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Foreword

The time seems ripe for a critical compendium of that segment of the biological universe we call viruses. Virology, as a science, having passed only recently through its descriptive phase of naming and numbering, has probably reached that stage at which relatively few new—truly new—viruses will be discovered. Triggered by the intellectual probes and techniques of molecular biology, genetics, biochemical cytology, and high resolution microscopy and spectroscopy, the field has experienced a genuine information explosion.

Few serious attempts have been made to chronicle these events. This comprehensive series, which will comprise some 6000 pages in a total of about 18 volumes, represents a commitment by a large group of active investigators to analyze, digest, and expostulate on the great mass of data relating to viruses, much of which is now amorphous and disjointed, and scattered throughout a wide literature. In this way, we hope to place the entire field in perspective, and to develop an invaluable reference and sourcebook for researchers and students at all levels.

This series is designed as a continuum that can be entered anywhere, but which also provides a logical progression of developing facts and integrated concepts.

Volume 1 contains an alphabetical catalogue of almost all viruses of vertebrates, insects, plants, and protists, describing them in general terms. Volumes 2–4 deal primarily, but not exclusively, with the processes of infection and reproduction of the major groups of viruses in their hosts. Volume 2 deals with the simple RNA viruses of bacteria, plants, and animals; the togaviruses (formerly called arboviruses), which share with these only the feature that the virion's RNA is able to act as messenger RNA in the host cell; and the reoviruses of animals and plants, which all share several structurally singular features, the

most important being the double-strandedness of their multiple RNA molecules.

Volume 3 addresses itself to the reproduction of all DNA-containing viruses of vertebrates, encompassing the smallest and the largest viruses known. The reproduction of the larger and more complex RNA viruses is the subject matter of Volume 4. These viruses share the property of being enclosed in lipoprotein membranes, as do the togaviruses included in Volume 2. They share as a group, along with the reoviruses, the presence of polymerase enzymes in their virions to satisfy the need for their RNA to become transcribed before it can serve messenger functions.

Volumes 5 and 6 represent the first in a series that focuses primarily on the structure and assembly of virus particles. Volume 5 is devoted to general structural principles involving the relationship and specificity of interaction of viral capsid proteins and their nucleic acids, or host nucleic acids. It deals primarily with helical and the simpler isometric viruses, as well as with the relationship of nucleic acid to protein shell in the T-even phages. Volume 6 is concerned with the structure of the picornaviruses, and with the reconstitution of plant and bacterial RNA viruses.

Volumes 7 and 8 deal with the DNA bacteriophages. Volume 7 concludes the series of volumes on the reproduction of viruses (Volumes 2–4 and Volume 7) and deals particularly with the single- and double-stranded virulent bacteriophages.

Volume 8, the first of the series on regulation and genetics of viruses, covers the biological properties of the lysogenic and defective phages, the phage-satellite system P 2–P 4, and in-depth discussion of the regulatory principles governing the development of selected lytic phages.

Volume 9 provides a truly comprehensive analysis of the genetics of all animal viruses that have been studied to date. These chapters cover the principles and methodology of mutant selection, complementation analysis, gene mapping with restriction endonucleases, etc. Volume 10 also deals with animal cells, covering transcriptional and translational regulation of viral gene expression, defective virions, and integration of tumor virus genomes into host chromosomes.

Volume 11 covers the considerable advances in the molecular understanding of new aspects of virology which have been revealed in recent years through the study of plant viruses. It covers particularly the mode of replication and translation of the multicomponent viruses and others that carry or utilize subdivided genomes; the use of proto-

plasts in such studies is authoritatively reviewed, as well as the nature of viroids, the smallest replicatable pathogens. Volume 12 deals with special groups of viruses of protists and invertebrates which show properties that set them apart from the main virus families. These are the lipid-containing phages and the viruses of algae, fungi, and invertebrates.

Volume 13 contains chapters on various topics related to the structure and assembly of viruses, dealing in detail with nucleotide and amino acid sequences, as well as with particle morphology and assembly, and the structure of virus membranes and hybrid viruses. The first complete sequence of a viral RNA is represented as a multicolored foldout.

Volume 14 contains chapters on special and/or newly characterized vertebrate virus groups: bunya-, arena-, corona-, calici-, and orbiviruses, icosahedral cytoplasmic deoxyriboviruses, fish viruses, and hepatitis viruses.

This volume deals with immunological reactions to viruses. Several subsequent volumes will deal with virus–host relationships and with methodological aspects of virus research.

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CHAPTER 1

Immune Responses, Immune Tolerance, and Viruses

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1. INTRODUCTION

Exactly how viruses or other microbes can persist in the host they invade is one of the most challenging problems in animal virology and an intellectual puzzle in biology. Because the immune system is responsible for purging these agents from the host, their continued presence has long been equated with partial or total malfunction of the appropriate immune response.

Reflecting on the early observations of Eric Traub (1936*a*,*b*) concerning natural infections of mice with lymphocytic choriomeningitis virus (LCMV) as well as examination of chimeric cattle by Ray Owen (1945), Burnett and Fenner postulated (1949, 1959) nearly three decades ago that clonal elimination of immunocompetent cells is the basis for immunological tolerance to viruses and self antigens, respectively. Immunological tolerance is defined as the state of specific refractoriness in responding to an antigen (virus) following a prior exposure to that antigen (virus). The concept that individuals can become immunologically tolerant to viruses, especially LCMV and retroviruses, was championed by many investigators during the 1950s and 1960s (Burnett and Fenner, 1949; Hotchin and Cinits, 1958; Volkert and Larsen, 1965; Traub, 1960; Gross, 1961). This conclusion followed observations that,

after exposure to virus *in utero* or at birth, adult animals retained infectious virus throughout life, but free antibody(ies) to that virus was not detected in their circulations.

Reappraisal of this conclusion has paralleled the outburst of recent knowledge defining the subcomponent cell populations of the immune system and their interactions in generating immune responses, coupled with new technology for measuring immune response products, especially in the presence of virus. Although several unanswered questions remain, the overwhelming evidence indicates that immune responses accompany most, if not all, viral infections, and that classical immunological tolerance does not occur.

This chapter will present the evidence that has accumulated regarding the generation of antiviral immune responses during virus persistence. Toward understanding such responses to viruses, we will first describe immune responses to antigens other than virus as presently understood; then we will dissect immune responses and discuss ways of investigating immune tolerance.

2. IMMUNE SYSTEM

2.1. Organization, Interaction, and Genetic Control

Conceptually, over the last decade a revolution has occurred in our understanding of the biology and function of the immune system. It is now abundantly clear (1) that both the making of antibody and the mediation of immune responses by cells are end products of cellular collaboration, which is (2) associated with unique subsets of both thymus-derived (T) and bone-marrow-derived (B) lymphocytes, and (3) that regulation is under genetic control. The end result is measured as a specific immune response initiated by a specific antigen and associated with unique interactions among macrophages, T-cell subsets, and B cells. The immune product results from a series of minute regulations from a network of unique cells (Jerne, 1974) involving such events as cell–cell interactions and release of mediators that can enhance or suppress the immune response. Alterations in expected immune responses following exposure to antigens may occur at any one of several stages. Studies in which replicating agents are the antigens may lead to information concerning generation and control of immune responses. Further, viruses themselves, when they infect immunocompetent cells, may be used as probes toward understanding each step in lymphocyte differentiation and function.

Cell subsets of the immune system are often distinguished from one another in terms of their functions or the unique cell-surface determinants they carry (Raff, 1977; Mitchell, 1977; Gershon, 1974; Katz, 1977; Greaves *et al.*, 1974). On the basis of several properties, particularly their content of unique cell-surface determinants that bind specific reagents, reasonably homogeneous subsets can be segregated from the total populations of immune cells so that their functions and interactions can be analyzed. Evidence for cellular collaboration has been documented for T-cell–B-cell, T-cell–T-cell, T-cell–macrophage, and B-cell–macrophage interreactions. Cellular collaboration is exemplified as follows: antigen-specific helper factors are released from T lymphocytes bearing Ly1 antigens on their surfaces (helper T cells). These factors, under genetic restraints, bind to macrophages. In turn, macrophages induce a unique clone of B cells, again under genetic restriction, to proliferate and differentiate into plasma cells, which release specific antibody. Modulating effects between T-helper lymphocytes and T-suppressor lymphocytes are important in regulating such responses (reviewed in Katz, 1977).

The genetic control over antibody synthesis (McDevitt and Landy, 1972) has been noted at the level of B cells (Benacerraf and McDevitt, 1972; Taussig *et al.*, 1974), T cells (Benacerraf and McDevitt, 1972), and macrophages (Weiner and Bandieri, 1974). In addition, several cytotoxic T-cell responses to virus infections (Cole *et al.*, 1972; Doherty and Zinkernagel, 1974; Zinkernagel and Doherty, 1973) and to products of tissue- and adjuvant-induced autoimmune diseases, i.e., allergic encephalomyelitis (reviewed by Paterson, 1977; Gonatas and Howard, 1974; Williams and Moore, 1973), are under genetic restriction and regulation. Thus the control of immune responses is manifested in a number of different ways during virus infections and autoimmune responses.

2.2. Measurement of Immune Responsiveness: Antigen-Binding Cells

Evaluating experiments on alterations of immune function (enhancement or suppression) requires knowledge of the strengths and limitations of techniques used to measure them. Total populations and subpopulations of immunocompetent cells can be measured on the basis of functional assays and unique cell-surface markers (Katz, 1977). However, some of these markers may change during differentiation. Hence detection and description of cell surface markers on lymphocytes

are, in part, relative to the conditions of the assay. Despite the limitations, a vast and informative body of data now exists (Greaves *et al.*, 1974; Katz, 1977) and T-cell-specific helper cells, suppressor cells, and cytotoxic cells can be marked, segregated, and isolated. Antigen-specific immunocompetent cells can be detected by using radiolabeled antigens (Nossal and Ada, 1971) that react with specific receptors on T and/or B cells or their subpopulations. Hence antigen-specific T and/or B cells can be identified within a population and quantitated. In an extension of this technique, one uses antigen radiolabeled at a high specific activity so that cells binding this antigen can be eliminated on the basis of radioactive injury. Thereafter, the function usually performed by these cells is no longer present. The use of such an assay to cause suicide of antigen-specific cells in combination with reconstitution experiments has been helpful in charting specific immune response interactions.

2.3. Measurement of Immune Responsiveness: B-Cell Products (Antibodies)

When antibodies form and circulate free of antigen, they are easily detected by any of a wide variety of techniques (Lennette and Schmidt, 1979; Bloom and David, 1976). These consist of both primary (radiobinding assays, fluorescent binding assays) and secondary (complement fixation, hemagglutination inhibition, lysis of infected cells in the presence of complement or lymphocytes bearing Fc receptors) binding assays. In contrast, when antibodies and antigen are both present, antibody may not be detectable by such assays; instead one must measure antigen–antibody complexes (Oldstone and Perrin, 1979; Theofilopoulos and Dixon, 1976; Zubler and Lambert, 1976). When antibodies complex with antigen, their quantitation is difficult unless antibody is measured before it reaches the antigen. This is best done by quantitating the numbers of antibody-secreting cells in a Jerne plaque assay (Jerne and Nordin, 1963).

2.4. Measurement of Immune Responsiveness: T-Cell Activities

T-suppressor- and T-helper-specific functions are measured in biological systems in which enhanced or suppressed immunological activity is quantitated. For example, some *in vitro* assays use pokeweed mitogen to stimulate B cells in the presence of added T-helper or T-sup-

pressor cells. The amount of immunoglobulin made and released in the fluid phase or the number of plasma cells generated in culture is quantitated (Moretta *et al.*, 1977; Oldstone *et al.*, 1977). The activity of cytotoxic T cells can be quantitated by the use of a ^{51}Cr release assay (Brunner *et al.*, 1976). Target cells tested are radiolabeled with ^{51}Cr and reacted with different amounts of primed cytotoxic T cells. The number of cytotoxic T cells present in the reaction correlates directly with the degree to which the lysed cells release ^{51}Cr.

3. IMMUNE RESPONSES IN NONVIRAL SYSTEMS

3.1. Immune Regulation

The tuning of the immune system is the result of a balance between factors that enhance and suppress the immune system. Lack of control leading to overbearing immune enhancement occurs, for example, with polyclonal activation of immune cells, which can lead to autoallergic reactions and resultant disease. This is not the subject of this chapter. The opposite extreme, immunological tolerance, will be considered in detail.

By definition, immune tolerance refers to the lack of response to an antigen previously contacted. Conceptually immune tolerance can occur via divergent mechanisms which may have their effects on B cells, T cells, and/or macrophages. Although the end stage is unresponsiveness, the routes to that end differ. Immune tolerance results from either central unresponsiveness or peripheral unresponsiveness.

3.2. Central Tolerance

Central tolerance or unresponsiveness refers to the irreversible loss of competent lymphocytes and can occur by clonal elimination or clonal dysfunction. Burnett originally postulated that a mechanism of clonal elimination could provide a biological basis for cell tolerance (1959). The scheme indicates that during T-cell, B-cell, or macrophage maturation, interaction with specific antigen may occur. The antigen binds with specific antigenic receptors on the cell surface, leading to the inactivation and clonal elimination of these potentially immune functioning cells. Thus far the most meaningful experiments in this vein involve B-cell tolerance, perhaps owing to the ease in measuring the final product, i.e., antibodies. Experimental evidence for central

unresponsiveness has been provided in several laboratories (Chiller and Weigle, 1975; Katz and Benacerraf, 1974) by showing the deletion of specific antigen-binding cells. Although no antigen-binding cells should be detected during central tolerance (clonal deletion), they may similarly fail to form during peripheral unresponsiveness because of ligand interaction (see below). Before tolerance can be broken and immune responsiveness restored, new cells must regenerate from precursor stem-cell pools. Clonal deletion implies three major points. First, specific antigen-binding B or T lymphocytes must be absent. Second, in most systems studied, immunity is lacking during the induction period of tolerance. Third, maintenance of tolerance is dependent directly on the continued presence of antigen rather than indirectly on antigen-induced regulation factors such as suppressor cells or antigen–antibody complexes.

Clonal dysfunction indicates that the clones are not eliminated but rather lack normal activity that would produce immune responsiveness. Viruses are permissive for and can persist in cells of the immune system (Notkins *et al.*, 1970; Doyle and Oldstone, 1978; Popescu *et al.*, 1977). Could viruses alter lymphocyte function(s)? Although it is not known whether such lymphocyte dysfunction occurs, it is clear that viruses can persist in some differentiated cells (neuroblastomas) and alter their luxury (differentiation) function without altering their vital function (growth, cloning efficiency, survival). Differentiated functions of neuroblastoma cells (making the transferase and/or esterase needed to synthesize or degrade acetylcholine) can be uniquely impaired during persistent LCMV infection, both *in vitro* and *in vivo* without altering the vital functions of cultured cells or those in an intact animal (Oldstone *et al.*, 1977*a*). However, despite the many clinical observations of impaired immune responses during virus infections (von Pirquet, 1908; Notkins *et al.*, 1970), there is no direct evidence yet that viruses infecting lymphocytes or macrophages can cause their dysfunction without their death.

3.3. Peripheral Tolerance

Peripheral unresponsiveness indicates that cells competent for immune responses are present but cannot be induced to respond. Any of several mechanisms could produce peripheral tolerance. For example, ligands may induce inactivation of immunocompetent lymphocytes by receptor blockade, and not by their deletion. Antibodies generated to idiotypic or allotypic determinants of cell-surface