40–80, 1972.
53. Clayberger, C., Dekruyff, R. H., and Cantor, H.: Immunoregulatory activities of autoreactive T cells: an I-A-specific T cell clone mediates both help and suppression of antibody responses. J. Immunol. **132:** 2237–2243, 1984.
54. Click, R. E., Benck, L., and Alter, B. J.: Enhancement of antibody synthesis in vitro by mercaptoethanol. Cell. Immunol. **3:** 156–160, 1972.
55. Conolly, M. E., and Greenacre, J. K.: The beta-adrenoceptor on the

human lymphocyte and human lung parenchyma. Br. J. Pharmacol. **59:** 17–23, 1977.
56. Crabtree, G. R., Gillis, S., Smith, K. A., and Munck, A.: Mechanisms of glucocorticoid-induced immunosuppression: inhibitory effects on expression of Fc receptors and production of T cell growth factor. J. Steroid Biochem. **12:** 445–449, 1980.
57. Cross, R. J., Markesbery, W. R., Brooks, W. H., and Rozman, T. L.: Hypothalamic-immune interactions: neuromodulation of natural killer activity by lesioning of the anterior hypothalamus. Immunology **51:** 399–405, 1984.
58. Daly, M. J., and Levy, G. P.: The sub-classification of β-adrenoceptors: evidence in support of the dual β-adrenoceptor hypothesis. *In* Trends in Autonomic Pharmacology, ed. by D. Kalsner, Vol. 1, pp. 347–385, Urban and Schwarzenberg, Baltimore, 1979.
59. Davies, A. O.: Regulation of beta-adrenergic receptors by steroid hormones. Annu. Rev. Physiol. **46:** 119–130, 1984.
60. DeFranco, A. L., Kung, J. T., and Paul, W. E.: Regulation of growth and proliferation in B cell subpopulations. Immunol. Rev. **64:** 161–182, 1982.
61. DeFreitas, E. C., Chesnut, R. W., Grey, H. M., and Chiller, J. M.: Macrophage-dependent activation of antigen-specific T cells requires antigen and a soluble monokine. J. Immunol. **131:** 23–29, 1983.
62. DelRey, A., Besedovsky, H. O., Sorkin, E., DaPrada, M., and Bondiolotti, G. P.: Sympathetic immunoregulation: difference between high- and low-responder animals. Am. J. Physiol. **242:** R30–R33, 1972.
63. DePelchin, A., and Letesson, J. J.: Adrenaline influence on the immune response. I. Accelerating or suppressor effects according to the time of application. Immunol. Lett. **3:** 199–205, 1981.
64. DePelchin, A., and Letesson, J. J.: Adrenaline influence on the immune response. II. Its effects through action on the suppressor T-cells. Immunol. Lett. **3:** 207–213, 1981.
65. Dorf, M. E., and Benacerraf, B.: Suppressor cells and immunoregulation. Annu. Rev. Immunol. **2:** 127–158, 1984.
66. Doxey, J. C., Frank, L. W., and Hersom, A. S.: Studies on the pre- and post-junctional activities of alpha-adrenoceptor agonists and their cardiovascular effects in anesthetized rats. J. Auto. Pharmacol. **1:** 157–169, 1981.
67. Draskoci, M., and Jankovic, B. D.: Involution of thymus and suppression of immune responses in rats treated with reserpine. Nature (Lond.) **202:** 408, 1964.
68. Dukor, P., Salvin, S. B., Dietrich, F. M., Gelzer, J., Hess, R., and Loustalot, P.: Effect of reserpine on immune reactions and tumor growth. Eur. J. Cancer **2:** 253–261, 1966.
69. Dutton, R. W., Wetzel, G. D., and Swain, S. L.: Partial purification and characterization of a BCGFII from EL4 culture supernatants. J. Immunol. **132:** 2451–2456, 1984.
70. Eardley, D. D., Hugenberger, J., McVay-Boudreau, L., Shen, F. W., Gershon, R. K., and Cantor, H.: Immunoregulatory circuits among T-cell sets. I. T-helper cells induce other T-cell sets to exert feedback inhibition. J. Exp. Med. **147:** 1106–1115, 1978.
71. Egwang, T. G., and Befus, A. D.: The role of complement in the induction and regulation of immune responses. Immunology **51:** 207–224, 1984.
72. Eichmann, K.: Expression and function of idiotypes on lymphocytes. Adv. Immunol. **26:** 195–254, 1978.
73. Erb, P., and Feldmann, M.: The role of macrophages in the generation of T-helper cells. II. The genetic control of the macrophage-T-cell interaction for helper cell induction with soluble antigens. J. Exp. Med. **142:** 460–472, 1975.
74. Ernstrom, U., and Sandberg, G.: Effects of adrenergic alpha- and beta-receptor stimulation on the release of lymphocytes and granulocytes from the spleen. Scand. J. Haematol. **11:** 275–286, 1973.
75. Ernstrom, U., and Soder, O.: Influence of adrenaline on the dissemination of antibody-producing cells from the spleen. Clin. Exp. Immunol. **23:** 131–140, 1975.
76. Exton, J. H.: Mechanisms involved in alpha-adrenergic phenomena. Am. J. Physiol. **248:** E633–E647, 1985.
77. Fain, J. N., and Gracia-Sainz, J. A.: Role of phosphatidylinositol turnover in alpha 1 and of adenylate cyclase inhibition of alpha 2 effects of catecholamines. Life Sci. **26:** 1183–1194, 1980.
78. Feller, D. J., and Bylung, D. B.: Comparison of alpha-2 adrenergic receptors and their regulation in rodent and porcine species. J. Pharmacol. Exp. Ther. **228:** 275–282, 1984.
79. Felten, D. L., Felten, S. Y., Carlson, S. L., Olschowka, J. A., and Livnat, S.: Noradrenergic and peptidergic innervation of lymphoid tissue. J. Immunol. **135:** 755s–765s, 1985.
80. Fraser, C. M., and Venter, J. C.: Monoclonal antibodies to beta-adrenergic receptors: use in purification and molecular characterization of beta receptors. Proc. Natl. Acad. Sci. USA **77:** 7034–7038, 1980.
81. Fujiwara, M., Muryobayashi, T., and Shimamoto, K.: Histochemical demonstration of monoamines in the thymus of rats. Jpn. J. Pharmacol. **16:** 493–494, 1966.
82. Galant, S. P., Underwood, S. B., Lundak, T. C., Groncy, C. C., and Mouratides, D. I.: Heterogeneity of human lymphocyte subpopulations to pharmacologic stimulation. I. Lymphocyte responsiveness to beta adrenergic agents. J. Allergy Clin. Immunol. **62:** 349–356, 1978.
83. Galindo, B., and Imaeda, T.: Electron microscope study of the white pulp of the mouse spleen. Anat. Rec. **143:** 399–415, 1962.
84. Garcia-Sainz, J. A., Hoffman, B. B., Li, S., Lefkowitz, R. J., and Fain, J. N.: Role of alpha 1 adrenoceptors in the turnover of phosphatidylinositol and the alpha 2 adrenoceptors in the regulation of cyclic AMP in hamster adipocytes. Life Sci. **27:** 953–961, 1980.
85. Gershon, R. K.: Immune regulation. Fed. Proc. **38:** 2051–2077, 1979.
86. Gilbert, K. M., and Hoffmann, M. K.: Lymphokine-induced suppressor B cells. Immunology **46:** 545–553, 1982.
87. Gilbert, K. M., and Hoffmann, M. K.: Suppressor B lymphocytes. Immunol. Today **4:** 253–255, 1983.
88. Gilbert, K. M., and Hoffman, M. K.: cAMP is an essential signal in the induction of antibody production by B cells, but inhibits helper function of T cells. J. Immunol. **135:** 2084–2089, 1985.
89. Gillis, S., and Mizel, S.: T cell lymphoma model for the analysis of interleukin-1 mediated T cell activation. Proc. Natl. Acad. Sci. USA **78:** 1133–1137, 1981.
90. Giron, L. T., Crutcher, K. A., and Davis, J. N.: Lymph nodes—a possible site for sympathetic neuronal regulation of immune responses. Ann. Neurol. **8:** 520–525, 1980.
91. Goodwin, J. S., and Ceuppens, J.: Regulation of the immune response by prostaglandins. J. Clin. Immunol. **3:** 295–315, 1983.
92. Green, D. R., Eardley, D. D., Kimura, A., Murphy, D. B., Yamauchi, K., and Gershon, R. K.: Immunoregulatory circuits which modulate responsiveness to suppressor cell signals: characterization of an effector cell in the contrasuppressor circuit. Eur. J. Immunol. **11:** 973–980, 1981.
93. Greengard, P.: Phosphorylated proteins as physiological effectors. Science (Wash. DC) **199:** 146–152, 1978.
94. Guillet, J. G., Kaveri, S. V., Durieu, O., Delavier, C., Hoebeke, J., and Strosberg, A. D.: Beta-adrenergic agonist activity of a monoclonal antiidiotypic antibody. Proc. Natl. Acad. Sci. USA **82:** 1781–1784, 1985.
95. Hadden, J. W., Hadden, E. M., and Middleton, E.: Lymphocyte blast transformation. I. Demonstration of adrenergic receptors in human peripheral lymphocytes. Cell. Immunol. **1:** 583–595, 1970.
96. Hadden, J. W.: Sympathetic modulation of immune response. N. Engl. J. Med. **285:** 178, 1971.
97. Hadden, J. W., Coffey, R. G., Ananthakrishnan, R., and Hadden, E. M.: Cyclic nucleotide and calcium in lymphocyte regulation and activation. Ann. NY Acad. Sci. **332:** 241–254, 1979.
98. Hall, N. R., and Goldstein, A. L.: Neurotransmitters and the immune system. *In* Psychoneuroimmunology, ed. by R. Ader, pp. 521–543, Academic Press, New York, 1981.
99. Hall, N. R., McClure, J. E., Hu, S-K., Tare, N. S., Seals, C. M., and Goldstein, A. L.: Effects of 6-hydroxydopamine upon primary and secondary thymus dependent immune responses. Immunopharmacology **5:** 39–48, 1982.
100. Harden, T. K.: Agonist-induced desensitization of the beta-adrenergic receptor-linked adenylate cyclase. Pharmacol. Rev. **35:** 5–32, 1983.
101. Hirata, F., and Axelrod, J.: Phospholipid methylation and biological signal transmission. Science (Wash. DC) **209:** 1082–1090, 1980.
102. Hoffeld, T. J.: Agents which block membrane lipid peroxidation enhance mouse spleen cell immune activities in vitro: relationship to the enhancing activity of 2-mercaptoethanol. Eur. J. Immunol. **11:** 371–376, 1981.
103. Hoffmann, M. K.: Macrophages and T cells control distinct phases of B cell differentiation in the humoral response in vitro. J. Immunol. **125:** 2076–2081, 1980.
104. Hoffman, M. K., Mizel, S. B., and Hirst, J. A.: IL1 requirement for B cell activation revealed by use of adult serum. J. Immunol. **133:** 2566–2568, 1984.
105. Howard, M., Farrar, J., Hilfiker, M., Johnson, B., Takatsu, K., Hamaoka, T., and Paul, W. E.: Identification of a T-cell derived B-cell growth factor distinct from interleukin 2. J. Exp. Med. **155:** 914–923, 1982.
106. Howard, M., and Paul, W. E.: Regulation of B-cell growth and differentiation by soluble factors. Annu. Rev. Immunol. **1:** 307–333, 1983.
107. Howard, M., Nakamiski, K., and Paul, W. E.: B cell growth and differentiation factors. Immunol. Rev. **78:** 185–210, 1984.
108. Ishizuka, M., Gafni, M., and Braun, W.: Cyclic AMP effects on antibody formation and their similarities to hormone-mediated events. Proc. Soc. Exp. Biol. Med. **134:** 963–967, 1970.
109. Ishizuka, M., Braun, W., and Matsumoto, T.: Cyclic AMP and immune responses. I. Influence of poly A:U and cAMP on antibody formation in vitro. J. Immunol. **107:** 1027–1035, 1971.
110. Ito, M., Sless, F., and Parrott, D. M.: Evidence for control of complement receptor rosette-forming cells by alpha- and beta-adrenergic agents. Nature (Lond.) **266:** 633–635, 1977.
111. Jackson, J. C., Cross, R. J., Walker, R. F., Markesbery, W. R., Brooks, W. H., and Roszman, T. L.: Influence of serotonin on the immune response. Immunology **54:** 505–512, 1985.
112. Jakobs, K. H., Aktories, K., and Schultz, G.: Inhibition of adenylate cyclase by hormone and neurotransmitters. Adv. Cyclic Nucleotide Res. **14:** 173–187, 1981.
113. Jandinski, J., Cantor, H., Tadakuma, T., Peavy, D. L., and Pierce, C. W.: Separation of helper T cells from suppressor T cells expressing different Ly components. I. Polyclonal activation: suppressor and helper

activities are inherent properties of distinct T-cell subclasses. J. Exp. Med. **143:** 1382–1390, 1976.
114. JEGASOTHY, B. V., NAMBA, Y., AND WAKSMAN, B. H.: Regulatory substances produced by lymphocytes. VII. IDS (inhibitor of DNA synthesis) inhibits stimulated lymphocyte proliferation by activation of membrane adenylate cyclase at a restriction point in late G1. Immunochemistry **15:** 551–555, 1978.
115. JERNE, N. K.: Towards a network theory of the immune system. Ann. Immunol. **125C:** 373–389, 1974.
116. JOHNSON, D. L., AND GORDON, M. A.: Characteristics of adrenergic binding sites associated with murine lymphocytes isolated from spleen. J. Immunopharmacol. **2:** 435–452, 1980.
117. JOHNSON, D. L., ASHMORE, R. C., AND GORDON, M. A.: Effects of beta-adrenergic agents on the murine lymphocyte response to mitogen stimulation. J. Immunopharmacol. **3:** 205–219, 1981.
118. JOHNSON, G. L., WOLFE, B. B., HARDEN, T. K., MOLINOFF, P. B., AND PERKINS, J. P.: Role of beta-adrenergic receptors in catecholamine-induced desensitization of adenylate cyclase in human astrocytoma cells. J. Biol. Chem. **253:** 1472–1480, 1978.
119. JOHNSON, H. M., BLALOCK, J. E., AND BARON, S.: Separation of mitogen-induced suppressor and helper cell activities during inhibition of interferon production by cyclic AMP. Cell. Immunol. **33:** 170–179, 1977.
120. JONES, L. M., AND MICHELL, R. H.: Stimulus-response coupling at alpha-adrenergic receptors. Biochem. Soc. Trans. **6:** 673–688, 1978.
121. JULIUS, M. H., CHILLER, J. M., AND SIDMAN, C. L.: Major histocompatibility complex restricted cellular interactions determining B cell activation. Eur. J. Immunol. **12:** 627–633, 1982.
122. KASAHARA, K., TANAKA, S., ITO, T., AND HAMASHIMA, Y.: Suppression of the primary immune response by chemical sympathectomy. Res. Commun. Chem. Pathol. Pharmacol. **16:** 687–694, 1977.
123. KATZ, D. H., AND BENACERRAF, B.: The regulatory influence of activated T cells on B cell responses to antigens. Adv. Immunol. **15:** 1–94, 1972.
124. KATZ, D. H., AND UNANUE, E. R.: Critical role of determinant presentation in the induction of specific responses in immunocompetent lymphocytes. J. Exp. Med. **137:** 967–990, 1973.
125. KENAKIN, T. P.: An in vitro quantitative analysis of the alpha adrenoceptor partial agonist activity of dobutamine and its relevance to inotropic selectivity. J. Pharmacol. Exp. Ther. **216:** 210–219, 1981.
126. KIM, B. S.: Mechanisms of idiotype suppression: role of anti-idiotype antibody. Surv. Immunol. Res. **1:** 126–132, 1982.
127. KISHIMOTO, A., TAKAI, Y., MORI, T., KIKKAWA, U., AND NISHIZUKA, Y.: Activation of calcium and phospholipid-dependent protein kinase by diacylglycerol, its possible relation to phosphatidylinositol turnover. J. Biol. Chem. **255:** 2273–2276, 1980.
128. KISHIMOTO, T., YOSHIZAKI, K., KIMOTO, M., OKADA, M., KURITANI, T., KIKUTANI, H., SHIMIZU, K., NAKAGAWA, T., NAKAGAWA, N., MIKI, Y., KISHI, H., FUKUNAGA, K., YOSHIKUBO, T., AND TAGA, T.: B cell growth and differentiation factors and mechanism of B cell activation. Immunol. Rev. **78:** 97–118, 1984.
129. KOENIG, S., AND HOFFMANN, M. K.: Bacterial lipopolysaccharide activates suppressor B lymphocytes. Proc. Natl. Acad. Sci. USA **76:** 4608–4612, 1979.
130. LANDS, A. M., ARNOLD, A., MCAULIFF, J. P., LUDUENA, F. P., AND BROWN, T. G.: Differentiation of receptor systems activated by sympathomimetic amines. Nature (Lond.) **214:** 597–598, 1967.
131. LANDS, A. M., LUDUENA, F. P., AND BUZZO, H. J.: Differentiation of receptors responsive to isoproterenol. Life Sci. **6:** 2241–2249, 1967.
132. LANGER, S. Z.: Presynaptic regulation of catecholamine release. Biochem. Pharmacol. **23:** 1793–1800, 1974.
133. LEFKOWITZ, R. J.: Heterogeneity of adenylate cyclase-coupled beta-adrenergic receptors. Biochem. Pharmacol. **24:** 583–590, 1975.
134. LEMKE, H., AND OPITZ, H-G.: Function of 2-mercaptoethanol as a macrophage substitute in the primary immune response in vitro. J. Immunol. **117:** 388–395, 1976.
135. LIEBSON, H. J., MARRACK, P., AND KAPPLER, J.: B cell helper factors. II. Synergy among three helper factors in the response of T cell- and macrophage-depleted B cells. J. Immunol. **129:** 1398–1402, 1982.
136. MACMANUS, J. P., WHITFIELD, J. F., AND YOUDALE, T.: Stimulation by epinephrine of adenyl cyclase activity, cyclic AMP formation, DNA synthesis, and cell proliferation in populations of rat thymic lymphocytes. J. Cell. Physiol. **77:** 103–116, 1970.
137. MAKINO, S., AND REED, C. E.: Epinephrine inhibition of hemolytic plaque forming cells (PFC) following in vitro immunization. Fed. Proc. **29:** 431, 1970.
138. MARRACK, P., GRAHAM, S. D., KUSHNIR, E., LIEBSON, H. J., ROEHM, N., AND KAPPLER, J. W.: Nonspecific factors in B cell responses. Immunol. Rev. **63:** 33–49, 1982.
139. MASEK, K., KADLECOV, O., AND PETROVICKY, A. P.: The effect of brain stem lesions on the immune response. Adv. Immunopharmacol. **2:** 443–450, 1983.
140. MCCALL, C. O., SANDERS, V. M., BLACK, L. E., AND MUNSON, A. E.: Modulation of the in vitro murine primary antibody response by supernatants from spleen cell cultures treated with adrenergic agonists (abstract). Fed. Proc. **44:** 1488, 1985.
141. MCGRATH, J. C.: Evidence for more than one type of post-junctional alpha-adrenoceptor. Biochem. Pharmacol. **31:** 467–484, 1982.
142. MCPHERSON, G. A., AND SUMMERS, R. J.: Characteristics and localization of ^{3}H-clonidine binding in membranes prepared from guinea-pig spleen. Clin. Exp. Pharmacol. Physiol. **9:** 77–87, 1982.
143. MELCHERS, F., AND ANDERSSON, J.: B cell activation: three steps and their variations. Cell **37:** 715–720, 1984.
144. MELCHIORRE, C.: Selectivity of alpha-1 and alpha-2 adrenergic agonists and antagonists. Farmaco, Ed. Sci. **35:** 535–550, 1980.
145. MELMON, K. L., BOURNE, H. R., WEINSTEIN, Y., SHEARER, G. M., KRAM, J., AND BAUMINGER, S.: Hemolytic plaque formation by leukocytes in vitro. J. Clin. Invest. **53:** 13–21, 1974.
146. MELMON, K. L., WEINSTEIN, Y., SHEARER, G. M., BOURNE, H. R., AND BAUMINGER, S.: Separation of specific antibody-forming mouse cells by their adherence to insolubilized endogenous hormones. J. Clin. Invest. **53:** 22–30, 1974.
147. MICHELL, R. H.: Inositol phospholipids and cell surface receptor function. Biochim. Biophys. Acta **415:** 81–147, 1975.
148. MILES, K., QUINTANS, J., CHELMICKA-SCHORR, E., AND ARNASON, B.: The sympathetic nervous system modulates antibody response to thymus-independent antigens. J. Neuroimmunol. **1:** 101–105, 1981.
149. MILES, K., ATWEH, S., OTTEN, G., ARNASON, B. G. W., AND CHELMICKA-SCHORR, E.: Beta-adrenergic receptors on splenic lymphocytes from axotomized mice. Int. J. Immunopharmacol. **6:** 171–177, 1984.
150. MINNEMAN, K. P., HEGSTRAND, L. R., AND MOLINOFF, P. B.: Simultaneous determination of beta 1 and beta 2-adrenergic receptors in tissues containing both receptor subtypes. Mol. Pharmacol. **16:** 34–46, 1979.
151. MISHELL, R. I., AND DUTTON, R. W.: Immunization of dissociated spleen cell cultures from normal mice. J. Exp. Med. **126:** 423–442, 1967.
152. MIZEL, S. B.: Interleukin 1 and T cell activation. Immunol. Rev. **63:** 51–72, 1982.
153. MOLLER, G., editor: Interleukins and lymphocyte activation. Immunol. Rev. **63:** 5–209, 1982.
154. MORAN, N. C.: Pharmacological characterization of adrenergic receptors. Pharmacol. Rev. **18:** 503–512, 1966.
155. MORGAN, N. G., WAYNICK, L. E., AND EXTON, J. H.: Characterisation of the alpha-1 adrenergic control of hepatic cAMP in male rats. Eur. J. Pharmacol. **96:** 1–10, 1983.
156. MOSIER, D. E.: A requirement for two cell types for antibody formation in vitro. Science (Wash. DC) **158:** 1573–1575, 1967.
157. MOSIER, D. E., AND COPPLESON, L. W.: A three-cell interaction required for the induction of the primary immune response in vitro. Proc. Natl. Acad. Sci. USA **61:** 542–547, 1968.
158. MOSIER, D. E.: Cell interactions in the primary immune response in vitro. A requirement for specific cell clusters. J. Exp. Med. **129:** 351–362, 1969.
159. MURAD, F., CHI, Y. M., RALL, T. W., AND SUTHERLAND, E. W.: The effect of catecholamines and choline esters on the formation of adenosine 3′,5′-phosphate by preparations from cardiac muscle and liver. J. Biol. Chem. **237:** 1233–1238, 1962.
160. MURAGUCHI, A., MIYAZAKI, H., KEHRL, J. H., AND FAUCI, A. S.: Inhibition of human B cell activation by diterpine forskolin: interference with B cell growth factor-induced G1 to S transition of the B cell cycle. J. Immunol. **133:** 1283–1287, 1984.
161. NAHORSKI, S. R.: Heterogeneity of cerebral beta-adrenoceptor binding sites in various vertebrate species. Eur. J. Pharmacol. **51:** 199–209, 1978.
162. NAHORSKI, S. R., AND WILCOCKS, A. L.: Identification of beta adrenoreceptor subtypes in various regions of the rat brain. *In* Recent Advances in the Pharmacology of Adrenoceptors, ed. by E. Szabadi, G. M. Bradshaw, and P. Bevan, pp. 347–348, P. North Holland, Amsterdam, 1978.
163. NAKAKI, T., NAKADATE, T., YAMAMOTO, S., AND KATO, R.: Inhibition of dibutyryl cyclic AMP-induced insulin release by alpha-2 adrenergic stimulation. Life Sci. **32:** 191–195, 1983.
164. NAKANISHI, K., HOWARD, M., MURAGUCHI, A., FARRAR, J., TAKATSU, K., HAMAOKA, T., AND PAUL, W. E.: Soluble factors involved in B cell differentiation: identification of two distinct T cell-replacing factors (TRF). J. Immunol. **130:** 2219–2224, 1983.
165. NAKANISHI, K., MALEK, T. R., SMITH, K. A., HAMAOKA, T., SHEVACH, E. M., AND PAUL, W. E.: Both interleukin 2 and a second T cell-derived factor in EL-4 supernatant have activity as differentiation factors in IgM synthesis. J. Exp. Med. **160:** 1605–1621, 1984.
166. NAKAZAWA, H., HOBDAY, J., TOWNLEY, R., AND CHAPERON, E.: Effect of beta-adrenergic blockade, pertussis vaccine, and Freund's adjuvant on reaginic antibody response in mice. Int. Arch. Allergy Appl. Immunol. **53:** 197–205, 1977.
167. NETA, R., AND SALVIN, S. B.: Specific suppression of delayed hypersensitivity: the possible presence of a suppressor B cell in the regulation of delayed hypersensitivity. J. Immunol. **113:** 1716–1725, 1974.
168. NOELLE, R., KRAMMER, P. H., OHARA, J., UHR, J. W., AND VITETTA, E. S.: Increased expression of Ia antigens on resting B cells: an additional role for B-cell growth factor. Proc. Natl. Acad. Sci. USA **81:** 6149–6153, 1984.
169. OHMORI, H., AND YAMAMOTO, I.: Mechanism of augmentation of the antibody response in vitro by 2-mercaptoethanol in murine lymphocytes. I. 2-Mercaptoethanol induced stimulation of the uptake of cystine, an essential amino acid. J. Exp. Med. **155:** 1277–1290, 1982.
170. OHMORI, H., AND YAMAMOTO, I.: Mechanism of augmentation of the antibody response in vitro by 2-mercaptoethanol in murine lymphocytes.

II. A major role of the mixed disulfide between 2-mercaptoethanol and cysteine. Cell. Immunol. **79:** 173–185, 1983.
171. OLIVER, K., NOELLE, R. J., UHR, J. W., KRAMMER, P. H., AND VITETTA, E. S.: B cell growth factor (BCGFI or BSFp1) is a differentiation factor for resting B cells and may not induce cell growth. Proc. Natl. Acad. Sci. USA **82:** 2465–2467, 1985.
172. OPITZ, H. G., OPITZ, U., LEMKE, H., HEWLETT, G., SCHREML, W., AND FLAD, H. D.: The role of fetal calf serum in the primary immune response in vitro. J. Exp. Med. **145:** 1029–1038, 1977.
173. OPITZ, H. G., LEMKE, H., AND HEWLETT, G.: Activation of T-cells by a macrophage or 2-mercaptoethanol activated serum factor is essential for induction of a primary immune response to heterologous red cells in vitro. Immunol. Rev. **40:** 53–77, 1978.
174. ORTEZ, R. A.: Cyclic AMP-dependent content of murine splenic T and B lymphocytes and non-lymphoid tissues. Immunol. Commun. **11:** 227–237, 1982.
175. PARDEE, A. B.: A restriction point for control of normal animal cell proliferation. Proc. Natl. Acad. Sci. USA **71:** 1286–1290, 1974.
176. PARKER, C. W., WEDNER, H. J., AND SULLIVAN, T. J.: Cyclic AMP and the immune response. Adv. Cyclic Nucleotide Res. **4:** 1–79, 1974.
177. PARKER, C. W.: Role of cyclic nucleotides in regulating lymphocytes. Ann. NY Acad. Sci. **332:** 255–261, 1979.
178. PARKER, D. C.: Stimulation of mouse lymphocytes by insoluble anti-mouse immunoglobulins. Nature (Lond.) **258:** 361–363, 1975.
179. PAUWELS, R., BAZAN, H., PLATTEAU, B., AND VAN DER STRAETEN, M.: Influence of adrenergic drugs on IgE production. Int. Arch. Allergy Appl. Immunol. **61:** 347–351, 1980.
180. PEARLMAN, D. S.: The effect of adrenergic agents on the antibody response in vitro (abstract). J. Allergy Clin. Immunol. **47:** 109–110, 1971.
181. PERKINS, J. P., TOEWS, M. L., AND HARDEN, T. K.: Regulation of beta-adrenergic receptors during exposure of astrocytoma cells to catecholamines. Adv. Cyclic Nucleotide Protein Phosphorylation Res. **17:** 37–46, 1984.
182. PERSSON, H., AND OLSSON, T.: Some pharmacological properties of terbutaline (INN), 1-(3,5-dihydroxyphenyl)-2-(*t*-butylamino)ethanol. A new sympathimimetic beta-receptor stimulating agent. Acta Med. Scand. **512:** 11–19, 1970.
183. PERSSON, K., AND PERSSON, K.: The metabolism of terbutaline in vitro by rat and human liver O-methyltransferases and monoamine oxidases. Xenobiotica **2:** 375–382, 1972.
184. PIERPAOLI, W., AND MAESTRONI, G. J. M.: Pharmacological control of the hormonally modulated immune response. II. Blockade of antibody production by a combination of drugs acting on neuroendocrine functions. Its prevention by gonadotropins and corticotropin. Immunology **34:** 419–430, 1978.
185. PLESCIA, O. J., YAMAOTO, I., AND SHIMAMURA, T.: Cyclic AMP and immune responses: changes in the splenic level of cyclic AMP during the response of mice to antigen. Proc. Natl. Acad. Sci. USA **72:** 888–891, 1975.
186. POCHET, R., DELESPESSE, G., GAUSSET, P. W., AND COLLET, H.: Distribution of beta-adrenergic receptors on human lymphocyte subpopulations. Clin. Exp. Immunol. **38:** 578–584, 1979.
187. PURE, E., ISAKSON, P. C., TAKATSU, K., HAMAOKA, T., SWAIN, S. L., DUTTON, R. W., DENNERT, G., UHR, J. W., AND VITETTA, E. S.: Induction of B cell differentiation by T cell factors. I. Stimulation of IgM secretion by products of a T cell hybridoma and a T cell line. J. Immunol. **127:** 1953–1958, 1981.
188. RALL, T. W., SUTHERLAND, E. W., AND BERTHET, J.: The relationship of epinephrine and glucagon to liver phosphorylase. IV. Effect of epinephrine and glucagon on the reactivation of phosphorylase in liver homogenates. J. Biol. Chem. **224:** 463–475, 1957.
189. RALL, T. W.: Role of adenosine 3′,5′-monophosphate (cyclic AMP) in actions of catecholamines. Pharmacol. Rev. **24:** 399–410, 1972.
190. RASMUSSEN, H.: Calcium and cAMP as Synarchic Messengers. John Wiley & Sons, New York, 1981.
191. REED, C. E., BENNER, M., LOCKEY, S. D., ENTA, T., MAKINO, S., AND CARR, R. H.: On the mechanism of the adjuvant effect of Bordetella pertussis vaccine. J. Allergy Clin. Immunol. **49:** 174–182, 1972.
192. REILLY, F. D., MCCUSKEY, R. S., AND MEINEKE, H. A.: Studies of the hemopoietic microenvironment. VIII. Adrenergic and cholinergic innervation of the murine spleen. Anat. Rec. **185:** 109–118, 1976.
193. REILLY, F. D., MCCUSKEY, P. A., MILLER, M. L., MCCUSKEY, R. S., AND MEINEKE, H. A.: Innervation of the periarteriolar lymphatic sheath of the spleen. Tissue & Cell **11:** 121–126, 1979.
194. ROBISON, G. A., BUTCHER, R. W., AND SUTHERLAND, E. W., eds: Cyclic AMP, pp. 5–531. Academic Press, New York, 1971.
195. ROEHM, N. W., LEIBSON, H. J., ZLOTNIK, A., KAPPLER, J., MARRACK, P., AND CAMBIER, J. C.: Interleukin-induced increase in Ia expression by normal mouse B cells. J. Exp. Med. **160:** 679–694, 1984.
196. ROSENBERG, Y. J.: Isotype-specific T cell regulation of immunoglobulin expression. Immunol. Rev. **67:** 33–58, 1982.
197. ROSENTHAL, A. S., AND SHEVACH, E. M.: Function of macrophages in antigen recognition by guinea pig T lymphocytes. I. Requirement for histocompatible macrophages and lymphocytes. J. Exp. Med. **138:** 1194–1212, 1973.
198. RUFFOLO, R. R., SPRADLIN, T. A., POLLOCK, G. D., WADDELL, J. E., AND MURPHY, P. J.: Alpha and beta adrenergic effects of the stereoisomers of dobutamine. J. Pharmacol. Exp. Ther. **219:** 447–452, 1981.
199. RUFFOLO, R. R., AND YADEN, E. L.: Vascular effects of the stereoisomers of dobutamine. J. Pharmacol. Exp. Ther. **224:** 46–50, 1983.
200. RUFFOLO, R. R.: Interactions of agonists with peripheral alpha-adrenergic receptors. Fed. Proc. **43:** 2910–2916, 1984.
201. RUGG, E., BARNETT, D. B., AND NAHORSKI, S. R.: Coexistence of beta 1 and beta 2 adrenoceptors in mammalian lung: evidence from direct binding studies. Mol. Pharmacol. **14:** 996–1005, 1978.
202. SANDERS, V. M., AND MUNSON, A. E.: Beta adrenoceptor mediation of the enhancing effect of norepinephrine on the murine primary antibody response in vitro. J. Pharmacol. Exp. Ther. **230:** 183–192, 1984.
203. SANDERS, V. M., AND MUNSON, A. E.: Kinetics of the enhancing effect produced by norepinephrine and terbutaline on the murine primary antibody response in vitro. J. Pharmacol. Exp. Ther. **231:** 527–531, 1984.
204. SANDERS, V. M., AND MUNSON, A. E.: Transfer of the beta-2 adrenoceptor induced enhancement of the antibody response by supernatants from terbutaline exposed spleen cells. J. Leuk. Biol. Abstr. **36:** 406, 1984.
205. SANDERS, V. M., AND MUNSON, A. E.: Role of alpha adrenoceptor activation in modulating the murine primary antibody response in vitro. J. Pharmacol. Exp. Ther. **232:** 395–400, 1985.
206. SANDERS, V. M., MCCALL, C. O., HORSTMAN, D. A., EICHLER, T. J., CAMPBELL, K. S., AND MUNSON, A. E.: Kinetics of the alpha-2 adrenoceptor-induced inhibition of the murine primary antibody response in vitro (abstract). Fed. Proc. **44:** 1491, 1985.
207. SCHEID, M. P., GOLDSTEIN, G. AND BOYSE, E. A.: The generation and regulation of lymphocyte populations. Evidence from differentiative induction systems in vitro. J. Exp. Med. **147:** 1727–1743, 1978.
208. SCHIMPL, A., AND WECKER, E.: Replacement of T-cell function by a T-cell product. Nat. New Biol. **237:** 15–17, 1972.
209. SCHREIER, M. H., ANDERSSON, J., LERNHARDT, W., AND MELCHERS, F.: Antigen-specific T-helper cells stimulate H-2-compatible and H-2-incompatible B-cell blasts polyclonally. J. Exp. Med. **151:** 194–203, 1980.
210. SHEARER, G. M., MELMON, K. L., WEINSTEIN, Y., AND SELA, M.: Regulation of antibody response by cells expressing histamine receptors. J. Exp. Med. **136:** 1302–1307, 1972.
211. SHEK, P. N., AND SABISTON, B. H.: Neuroendocrine regulation of immune processes: change in circulating corticosterone levels induced by the primary antibody response in mice. Int. J. Immunopharmacol. **5:** 23–33, 1983.
212. SHENKER, B. J., AND GRAY, I.: Cyclic nucleotide metabolism during lymphocyte transformation. I. Enzymatic mechanisms in changes in cAMP and cGMP concentration in BALB/c mice. Cell. Immunol. **43:** 1–22, 1979.
213. SHENKER, B. J., AND GRAY, I.: Cyclic nucleotide metabolism during lymphocyte transformation. II. Comparative studies in the nude BALB/c mouse. Cell. Immunol. **43:** 23–29, 1979.
214. SHERR, D. H., AND DORF, M. E.: An idiotype-specific helper population that bears immunoglobulin, Ia, and Lyt-1 determinants. J. Exp. Med. **159;** 1189–1200, 1984.
215. SHIMAMURA, T., HASHIMOTO, K., AND SASAKI, S.: Feedback suppression of the immune response in vivo. I. Immune B cells induce antigen-specific suppressor T cells. Cell. Immunol. **68:** 104–113, 1982.
216. SHIMAMURA, T., HABU, S., HASHIMOTO, K., AND SASAKI, S.: Feedback suppression of the immune response *in vivo*. III. Lyt-1+ B cells are suppressor inducer cells. Cell. Immunol. **83:** 221–224, 1984.
217. SIBLEY, D. R., NAMBI, P., PETERS, J. R., AND LEFKOWITZ, R. J.: Phorbol diesters promote beta-adrenergic receptor phosphorylation and adenylate cyclase desensitization in duck erythrocytes. Biochem. Biophys. Res. Commun. **121:** 973–979, 1984.
218. SIBLEY, D. R., PETERS, J. R., NAMBI, P., CARON, M. G., AND LEFKOWITZ, R. J.: Desensitization of turkey erythrocyte adenylate cyclase. Beta adrenergic receptor phosphorylation is correlated with attenuation of adenylate cyclase activity. J. Biol. Chem. **259:** 9742–9749, 1984.
219. SINCLAIR, N. R.: Mechanisms of immunoregulation. Immunoregulation by antibody and antigen-antibody complexes. Trans. Proc. **10:** 349–353, 1978.
220. SINGH, U., AND OWEN, J. J. T.: Studies on the maturation of thymus stem cells. The effects of catecholamines, histamine, and peptide hormones on the expression of T cell alloantigens. Eur. J. Immunol. **6:** 59–62, 1976.
221. SINGH, U., MILLSON, D. S., SMITH, P. A., AND OWEN, J. J. T.: Identification of beta adrenoceptors during thymocyte ontogeny in mice. Eur. J. Immunol. **9:** 31–35, 1979.
222. STADEL, J. M., STRULOVICI, B., NAMBI, P., LAVIN, K., BRIGGS, M. M., CARON, M. G., AND LEFKOWITZ, R. J.: Desensitization of the beta-adrenergic receptor of frog erythrocytes: recovery and characterization of the down regulated receptors in sequestered vesicles. J. Biol. Chem. **258:** 3032–3038, 1983.
223. STARKE, K.: Alpha-adrenoceptor subclassification. Rev. Physiol. Biochem. Pharmacol. **88:** 199–236, 1981.
224. STEEG, P. S., MOORE, R. N., AND OPPENHEIM, J. J.: Regulation of murine macrophage Ia-antigen expression by products of activated spleen cells. J. Exp. Med. **152:** 1734–1744, 1980.
225. STILES, G. L., STRASSER, R. H., CARON, M. G., AND LEFKOWITZ, R. J.: Mammalian beta-adrenergic receptors. Structural differences in beta 1

and beta 2 subtypes revealed by peptide maps. J. Biol. Chem. **258:** 10689–10694, 1983.
226. STILES, G. L., CARON, M. G., AND LEFKOWITZ, R. J.: Beta-adrenergic receptors: biochemical mechanisms of physiological regulation. Physiol. Rev. **64:** 661–743, 1984.
227. STROM, T. B., AND CARPENTER, C. B.: Cyclic nucleotides in immunosuppression—neuroendocrine pharmacologic manipulation and in vivo immunoregulation of immunity acting via second messenger systems. Transplant. Proc. **12:** 304–310, 1980.
228. STROM, T. B., LANE, M. A., AND GEORGE, K.: The parallel, time-dependent bimodal change in lymphocyte cholinergic binding activity and cholinergic influence upon lymphocyte-mediated cytotoxicity after lymphocyte activation. J. Immunol. **127:** 705–710, 1981.
229. SWAIN, S. L., DENNERT, G., WARNER, J. F., AND DUTTON, R. W.: Culture supernatants of a stimulated T-cell line have helper activity that acts synergistically with interleukin 2 in the response of B cells to antigen. Proc. Natl. Acad. Sci. USA **78:** 2517–2521, 1981.
230. SWAIN, S. L., HOWARD, M., KAPPLER, J., MARRACK, P., WATSON, J., BOOTH, R., WETZEL, G. D., AND DUTTON, R. W.: Evidence for two distinct classes of murine B cell growth factors with activities in different functional assays. J. Exp. Med. **158:** 822–835, 1983.
231. TEH, H., AND PAETKAU, V.: Biphasic effect of cyclic AMP on an immune response. Nature (Lond.) **250:** 505–507, 1974.
232. TEH, H-S, AND PAETKAU, V.: Regulation of immune responses. II. The cellular basis of cylic AMP effects on humoral immunity. Cell. Immunol. **24:** 220–229, 1976.
233. THOENAN, H., AND TRANZER, J. P.: The pharmacology of 6-hydroxydopamine. Annu. Rev. Pharmacol. **13:** 169–180, 1973.
234. TUTTLE, R. R., AND MILLS, J.: Dobutamine: development of a new catecholamine to selectively increase cardiac contractility. Circ. Res. **36:** 185–196, 1975.
235. UHR, J. W., AND MOLLER, G.: Regulatory effect of antibody on the immune response. Adv. Immunol. **8:** 81–127, 1968.
236. UNANUE, E. R.: The regulation of lymphocyte functions by the macrophage. Immunol. Rev. **40:** 227–255, 1978.
237. VITETTA, E. S., BROOKS, K., CHEN, Y-W., ISAKSON, P., JONES, S., LAYTON, J., MISHRA, G. C., PURE, E., WEISS, E., WARD, C., YUAN, D., TUCKER, P., UHR, J. W., AND KRAMMER, P. H.: T cell-derived lymphokines that induce IgM and IgG secretion in activated murine B cells. Immunol. Rev. **78:** 137–157, 1984.
238. WAHL, S. M., WILTON, J. M., ROSENTREICH, D. L., AND OPPENHEIM, J. J.: The role of macrophages in the production of lymphokines by T and B lymphocytes. J. Immunol. **114:** 1296–1301, 1975.
239. WALDMAN, T. A., GOLDMAN, C. K., ROBB, R. J., DEPPER, J. M., LEONARD, W. J., SHARROW, S. O., BONGIOVANNI, K. F., KORSMAYER, S. J., AND GREENE, W. C.: Expression of interleukin 2 receptors on activated human B cells. J. Exp. Med. **160:** 1450–1466, 1984.
240. WANG, T., SHEPPARD, J. R, AND FOKER, J. E.: Rise and fall of cyclic AMP required for onset of lymphocyte DNA synthesis. Science (Wash. DC) **201:** 155–157, 1978.
241. WATSON, J., EPSTEIN, R., AND COHN, M.: Cyclic nucleotides as intracellular mediators of the expression of antigen-sensitive cells. Nature (Lond.) **246:** 405–409, 1973.
242. WATSON, J.: The influence of intracellular levels of cyclic nucleotides on cell proliferation and the induction of antibody synthesis. J. Exp. Med. **141:** 97–111, 1975.
243. WEBBER, R. H., DEFELICE, R., FERGUSON, R. J., AND POWELL, J. P.: Bone marrow response to stimulation of the sympathetic trunk in rats. Acta Anat. **77:** 92–97, 1970.
244. WEDNER, H. J., AND PARKER, C. W.: Lymphocyte activation. Prog. Allergy **20:** 195–300, 1976.
245. WEDNER, H. J.: Cyclic nucleotides in the immune response. *In* Handbook of Experimental Pharmacology, Cyclic Nucleotides. Part II. Physiology and Pharmacology, ed. by J. W. Kebabian and J. A. Nathanson, pp. 763–785, Springer-Verlag, New York, 1982.
246. WEIGLE, W. O.: Cyclical production of antibody as a regulatory mechanism in the immune response. Adv. Immunol. **21:** 87–111, 1975.
247. WEINSTEIN, Y., MELMON, K. L., BOURNE, H. R., AND SELA, M.: Specific leukocyte receptors for small endogenous hormones. Detection by cell binding to insolubilized hormone preparations. J. Clin. Invest. **52:** 1349–1361, 1973.
248. WILLIAMS, J., PETERSON, R., SHEA, P., SCHMEDTJE, J., BAUER, D., AND FELTEN, D.: Sympathetic innervation of murine thymus and spleen: evidence for a functional link between the nervous and immune systems. Brain Res. Bull. **6:** 83–94, 1981.
249. WILLIAMS, L. T., SNYDERMAN, R., AND LEFKOWITZ, R. J.: Identification of beta adrenergic receptors in human lymphocytes by (−)^{3}H-alprenolol binding. J. Clin. Invest. **57:** 149–155, 1976.
250. WILLIAMSON, J. R., COOPER, R. H., AND HACK, J. B.: Role of calcium in the hormonal regulation of liver metabolism. Biochim. Biophys. Acta **639:** 243–295, 1981.
251. WOOD, C. L., ARNETT, C. D., CLARKE, W. R., TSAI, B. S., AND LEFKOWITZ, R. J.: Subclassification of alpha-adrenergic receptors by direct binding studies. Biochem. Pharmacol. **28:** 1277–1282, 1979.
252. WYBRAN, J.: Enkephalins and endorphins as modifiers of the immune system: present and future. Fed. Proc. **44:** 92–94, 1985.
253. YAMAMOTO, I., AND WEBB, D. R.: Antigen-stimulated changes in cyclic nucleotide levels in the mouse. Proc. Natl. Acad. Sci. USA **72:** 2320–2324, 1975.
254. YAMAZAKI, S., KATADA, T., AND UI, M.: Alpha 2-adrenergic inhibition of insulin secretion via interference with cAMP generation in rat pancreatic islets. Mol. Pharmacol. **21:** 648, 1982.
255. ZMUDA, J., AND FRIEDENSON, B.: Changes in intracellular glutathione levels in stimulated and unstimulated lymphocytes in the presence of 2-mercaptoethanol or cysteine. J. Immunol. **130:** 362–364, 1983.
256. ZUBLER, R. H., LOWENTHAL, J. W., ERARD, F., HASHIMOTO, N., DEVOS, R., AND MACDONALD, H. R.: Activated B cells express receptors for, and proliferate in response to, pure interleukin 2. J. Exp. Med. **160:** 1170–1183, 1984.