IMMUNOPHARMACOLOGY

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AND
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Overview of Symposium

MARVIN E. ROSENTHALE

As pointed out in a recent article by Michael Whitehouse (1973) the subject of Immunopharmacology now has a nomenclature problem. Is immunopharmacology the study of suppression or stimulation of the immune process by drugs, is it the use of immune effectors such as antibody to control the immune response, or is it the use of immune mechanisms to assay drugs and hormones? Recent symposia (Cotten and La Du, 1973; Schild, 1968; Symp. Fed. Proc., 1974) appear to think it is all of these and I think I must agree. Terms frequently used to separate these disciplines such as immunoregulation or immunoassay or immunotherapy must all be considered subdivisions of immunopharmacology, which I envision as the study of the use of drugs or immune effectors which modulate the immune response for a large variety of therapeutic or diagnostic purposes.

Now obviously, with such a broad outlook, the organization of a meeting in immunopharmacology could conceivably be so all inclusive as to be totally unmanageable. Accordingly, we have narrowed the scope of this meeting to experimental and clinical areas where the greatest therapeutic advances are possible and where the greatest number of unanswered questions exist, i.e., the effects of therapeutic substances on pathways of

immunologic-induced or immunologic-sustained disease. In this symposium we will also attempt to establish guidelines for the experimental selection and clinical utilization of immunoregulants. This is an ambitious undertaking for a two-day symposium but I'm certain that as you peruse the articles we have been fortunate enough to hear presented and now publish you will agree with me that we can anticipate considerable progress.

Recent advances in immunology have made possible for the first time a general overall description of the immune system, and its interactions. The identification of the individual elements concerned with immunologic pathways theoretically now allows pharmacologic manipulation at a number of stages. Moreover the use of nondiscriminatory immunologic inhibitors is slowly giving way to the use of drugs capable of specifically regulating immune mechanisms, which may account for the more frequent use of the term immunoregulant rather than immunosuppressant.

An overall scheme of immune pathways leading to tissue damage is outlined in Fig. 1.

The immune system can be grossly divided into 3 stages, recognitive, central, and effector, and into 2 levels, that are concerned with humoral (immediate) or cellular (delayed) responses.

The recognitive stage consists of maturation of bursal (B) or thymic (T) derived lymphoid elements and the phagocytosis, recognition and processing of antigens by these elements. Extirpation or blockade of lymphoid tissue by various methods can modulate this stage.

The central stage is characterized by the interaction between proliferation and joint action of these cells which results in a two-level immune response: the cellular response where cellular immunopathies may be brought about by pathophoric or activated lymphocytes, and the humoral response where production of and interaction between immunoglobulins may give rise to three broad classes of allergic disease states. Complex formation with subsequent activation of the biologic amplifier complement, also occurs here. A variety of substances described later in this volume are capable of modulating this stage. These substances include the more widely used immunosuppressant classes such as antimetabolites, alkylating agents, and protein synthesis inhibitors.

The final effector stage consists of effector cell migration, each type of cell contributing one or more of a variety of inflammagenic mediators and or mechanisms capable of altering permeability, activating other enzymes, and inducing further activation and migration of cells etc., all resulting in tissue destruction. A number of agents have demonstrated capabilities for inhibiting the migration of one or more of these cells as well as the release and activity of mediators. These include antiinflammatory agents, specific receptor inhibitors, substances which increase intracellular nucleotides, smooth muscle relaxants, steroids, etc.

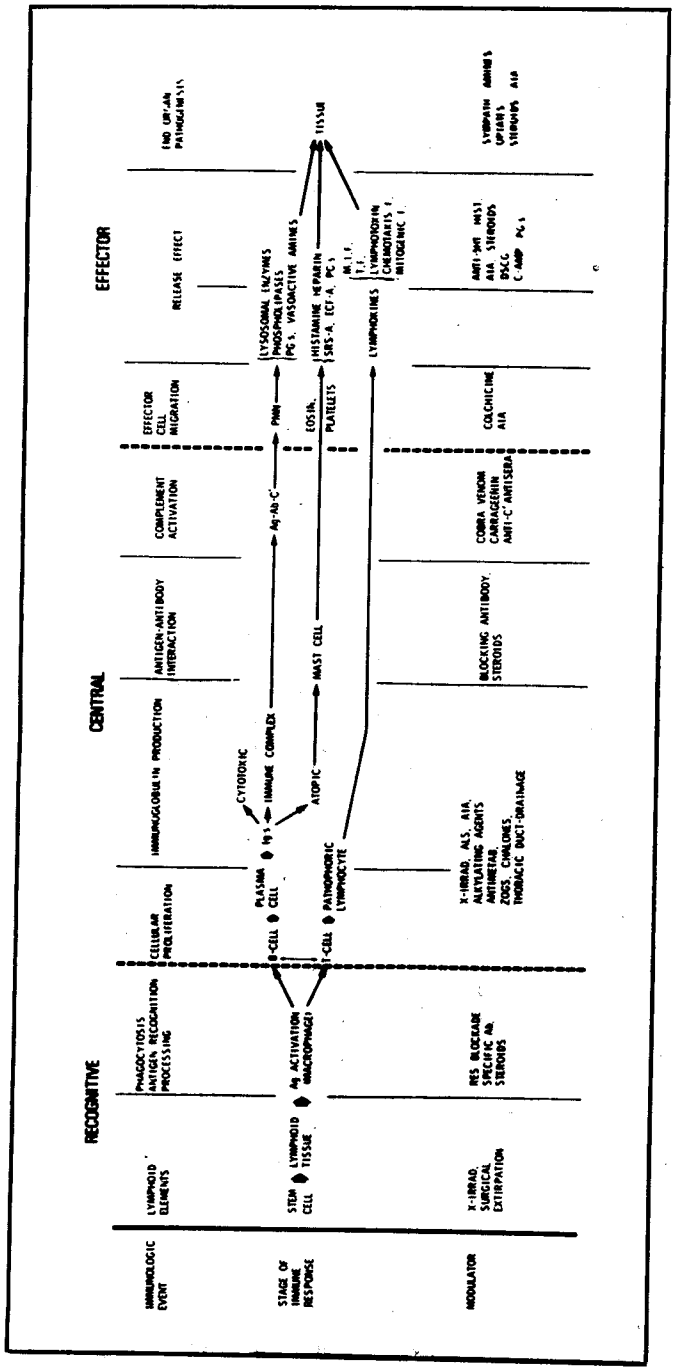

FIG. 1. *Immunopathological pathway: Ab antibody; Ag antigen; A.I.A. nonsteroidal antiinflammatory agents; ALS antilymphocyte serum; ANTI-5HT antiserotonin; B-cell bursal-derived cell; C' complement; C-AMP cyclic AMP; DSCG disodium cromoglycate; ECF-A eosinophile chemotactic factor A; EOSIN. esosinophiles; HIST. histamine; Ig immunoglobulin; M.I.F. migratory inhibiting factor; PGs prostaglandins; PMN polymorphonuclear leucocyte; T-cell thymic-derived cell; T.F. transfer factor; ZOGS cell surface poison.*

Not presented here is a complicated series of "autoinhibitory" or "feedback" mechanisms capable of regulating the "volume" of this response; an area which has for the most part been experimentally neglected until recently (Whitehouse, 1974). It should always be borne in mind that these mechanisms are inherently protective and that total or indiscriminant blockade resulting from "dirty" agents now in use disrupt normal physiological mechanisms as does immunopathologic disease. What is therefore needed are specific agents which can regulate or modify the abberrances at any point of the pathway until compensatory mechanisms intervene. Additionally, pharmacologic intervention must be selective and corrective to avoid the toxicity associated with the clinical use of immunologic agents.

With these thoughts in mind the organizers of this symposium have attempted to span a number of disciplines to present an overall look at a variety of aspects of drugs, the immune system, and their interactions. Like the immune system, we have divided our symposium into two major sections, an experimental section and a clinical section. These major sections will each be divided into two sessions, with the symposium thus comprised of four unique parts.

To set things in proper perspective, Dr. Becker's session (I) will cover the influence of drugs on the cellular and mediator functions of the immune response. The intracellular control of these functions and the cellular genetics involved will also be discussed as a prelude to session II where discussions lead by Dr. Chang will consider how the influence of drugs on the immune pathways, covered in session I can be exploited to design systems capable of searching out new agents.

Session III, moderated by Dr. Pearson, in some ways is analogous to session I, except that the emphasis will be on the effects of drugs on the various clinical parameters of the immune response. In session IV, Dr. Steinberg will present his observations on the clinical use of immunoregulants, in a session which we hope will highlight the unique nature and outstanding problems facing those working in this area.

Two years ago a number of the people present at this symposium took part in an international symposium, a section of which was devoted to immunological aspects of inflammation (Velo, Willoughby, and Giroud, 1974). The hope at that time was that the era of selective chemical manipulation of the immune system was on the horizon. I hope this volume will further summarize the progress thus far, and clarify the problems facing immunopharmacology.

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PART I Influence of Drugs on Immunopathologic Pathways

Introduction: Organophosphorus Inhibitors And Mechanisms of Cell Activation.

ELMER L. BECKER

The organizers of this Symposium are to be congratulated for providing a remarkably interesting and varigated set of topics for this intellectual banquet. In its variety and interest the first session on the "Influence of Drugs on Immunopathologic Pathways" provides a worthy start to the Symposium. This variety, welcome though it is, does make it difficult to provide a coherent Introduction which does justice to the scope of the papers in this session. However, many of the papers deal with the response of cells to a variety of immunologic stimuli. There now exists evidence that the first or one of the first steps in many, if not all, of these reactions is the activation of an esterase. Therefore, after first detailing the reactions where such evidence has been obtained, I propose to discuss very briefly the use of organophosphorus inhibitors in obtaining such evidence, then proceed to give examples of the employment of organophosphorus inhibitors in dissecting the details of the esterase step, and finally to consider the possibility of the use of such agents as drugs in the therapy of immunologic diseases.

IN VITRO REACTIONS INVOLVING ESTERASE ACTIVATION

Table I (modified from Becker and Henson, 1973) lists those reactions for which there is evidence that esterase activation is one of the necessary steps (Becker and Henson, 1973; Pruzansky and Patterson, 1974; Unanue et al., 1974). This list includes such diverse responses as the immunologically and nonimmunologically induced secretion of histamine and/or serotonin from mast cells, basophils, and platelets, chemotaxis, erythrophagocytosis, and induced lysosomal enzyme secretion by neutrophils, and the antibody induced movement of lymphocytes.

The evidence for esterase activation, in all of the above instances except one, is indirect. In general, it consists of the demonstration that rather specific organophosphorus inhibitors of serine esterases ("serine" because serine exists in the active site of the enzyme) such as DFP (diisopropyl phosphofluoridate) or phosphonate esters prevent the given reaction when present with the stimulus and cell but not when either one is reacted with the inhibitor alone. The assumption is made that these organophosphorus compounds are acting in each instance cited only as irreversible, phosphorylating inhibitors. The findings together with the assumption lead necessarily to the conclusion that the given cell contains a serine esterase existing in the cell in an enzymatically inert or proesterase form which is insusceptible to inactivation by the organophosphorus compound; on interaction of the cell and stimulus the proesterase is transformed into an active enzyme susceptible to inactivation by the organophosphorus inhibitor. This assumption is certainly not true for all cells under all conditions (Woodin and Wieneke, 1970; Ferluga et al. 1972; Woodin, 1974). However, in the reactions cited above, this assumption has been proved for the chemotaxis of polymorphonuclear leukocytes (Becker, 1971), the induced release of serotonin from platelets (Henson et al., 1974), and the antibody induced movement of lymphocytes (Becker and Unanue, unpublished data). Moreover, in one instance, there is direct biochemical evidence that the chemotactic response of rabbit neutrophils requires the activation of a proesterase, proesterase 1, which exists in or on the neutrophil in an enzymatically inert, phosphonate insusceptible form. Following interaction of chemotactic factor with the cell, proesterase 1 is changed into esterase 1, which is capable of hydrolyzing the aromatic amino acid ester, acetyl DL-phenylalanine-β-naphthyl ester and is highly susceptible to inhibition by phosphonate esters. (The references to and summary of the experimental basis for these conclusions is given in Becker) and Henson, (1973); a critical review of the evidence is found in Aldridge and Reiner, (1972). In addition,

Table I. Systems for which there is Evidence for a Requirement for Esterase Activation

1. Chemotactic response of rabbit neutorphils to C5a, C3a, $C\overline{567}$, and bacterial factor.
2. Erythrophagocytosis of $EAC\overline{423}$ by rabbit neutrophils.
3. Antigen-induced histamine release from rat peritoneal-mast cells induced by rat IgE antibody.
4. Complement-dependent, noncytoxic histamine release from rat peritoneal mast cells induced by rabbit antirat-IgG.
5. Histamine release from fat peritoneal mast cells induced by band 2 lysosomal protein from rabbit neutrophils.
6. Antigen-induced release of histamine from sensitized guinea pig lung slices.
7. Antigen-induced release of histamine from human lung slices sensitized with IgE antibody.
8. Serotonin release from rabbit platelets by zymosan-complement.
9. Noncytotoxic serotonin release from rabbit platelets by antiplatelet antibody.
10. Serotonin release from rabbit platelets by platelet-activating factor (PAF).
11. Serotonin release from rabbit platelets by collagen.
12. Movement of mouse lymphocytes induced by rabbit antimouse Ig.
13. Antigen-induced histamine release from human basophils sensitized with IgE antibody.

to esterase 1 being formed by the activation of proesterase 1, it also exists already preformed in the rabbit polymorphonuclear leukocyte (Becker and Ward, 1969). Partially purified fractions of preformed esterase 1 show high chemotactic activity completely inhibitable by 10^{-7}M p-nitrophenyl ethyl 5-chloropentylphosphonate (Tsung, Kegeles and Becker, unpublished data). This latter finding suggests that esterase 1 might be chemotactic; a result which might be anticipated, if its formation, as induced by chemotactic factors, is one of the steps in the biochemical sequence leading to the chemotactic response.

From analogy with known immunologically activated esterases, e.g., $C\overline{1}$, and from its activity as an amino acid esterase, the most reasonable supposition is that esterase 1 is a protease. The activation step, on the basis of the macromolecular nature of some of the known chemotactic factors, e.g., C567, C5a, and C3a, almost certainly involves the outer cell membrane, and a reasonable assumption is that proesterase 1 is on the cell membrane. However, much arduous work has yet to be done before any of these soft assumptions can be translated into hard biochemical fact.

SOME USES OF ORGANOPHOSPHORUS INHIBITORS FOR THE ANALYSIS OF IN VITRO IMMUNOLOGICAL REACTIONS.

Unanue et al. (1974) recently demonstrated that DFP inhibits the movement of mouse splenic lymphocytes induced by rabbit antimouse Ig if DFP is present when the antibody reacts with cells but not if DFP is incubated with either antibody or cells alone. He suggested that the antibody induced movement in these cells by activating an esterase. Subsequently, in unpublished work, Dr. Unanue and I have confirmed these findings and further shown that the reaction of antibody and mouse lymphocytes stops at the esterase activation step. The esterase formed from the putative proesterase is "frozen" at 4° C in the activated state, unable to induce the next step in the sequence until the temperature is raised. This conclusion is based on the finding that reacting antibody and lympnocytes, washing out the unbound antibody, incubating the cells with DFP, and then washing out the unbound DFP leads to inhibition of cell movement when all the steps except the demonstration of cell movement are carried out in the cold. The degree of inhibition under these conditions is as great as when DFP, antibody and cells are reacted together either at 37 or 40° C. Moreover, the inhibition obtained when the cells were treated in the multistep reaction in the cold depends on the time the activated cells and DFP are in contact and on the concentration of DFP. Substituting the poorly phosphorylating phenyl ethyl pentylphosphonate for DFP leads to no inhibition, whereas, the efficient phosphorylator, p-nitrophenyl ethyl butyl phosphonate, is highly inhibitory.

The work of Kaliner and Austen (1973) suggests that antigen induced histamine release in the cold from mast cells of human lung sensitized with IgE antibody proceeds to a significant degree beyond the esterase activation step. In contrast, the work of Pruzansky and Patterson (1974) suggests that in the antigen induced release from human basophils sensitized with IgE antibody, esterase activation does not occur at all in the cold but only at 37° C.

A series of phosphonate esters of the general structure have been synthesized. As the carbon atoms in the alkyl chains of the various R groups increase, the ability to inhibit various enzymes changes. For a given homologous series the patterns of activity, i.e., the inhibition profiles, differ among different enzymes and thus can be used to distinguish one serine esterase from another (Becker et al., 1963; Boone et al., 1964; Becker, 1967). Based on a similarity of inhibition profiles, it was concluded that antigen induced histamine release from rat mast cells sensitized with rat IgE antibody, histamine release from these same cells resulting from the action of cationic lysosomal proteins of the rabbit neutrophil or from the reaction of rabbit

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