Comprehensive Immunology 3

Immunopharmacology

Edited by JOHN W. HADDEN
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Introduction

Immunopharmacology: A New Discipline of Immense Potential

Among the looming triumphs of the biologic revolution is the rapidly developing understanding of the mechanisms of bodily defense. In the short span of 35 years, knowledge of immunologic machinery has progressed from crudest description to major understanding in cellular and molecular terms. Antibodies, immunoglobulins, and the complement system have been almost completely defined in detailed molecular terms. Organs, like thymus, spleen and lymph nodes—so long enigmatic black boxes—are beginning to be understood not only in cellular terms but in molecular, physiologic, and endocrinologic terms.

With this surging new information about the immune system comes the possibility of developing a pharmacology which can modulate and control immunologic functions. Immunopharmacology most broadly conceived must address (1) control of development and function of the cellular components of the immunologic apparatus; (2) facilitation and suppression of function of the immunologically competent cells of the several subclasses, like T helpers, suppressors, and effectors, and B effectors and suppressors; (3) manipulation and repair of the major biologic amplification systems, e.g., the complement system and kinin-kallikrein system, and (4) utilization, modulation, and inhibition of the galaxy of molecules generated by T lymphocytes, the lymphokines. This new pharmacology must deal with the fundamental effector mechanisms of immunity, namely inflammation, phagocytosis, vascular reactivity, and blood coagulation. Furthermore, immunopharmacology must address and manipulate cell–cell communication and interaction, so vital to control of the immunological apparatus.

This volume represents a major effort by leaders from outstanding laboratories who are feverishly working to develop the field of immunopharmacology. The editors have brought together much that is extant in this rapidly developing science. From the studies and perspectives collected in this volume one can recognize that a vigorous new discipline is taking shape.

Evidence that thymic hormones can drive stem cells through a succession of differentiative steps by selective gene action to the several classes and subclasses of functional T cells is already at hand (Komuro and Boyse, 1973; G. Goldstein *et al.*,

1977; Incefy and Good, 1976; Storrie *et al.*, 1976). As an example, both mouse and human precursors can be differentiated by thymopoietin (G. Goldstein *et al.*, 1977; Schlesinger and Goldstein, 1975) by processes which involve several steps, each of which utilizes RNA and protein synthesis, which seem to be separated by quantal proliferations (Touraine *et al.*, 1977). Hormones that can exercise powerful influences on lymphoid cellular development are rapidly becoming well defined molecules. They carry names like thymopoietin (Schlesinger and Goldstein, 1975), thymosin α_1 (A. L. Goldstein *et al.*, 1977), and Facteur serique thymique (Bach *et al* , 1977). Some of these molecules are relatively simple peptides which have been fully defined and which contain very short amino acid sequences (active sites) of extraordinary biologic activity. These short sequences and a variety of congeners that will be generated by enterprising chemists will become drugs for this new immunopharmacology. The immunopharmacologic development, I am sure, will present vistas of immense potential. I feel certain we will soon be speaking of an immunopharmacology that can control development and involution of the immunologic systems. It is difficult to predict how far this will go, but one can conceive of consequences that are immense. Control and manipulation of the development of antibody-producting cells has also begun, and the recent studies of Hämmerling *et al.*, (1976) employing the peptide molecule ubiquitin (Schlesinger *et al.*, 1975) to develop precursors to antibody-producing cells are encouraging. Similar progress in cellular and molecular terms can be seen in the studies of specific and nonspecific helper cells and suppressor molecules (Gershon, 1974; Tada, 1975; Kapp *et al.*, 1977; Waldmann *et al.*, 1974; Siegel *et al.*, 1976; Shou *et al.*, 1976; Schwartz *et al.*, 1977). The possibilities of using chemically defined adjuvants that can generate selectively helper T cells for IgG production while inducing suppressor T cells for IgE production promises at long last "immunization against allergy or unwanted immunity" as a product of the new immunopharmacology (Kishimoto and Ishizaka, 1975; Kishimoto *et al.*, 1976). Even defined sequences of the Fc portion of the IgE molecule already elucidated could represent exciting immunopharmaceuticals of great potential (Hamburger, 1975).

As impressive as they are, these extraordinary achievements may pale before pharmacological developments which are coming from molecular analysis of those powerful agents called lymphokines. Among these are some of the most active molecules known. Studies of the cellular and molecular control of lymphokine generation and release, for example, the molecular and cellular basis of their action on macrophages, are developing rapidly. Inquiry into and understanding of the language by which these fascinating molecules "talk" to the granular leukocytes, macrophages, and platelets represents a major challenge. It seems clear from several communications in this volume that substantial progress has been made in this important segment of the new discipline of immunopharmacology.

Furthermore, ingenious model systems have been developed to study in broad perspective as well as in fine detail the processes of immunopotentiation, immunomodulation, and immunostimulation. Better understanding of the controls that underlie activation to involvement in inflammation of platelets, macrophages, mast cells, and eosinophils, and stimulation of these cells to deliver or secrete their cellular production is developing rapidly. This surge too promises to provide real bases for a powerful pharmacology and, ultimately, for drug development.

Progress toward understanding of the molecular basis of cell–cell interactions and communication, the role of the cell membrane in cell interactions and communication, the details of hormonal influences on the physical state and chemistry of the membranes, the nature of the surface-to-nuclear signals generated by membrane perturbations that lead to either proliferation or differentiation and secretion, perhaps more than any of the developments in this field, proceeds so rapidly it is difficult to project its trajectory. Can the Yin-Yang concept of Goldberg *et al.*, (1974) concerning the cyclic nucleotides be harnessed to control cellular behavior? Will study of the prostaglandins really have a big payoff in cellular control? These are important questions to be answered by this research.

We can be certain that these fundamental pharmacological analyses will ultimately yield new and powerful means of prediction, manipulation, and control of the vital immunological apparatus. There is little question, from studying the chapters of this volume and the immense literature they reflect, that the field of immunopharmacology has a good start and extraordinary growth potential. It holds great promise for control of those vital immunological processes, which, in the aggregate, ensure our individuality. We can all look forward with excitement to the further development of this important new discipline.

Robert A. Good

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Contents

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Cyclic Nucleotides in Lymphocyte Proliferation and Differentiation

JOHN W. HADDEN

1. Introduction

The last fifteen years have seen a marked increase in the experimental evidence supporting the central roles played by cyclic nucleotides in the regulation of diverse processes in cells and tissues of organisms throughout the plant and animal kingdom. Beginning with the observations of Sutherland and Rall (1960) that indicated that cyclic 3′,5′-adenosine monophosphate (cAMP) mediates the intracellular action of epinephrine and glucagon to induce glycogenolysis in liver, the concept of the cAMP "second messenger" system has been generalized to virtually every cell of the mammalian organism, and the system has been linked to the induction and regulation of central cellular processes in these cells. In general, cAMP participates in those processes that involve the promotion of preprogrammed events consistent with the differentiated phenotype, i.e., the dominant functions for which that cell type is developed—for the liver, glucose production from glycogen stores; for the adrenal gland, steroid production; for fat tissue, lipolysis; and so on.

Since 1970, another candidate for a second messenger system has emerged: cyclic 3′,5′-guanosine monophosphate (cGMP). cGMP would appear to be as ubiquitously distributed in nature as cAMP, and the biological events to which it has been linked appear, in general, to oppose in function those linked to cAMP. The apparent contrasting roles of the two cyclic nucleotides, the only two consistently found in nature, was recently cast in a dualism hypothesis of biological regulation (Goldberg *et al.*, 1974). While admittedly tentative in its presentation, this hypothesis, in offering a balanced view of cellular regulation, has provided useful guidelines for experimental approaches.

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The initial concepts of the cyclic nucleotides as intracellular messengers derived principally from studies of how hormones or hormonelike agents that act at the cell surface induce a particular biological event through the intracellular production of the cyclic nucleotide. The cyclic nucleotide initiates the biological event inside the cell by activating one or another intracellular biochemical process. Since these first concepts were developed, it has become apparent that the cyclic nucleotides are involved in mediating a number of environmental influences and factors, in addition to hormones. Such diverse influences include temperature, pH, contact, nutrient availability, growth-promoting substances, growth inhibitors, differentiation-inducing factors, and a number of nonhormone factors involved in inflammation, intercellular communication, and environmental recognition. Indeed, the cyclic nucleotides have taken a central role in the emergence of a broad field of interdisciplinary interest in the cell surface as the translator of diverse environmental cues into intracellular instructions involved in events ranging from the regulation of cellular homeostasis to that of cellular proliferation and maturation. The emergence of the cell surface as a dominant issue in cellular regulation has introduced the concept of a balanced interaction between the cellular environment as translated by the cell surface and genetic determinants housed in the nucleus. This interactive concept has provided considerable impetus to the study of cyclic nucleotides in the regulation of nuclear processes related to the initiation and modification of genetic transcription. In no field has this concept of the regulatory roles played by cyclic nucleotides had more impact than in immunology. This chapter will attempt to deal with the development of cyclic nucleotide pharmacology as it pertains specifically to the proliferation and differentiation of lymphocytes related to the thymus, i.e., the thymus-derived T lymphocyte and its precursor, the prothymocyte.

2. Cyclic Nucleotide Biochemistry

As preface to the subject of this chapter, a review of certain aspects of cyclic nucleotide biochemistry appears relevant. cAMP and cGMP are both present in lymphocytes, and their levels depend on the source of the cells. In general, cAMP levels are detected at 1–60 pmol/mg protein and, with our techniques, approximate 11, 27, and 8 pmol/mg protein for lymphocytes from spleen, peripheral blood, and thymus, respectively. Similar values were reported by M. Bach (1975). cGMP levels for lymphoid tissues have varied considerably in literature reports, from 0.02 to 2 pmol/mg protein; with our methods, they average 0.5, 0.3, and 0.2 pmol/mg protein for spleen, peripheral blood, and thymus, respectively. These levels must be considered in light of the sample size and the methods used for cell purification for the extraction and purification techniques, and for cyclic nucleotide assay. Such considerations are important in evaluating the varying levels reported in the literature for these different lymphocyte populations. Based on measurements of comparable sample size (10^6 lymphocytes/ml), cAMP levels in lymphoid cells average 10- to 100-fold greater than those of cGMP.

The cyclic nucleotides are produced from their corresponding 5′-triphosphates, ATP and GTP, by specific cyclic nucleotide cyclases termed *adenylate cyclase* and *guanylate cyclase*, respectively. These enzymes differ in their optimum cation requirements—magnesium for adenylate cyclase and calcium and manganese for guanylate cyclase. They also differ in their degree of plasma membrane association.

The greater part of adenylate cyclase (approximately 90%) appears to be associated with the plasma membrane, while the remainder may be soluble. Recent experiments suggest that a small portion may be associated with the nuclear membrane and be activated differently from the plasma membrane cyclase (Parker *et al.*, 1974). Direct activators of adenylate cyclase in lymphocytes include β-adrenergic catecholamines such as isoproterenol and epinephrine, prostaglandin E_1 (PGE_1), and sodium fluoride. Guanylate cyclase, in contrast, appears to be mainly soluble and approximately 70% cytoplasmic (Deviller *et al.*, 1975), and the membrane-associated enzyme may be more easily solubilized than adenylate cyclase on disruption of cells. Like its activity in other tissues (Schultz *et al.*, 1975), guanylate cyclase activity of lymphoid cells is dependent on calcium (Katagiri *et al.*, 1976). No direct stimulant of guanylate cyclase of lymphocytes has been reported; however, indirect stimulation was observed with phytohemagglutinin (PHA) (Deviller *et al.*, 1975) and concanavalin A (Con A) (Katagiri *et al.*, 1976). The cyclic nucleotides are catabolized by the appropriate cyclic nucleotide phosphodiesterases to their respective 5′-monophosphates. The cAMP phosphodiesterase of lymphocytes is stimulated by imidazole (MacManus and Whitfield, 1971) and cGMP and inhibited by caffeine (Whitfield *et al.*, 1971). Although the cGMP phosphodiesterase was not examined directly, it is thought to be inhibited by imidazole (Hadden *et al.*, 1975a,b). In addition, a number of agents alter cyclic nucleotide levels in lymphocytes, but have not been specifically studied as to their biochemical actions.

The intracellular actions of the cyclic nucleotides in lymphocytes have received inadequate attention to date. The general assumption is that the action of cAMP in lymphocytes, as in other tissues, is mediated by activation of a protein kinase that, when liberated from its regulatory subunit, to which cAMP binds, phosphorylates various protein substrates and leads to their enzymatic activation. cAMP-dependent protein kinases have been isolated from lymphocytes, and have been demonstrated to phosphorylate protein substrates common to those phosphorylated by protein kinases from other tissues (Farago *et al.*, 1973; E. M. Johnson *et al.*, 1975b). Induced increases in cAMP yield glycogenolysis in lymphocytes (Hadden *et al.*, 1971a,c), a process that in liver has been established to result from protein-kinase-mediated phosphorylation and activation of phosphorylase, which catalyzes glycogen breakdown. Also, in experiments with intact lymphocytes, cAMP has been related to phosphorylation of nonnuclear proteins (Wedner and Parker, 1975). These observations indicate that the action of cAMP in lymphocytes is linked to protein kinase activation and the phosphorylation of protein substrates. A number of observations indicate that cAMP may modulate processes that do not involve a protein kinase. These processes include the inhibition of phosphorylation of nuclear proteins (E. M. Johnson and Hadden, 1975), the inhibition of glucose and potassium transport (Hadden *et al.*, 1971b; Coffey *et al.*, 1975a), the enhancement of RNA synthesis (Cross and Ord, 1971; Hirschhorn *et al.*, 1970; Webb *et al.*, 1973a) and of gluconeogenesis (Hadden *et al.*, 1971a), and the inhibition of phosphoribosyl pyrophosphate (PRPP) synthetase activity (Chambers *et al.*, 1974). A variety of potential actions of cAMP may be possible, and the conservative hypothesis that cAMP-dependent protein kinase represents the only mechanism appears to be unnecessarily restrictive. Much investigation is warranted, particularly in the area of direct demonstration of enzymatic pathways influenced by cAMP in lymphocytes.