ADVANCES IN

Pharmacology and Chemotherapy

VOLUME 10

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Pharmacology and Chemotherapy

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I. Introduction

In this chapter the author will try to correlate the results of three large fields of research, which, though originating at about the same time and

developing at about the same rate, have advanced in a parallel and independent fashion. Referred to are the fields of immunology and serology, pharmacology and chemotherapy, and pathology. If one reads present-day textbooks on immunology, pathology, or chemotherapy, the information gained is somewhat unilateral despite obvious effects of one field on one or both of the others. The only exception may be the fast-growing subspecialty of transplantation research.

No complete study of immunology, chemotherapy, or immunopathology is provided in this chapter; the reader may refer to the many already available review articles (Miescher and Müller-Eberhard, 1968; Letterer, 1967; Gell and Coombs, 1968; Diener, 1970; Steffen, 1968; Morrison, 1960; Sellei *et al.*, 1970; Wilmans, 1964; Abramoff and LaVia, 1970; Mandel, 1959). Instead, the author will introduce a synthetic approach to the problem of immunosuppression and reintroduce at the same time the value of careful morphological investigations.

II. Molecular Biology and Morphology of Antibody Formation

Before concentrating on the main subject—the morphology of immunosuppression—a summarizing review needs to be given of the current knowledge on the normal immune response. Readers interested in detailed descriptions of this subject may refer to the "Biology of the Immune Response" by Abramoff and LaVia (1970), or for the morphology and pathology, to Letterer (1967), Cottier *et al.* (1969), and Turk (1970).

Violation of the integrity of the human or animal body by foreign substances of a certain nature is followed by a specific reaction of the injured body called an *immune reaction*. Foreign substances that are able to elicit an immune reaction are named *antigens*; if a known antigen in a given organism induces an immune reaction, this substance may be well called an *immunogen*; if it introduces tolerance, it may be called *tolerogen*. Both tolerance and immunity (i.e., allergy) can be caused by the same antigen at different dose levels, at different routes of administration, or at different ages of the recipient. The specific reaction of the affected individual toward the antigen is carried out by antibodies. Antibodies are proteins with a variable fraction of their molecule that, on induction, is synthesized in a way to fit specifically with a certain segment of the antigen. This antigenic segment is called the *specific determinant*. A few examples of various antigenic determinants are given in Table I, and an example of the composition of an antibody molecule is given in Fig. 1. The cellular and molecular events that are initiated by the entrance of an antigen into the living organism are summarized in Fig. 2.

TABLE I

SIZES OF VARIOUS ANTIGENIC DETERMINANTS

Antigen	Determinant	Size in most extended form (Å)	Molecular weight
Dextran	Isomaltohexaose	34 × 12 × 7	990
Silk fibroin	Gly [gly_3ala_3] tyr	27	632
	Dodecapeptide mixture	44	1000
$G_{60}A_{40}$, $G_{60}A_{30}T_{10}$ and $G_{42}L_{28}A_{30}$	Hexaglutamic acid	36 × 10 × 6	792
Polyalanyl bovine serum albumin	Pentaalanine	25 × 11 × 6.5	373
Polylysyl rabbit serum albumin	Penta- (or hexa-) lysine	27 × 17 × 6.5	659
Polylysyl phosphoryl bovine serum albumin	Pentalysine	27 × 17 × 6.5	659

From Kabat (1966), reprinted with the permission of the author and of Williams & Wilkins, Baltimore, Maryland.

Phagocytosis has been known since Metschnikoff (1892) as the first visible reaction of the host toward an administered antigen. Extensive studies of Nossal, Ada, and co-workers have shown that, in lymphoreticular tissues, antigenic materials are fixed by phagocytic sinus endothelial cells, by cortical and medullary histiocytes, and by dendritic reticulum cells of the follicle (Nossal *et al.*, 1964; Ada *et al.*, 1967; Lang and Ada, 1967; McDevitt, 1968). However, dendritic reticulum cells apparently do not phagocytize antigen but absorb it to their surface and so probably allow a close contact of antigenic determinant sites with immunoreactive lymphoid cells (Szakal and Hanna, 1968; Schoenberg *et al.*, 1964). Antigen may persist 2–6 weeks in these various phagocytes and dendritic reticulum cells after a single primary injection. Within phagocytes, complex antigens then are broken down to simple antigenic molecules (Gill and Cole, 1965). This step of antigen processing appears important for the development of a primary immune response, although it may not be essential for all antigens (Pribnow and Silverman, 1967; Frei *et al.*, 1965; Feldman and Gallily, 1967). From these phagocytes information for the synthesis of specific antibodies is passed to immunoreactive lymphoid cells via the transfer of ribonucleic acid (RNA) or RNA–antigen complexes (Pinchuck *et al.*, 1968; Fishman and Adler, 1963a,b; Friedman *et al.*, 1965; Askonas and Rhodes, 1965; Gottlieb *et al.*, 1967). Since both ribonuclease (RNase) and pronase can destroy the activity of RNA extracted from sensitized animals,

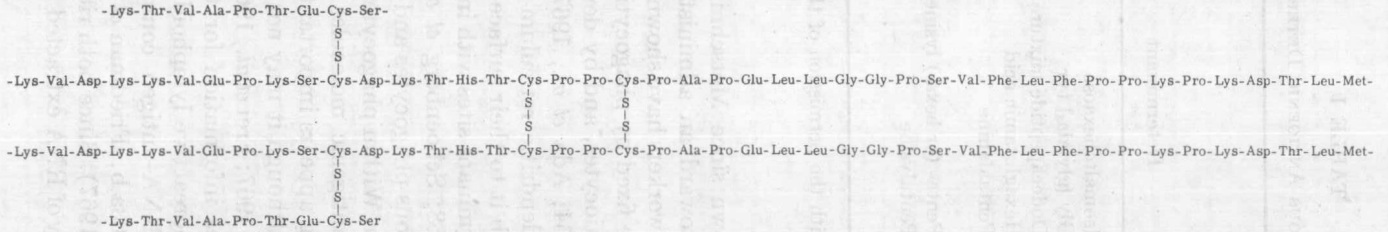

FIG. 1. Example of the chemical composition of an antibody molecule (fragment). From Steiner and Porter (1967). Reprinted from *Biochemistry* **6**, 3957–3970. Copyright 1967 by the American Chemical Society. Reprinted by permission of the copyright owner.

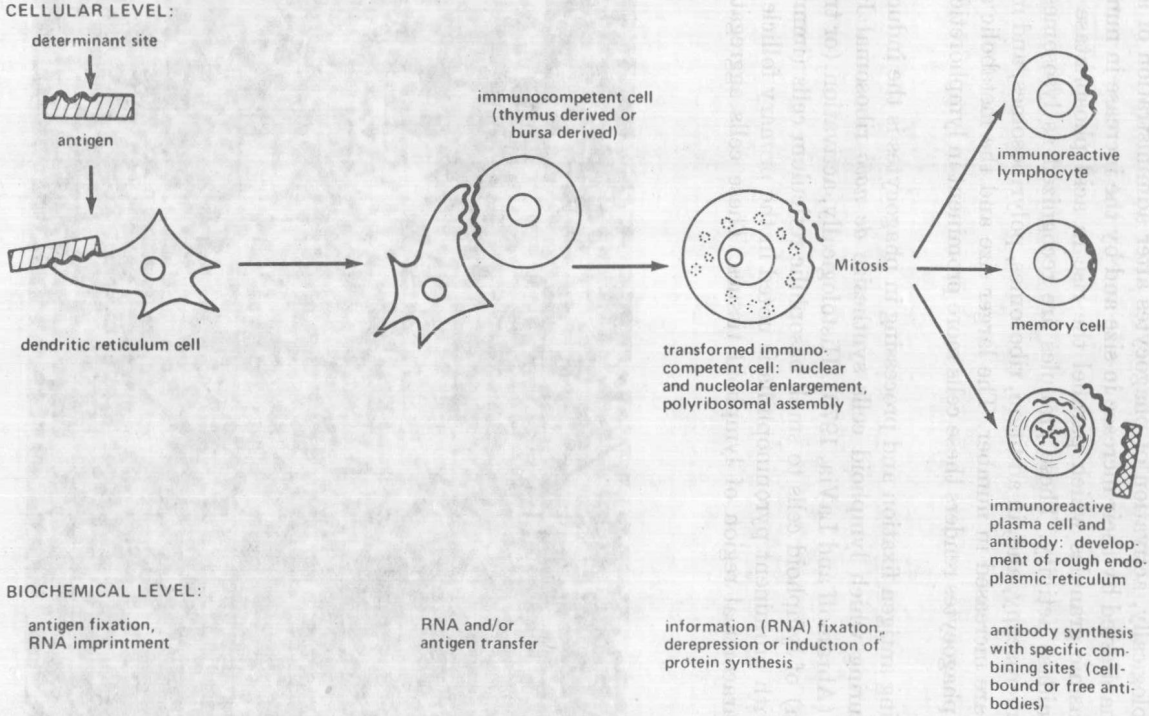

FIG. 2. Biology of antibody formation.

a complex of RNA and of antigen or antigen fragments may be essential for the induction of antibody synthesis.

Morphologically, activation of phagocytes after administration of antigents is manifested by their increase in size and by the increase in number of cytoplasmic granules which parallel the rise in acid phosphatase and β-glucuronidase activity. These granules are recognized as lysosomes by electron microscopy, and, in addition, ribosomes, polyribosomes, and mitochondria are increased in number. The larger size and the metabolic activation of phagocytes renders these cells more prominent in lymphoreticular tissues.

Following antigen fixation and processing in phagocytes is the induction period, during which lymphoid cells synthesize *de novo* ribosomal RNA (rRNA) (Abramoff and LaVia, 1970). Histologically, activation (or transformation) of lymphoid cells to small basophilic reticulum cells (immunoblasts) with prominent pyroninophilia is noted in the primary follicle and in the paracortical region of lymphoid tissues. These cells aggregate to

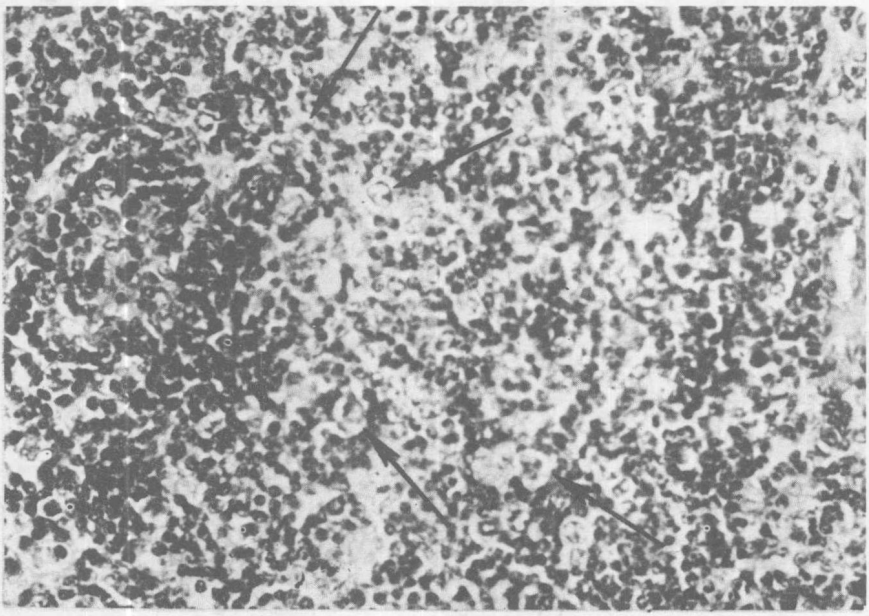

FIG. 3. Paracortical region of a mouse lymph node draining the site of injection of tubercle bacteria in adjuvant. Note increased number of activated reticulum cells, (immunoblasts arrows). H&E; magnification ×375.

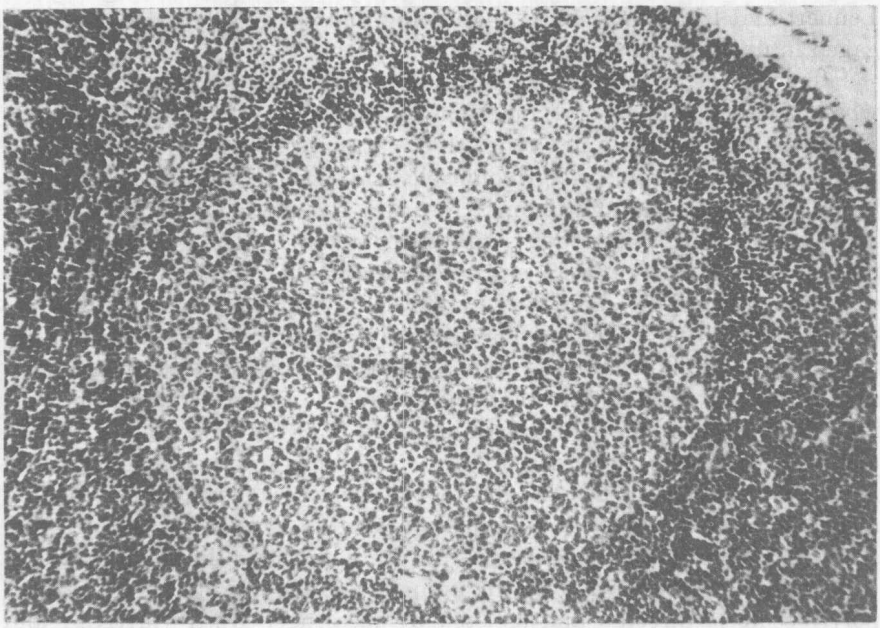

FIG. 4. Secondary follicle of a mouse lymph node draining the site of injection of bovine serum albumin. Note the division of the secondary follicle into the pale and the basophilic parts. H&E; magnification ×150.

form in the primary follicle a secondary follicle also called the *germinal center* (Figs. 3 and 4). This secondary follicle formation often is preceded by a transient dissociation of a preexistent secondary follicle (Congdon, 1962, 1964). Probably this dissociation is closely related to the period of induction, while reaggregation and *de novo* formation of secondary follicles represents the first step in antibody formation. Not in all cases of antigenic stimulation, however, is follicular dissociation obvious (Mariani *et al.*, 1971; Congdon, 1969). By electron microscopy, activated lymphoid cells, i.e., small basophilic reticulum cells, show increased numbers of polyribosomes but only a little rough endoplasmic reticulum. Their nuclei are enlarged and contain prominent nucleoli. Some authors subclassify these cells according to their cytological details into basophilic stems cells, germinoblasts, and germinocytes (Lennert, 1961; Mori and Lennert, 1969), although these may only represent variant states of activity of the same type of cell. Histochemically, cells in the secondary follicle characteristically contain a high concentration in 5′-nucleotidase (Braunstein *et al.*, 1958;

Lennert and Rinneberg, 1961), indicating that these cells are actively engaged in nucleic acid metabolism.

Following the induction period is the period of actual antibody synthesis which follows the general biochemical pathways of protein synthesis (Kabat, 1968; Mahler and Cordes, 1968): transcription of information for protein synthesis from deoxyribonucleic acid (DNA) to RNA, and translation of this information from RNA into the basic polypeptide chain. Each cell capable of antibody synthesis contains the DNA-encoded information for protein synthesis, and, according to the clonal selection theory of Jerne (Jerne, 1955) and Burnet (Burnet, 1959), may even contain the information for a single specific antibody. Antigenic stimulation is interpreted as selection and activation of these cells to produce their precoded antibody congruent to the inducing antigen. Another older theory, the instruction theory of Breinl and Haurowitz (1930) and Haurowitz (1965), also may be still valuable. According to this theory, only the information for protein synthesis is DNA encoded. The antigenic determinant, probably attached to RNA, serves as a template to modify nonspecific transcription and impose the synthesis of the specific group in the antibody molecule.

Amino acids are assembled to immunoglobulin chains at the site of polyribosomes, directed and assisted by messenger RNA (mRNA) and transfer RNA (tRNA) (Mahler and Cordes, 1968; Williamson and Askonas, 1967). It appears possible that the size of polyribosomal units is directly related to the size of the immunoglobulin chain (Kuff and Roberts, 1967). As in the synthesis of other proteins, polyribosomes involved in immunoglobulin synthesis appear membrane-bound, i.e., they are a component of rough endoplasmic reticulum (DePetris and Karlsbad, 1965; LaVia *et al.*, 1968). The release of antibody globulins from these membranes and from the cell is finally preceded by the addition of a carbohydrate group (Melchers and Knopf, 1969; Swenson and Kern, 1968).

Histological changes in lymphoreticular tissues during the period of antibody synthesis consist of fully developed secondary follicles, various numbers of pyroninophilic reticulum cells in the paracortical region, and differentiation of lymphoid cells to plasma cells in the medullary cords of lymph nodes (Fig. 5) (Ringertz and Adamson, 1950; Movat and Fernando, 1965; Krüger, 1967b; Krüger and Harris, 1970; Harris and Harris, 1956; Congdon and Makinodan, 1961). Histochemically, markedly elevated activities of glucose-6-phosphate dehydrogenase and alkaline phosphatase are noted in basophilic reticulum cells (immunoblasts) (Turk, 1967). Basophilic reticulum cells contain abundant aggregated cytoplasmic ribosomes, and plasma cellular differentiation in the medullary cords is paralleled by the marked increase in rough endoplasmic reticulum. Accordingly, intra-

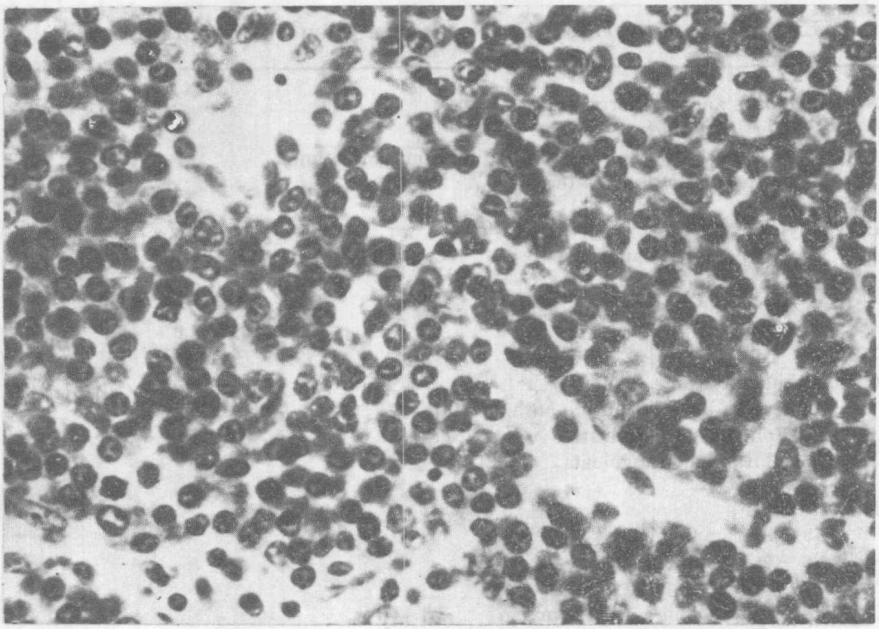

FIG. 5. Medullary cords of a mouse lymph node draining the injection site of bovine serum albumin. Note increased number in plasma cells. H&E; magnification ×675.

cellular antibody has been shown in basophilic reticulum cells of the secondary follicle (Pernis, 1967; Young and Friedman, 1967) and in cisternae lined by endoplasmic reticulum of plasma cells as well as their less well-differentiated precursors (DePetris and Karlsbad, 1965; Leduc *et al.*, 1968; Avrameas and Lespinats, 1967; DePetris *et al.*, 1963). Differentiation to antibody-producing cells usually is accompanied by cell proliferation in lymphoreticular tissues, the extent of which appears to depend upon the "strength" and on the dose of the antigen.

It is not yet well understood what the relationship is of the above-described mechanisms of antibody formation to the development of immune lymphocytes eliciting a cellular immune response. It appears that macrophages also play a role in cellular immunity (Dumonde, 1967) and that cell activation or transformation to pyroninophilic reticulum cells in the paracortical region of lymph nodes precedes the state of delayed hypersensitivity (Krüger and Harris, 1970; Oort and Turk, 1965). It has been suggested that immune lymphocytes may carry antibody-like substances

TABLE II

LIST OF IMMUNOSUPPRESSANTS AND CANCER CHEMOTHERAPEUTIC AGENTS

A. Hormones and antihormones
   1. Corticosteroids
   2. Corticosteroid antagonists (Metopirone, Mitotane)
   3. Estrogens and progesterone

B. Alkylating agents
   1. Nitrogen mustards
   2. Ethyleneimines
   3. Esters of alkylsulfonic acid
   4. Epoxides

C. Antimetabolites
   1. Pyrimidine and purine antagonists
   2. Folic acid antagonists
   3. Glutamine antagonists

D. Antibiotics
   1. Chloramphenicol
   2. Actinomycins
   3. Mitomycin C
   4. Daunomycin and adriamycin
   5. Mithramycin
   6. Bleomycin
   7. Puromycin
   8. Azotomycin
   9. Neocarzinostatin
   10. Streptonigrin

E. Enzymes
   1. L-Asparaginase
   2. Ribonuclease

F. Mitotic Inhibitors
   1. Colchicine and derivatives
   2. Podophyllin derivatives
   3. *Vinca rosea* alkaloids

G. Polyanions
   1. Pyran copolymers
   2. Polyinosinic acid–polycytidylic acid (poly I:C)

H. Miscellaneous Substances
   1. Methylhydrazine derivatives
   2. Mycophenolic acid
   3. BCNU [1,3-bis(2-chloroethyl)-1-nitrosourea]
   4. Newer alkylating agents