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CHAPTER 7

Virus Infections and the Immune Responses They Elicit

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I. Introduction

The immune responses elicited by animal viruses are of historical interest as well as current practical importance. The science of immunology can be said to have been founded by Edward Jenner, who in his well known "An enquiry into the causes and effects of the variolae vaccinia," published in 1798, described the inoculation of variolous matter into the arm of Mary Barge, who had previously had cow pox. He noted: "It is remarkable that variolous matter, when the system is disposed to reject it, should excite inflammation more speedily than when it produces the Small Pox." This is the first description of delayed hypersensitivity, and reactions to vaccination and rashes produced by virus infections were taken as examples of allergic reactions by von Pirquet (1907) in his pioneering study of allergy.

Since most virus infections cannot yet be treated by chemotherapy, immunoprophylaxis and isolation of cases have been the only effective ways to control virus diseases. The important epidemiologic consequences of the use of vaccines against smallpox, yellow fever, and poliovirus are well known, and it seems likely that vaccines against measles and rubella virus will also reduce the incidence of these diseases. In the veterinary field, vaccines against canine distemper, canine hepatitis, hog cholera, and Newcastle disease and Marek's disease of chickens have been highly effective. Thus, the immunogenicity of animal viruses is of considerable practical impor-

tance, and an enormous amount of information has accumulated on antibody formation in a wide range of animal forms inoculated with viruses or virus vaccines.

As expected, small viruses with low contents of nucleic acid and relatively simple structures have few antigens while large viruses, with greater contents of nucleic acid and complex structures, such as herpesviruses and poxviruses, have many antigens. With small viruses and viruses of intermediate size, such as adenoviruses or myxoviruses, the relationship between major antigens and the subunits assembled into intact virions is better understood. In this chapter only a few representative examples can be selected for discussion, to illustrate certain general principles concerning the immunogenicity of animal viruses and in what way they resemble or differ from responses to other antigens. Brief descriptions of the structures and known antigens of selected viruses will be followed by an account of the humoral and secretory responses elicited by them.

Unexpected results were obtained in the course of studies of immune responses to influenza viruses sharing some antigens but differing in others. It was found that following exposure to one such virus, infection by the second virus often elicited a stronger immune response against the first virus than the second. This phenomenon of "original antigenic sin" was one of the first examples of immunologic memory to be studied systematically, and only recently has the underlying cellular mechanism been investigated. The problem of memory formation in T and B lymphocytes to thymus-dependent and thymus-independent antigens is currently under vigorous investigation and observations on the sequential responses to cross-reacting viral antigens may be pertinent to this problem. The thymus dependence of viral antigens is also under study and results will be presented.

The most commonly studied immune responses to viruses are neutralizing antibodies in serum. The high sensitivity and precision of assays based on neutralization of plaque-forming viruses make this an excellent model system, and much information of general interest has emerged from its use. Neutralization itself has proved to be a complex phenomenon which is not yet fully understood. Among the features that have been studied and will be described are the sequence of appearance and neutralizing capacity of different classes of immunoglobulins, the avidity of antibodies, the additional effects of complement components, and the existence of infectious complexes of viruses and antibody.

Macrophages are implicated at many points in immune responses, especially in antigen processing and presentation and possibly in B and T lymphocyte interactions. They can interact directly with viruses and microorganisms and can mediate cytotoxic reactions against tumor cells and cells infected with viruses or intracellular parasites. The capacity of macrophages to support virus replication and thus spread infection, or to degrade virus and aid in recovery from infection will be discussed. The ontogeny of this virucidal capacity will be reviewed with emphasis on the influence of the thymus, either hormonally or through T lymphocytes and their products.

The interaction of sensitized lymphocytes with infected cells requires cell surface changes recognizable by the lymphocytes. Virus-induced surface changes have been described during infection for most viruses studied. Some of these represent new proteins or glycoproteins encoded in the virus genome, while others result from rearrangement of host material or derepression of host genes. The appearance of "new" host antigens or receptors (including Fc receptors) and the diminution of normally present antigens (including histocompatibility antigens) have been reported. Antigenic modulation, capping, and other phenomena previously described for nonviral cell surface antigens are also found with viral antigens and will be described.

Delayed hypersensitivity has been observed in many virus infections, and more recently *in vitro* studies have confirmed the existence of cell-mediated immune responses to viral antigens. These were regarded as little more than curiosities until children born with selective deficiencies of T cell function were found to be unduly susceptible to vaccinia virus, herpesviruses, and measles virus infections, while children with severe hypogammaglobulinemia generally are not. Observations of experimental animals confirm this importance of cell-mediated immunity in protection against certain virus infections but not others (reviewed by Allison and Burns, 1972; Allison, 1974). Thus, for some viruses, eliciting a powerful cell-mediated response is an important aspect of immunogenicity. A summary will be presented of the evidence for cell-mediated immune responses for viral antigens, particularly of observations made *in vitro*.

The fact that infections such as measles, varicella, and mumps usually occur only once had been known to medical practitioners for centuries, and when these were identified as caused by viruses it became clear that viruses are able to induce long-lasting immunity.

Two explanations have been put forward for prolonged antibody production in the absence of reinfection: persistence of virus in the host (Olitsky and Long, 1929a,b) and persistence of clones of immunocompetent cells (Burnet, 1959). Both explanations are true. The latter phenomenon is well known and evidence for virus persistence will be presented.

The observations of Traube on lymphocytic choriomeningitis infections in mice provided some of the evidence that led Burnet to postulate the existence of immunologic tolerance. In mice congenitally infected with this virus, antibody is not demonstrable in the serum, but during the last few years it has been shown that complexes of antibody and virus-specific antigen accumulate in the kidneys, giving rise to an immunopathological glomerulonephritis. Similar observations have been made with murine leukemogenic viruses. Thus the animals are not completely unresponsive to viral antigens. Cooperative phenomena, perhaps of a hapten-carrier nature and possibly responsible for some autoimmune phenomena, will be discussed.

In the same monograph in which he described reactions to vaccination and rashes produced by virus infections as allergic reactions, von Pirquet (1907) drew attention to the depression of reactions to tuberculin which occur during measles infections, the first report of suppression of an immune response by a virus. Certain virus infections (as with LDV, measles virus, the thymic agent) can dramatically affect the immune system. In addition to having important clinical consequences, these infections should be considered when interpreting laboratory studies of immune responses. Intercurrent virus infections occur in most animal colonies (especially Sendai virus, minute virus of mice, mouse pneumonitis virus). Mouse hepatitis virus can behave much like an endogenous virus, being undetectable until animals are immunosuppressed, and is frequently found in nude mice. Most passaged tumors carry LDV and other viruses. The effects of some of these virus infections on immune responses will be discussed.

II. Viral Antigens

A. Introduction

Viruses vary considerably in their structural and antigenic complexity. In a few cases their structures are known and their antigens

well characterized and related to the virion structure, e.g., with adenovirus and influenza virus described below. With small viruses such as the enteroviruses, the antigenic composition is relatively simple. In each of the three types of poliovirus, for instance, two antigens are distinguishable by immunodiffusion and complement fixation (CF). Infectious virions containing RNA have a D (dense) antigen whereas noninfectious particles have a C (coreless) antigen (Hummeler *et al.*, 1962). The D antigen can be converted to C antigen by denaturation, so that the C antigen can be regarded as a protein that has not attained or retained the D configuration brought about in the complete virion by association with the RNA-containing core. A third antigen, the S antigen, can be obtained by guanidine degradation of the virus and is probably a protein precursor of the virus coat (Scharff and Lewinton, 1963). Humans infected with poliovirus develop type-specific antibodies against C and D antigens, but only the latter neutralize the virus. In the following sections, the structures and antigens of more complicated viruses—adenovirus, influenza virus, paramyxoviruses, togaviruses, rhabdoviruses, and the herpesviruses—will be described.

B. Herpesviruses

Herpesviruses form a family of large enveloped viruses of 1800–2000 Å size with genomes of double-stranded DNA having G + C contents ranging for different members from 33% to 72% (Plummer *et al.*, 1969). The herpesviruses naturally infecting man tend to produce latent infections and focal cytopathology and are divided into four groups: (1) herpes simplex (HSV), (2) cytomegaloviruses (CMV), (3) varicellazoster (V-Z), and (4) Epstein–Barr (EB) virus. All of these viruses multiply in the cell nucleus where the naked icosohedral nucleocapsids can be seen and acquire their envelopes when budding through the inner lamella of the nuclear membrane or during their egress through the membranes of the endoplasmic reticulum. Shortly after infection, host DNA and protein synthesis cease as that of the virus commences. Viral membrane protein synthesis begins before viral DNA synthesis, and glycosylation of the virus proteins occurs in the membrane and is accomplished by host enzymes. HSV virions contain at least 24 virus-specific proteins and, although earlier studies showed HSV could be agglutinated by antisera made against the uninfected host cell (Watson and Wildy, 1963), recent studies reveal essentially no host proteins in the virion (see review by Roizman and Heine, 1972).

Most viral structural proteins, including glycoproteins, are made during the first two hours of infection before the viral DNA replication begins. Many structural proteins for HSV and EB virus, and perhaps all herpesviruses, thus represent the products of "early function" genes, and this situation differs from that usually found with bacteriophages and the papova viruses, where structural proteins are coded for by "late" genes that are transcribed after viral DNA replication. The protein portions of the membrane glycoproteins are virus-coded (Olshevsky and Becker, 1972) and genetic variants of these proteins determine such membrane phenomena as the "social behavior" of HSV, i.e., whether infected cells fuse with contiguous cells or clump. Two types of HSV are recognized and evidence has been presented for a type-specific glycoprotein, present in infected cells and on the virion surface, that can interact with antibody and result in virus neutralization (Cohen *et al.*, 1972; Powell *et al.*, 1974).

C. Adenoviruses

The adenoviruses are DNA viruses having icosohedral symmetry. There are at least 33 human types (Blacklow *et al.*, 1969), and Rosen has divided them into 3 subgroups (see Table I) according to hemagglutination (HA) characteristics (Rosen, 1960). Norrby (1969a) and Schlesinger (1969) have reviewed the structure and biology of adenoviruses. About 18% of the virus protein is associated with DNA in a central nucleoid (Laver *et al.*, 1967) which is surrounded by an outer protein coat, the capsid. The capsid contains 252 polygonal capsomeres—240 hexons forming triangular facets and 12 apices made up of pentons (Figs. 1 and 2). Each penton consists of a vertex

TABLE I

Classification of Adenoviruses

Subgroup	Serotypes	Hemagglutination	
		Rhesus	Rat
I	3,7,11,14,16,20,21,25,28	+	0
II	8,9,10,13,15,17,19,22,23,24,26,27,29,30	±	+
III	1,2,4,5,6,12,18	0	±[a]

[a] Partial agglutination, enhanced by heterotypic immune sera.

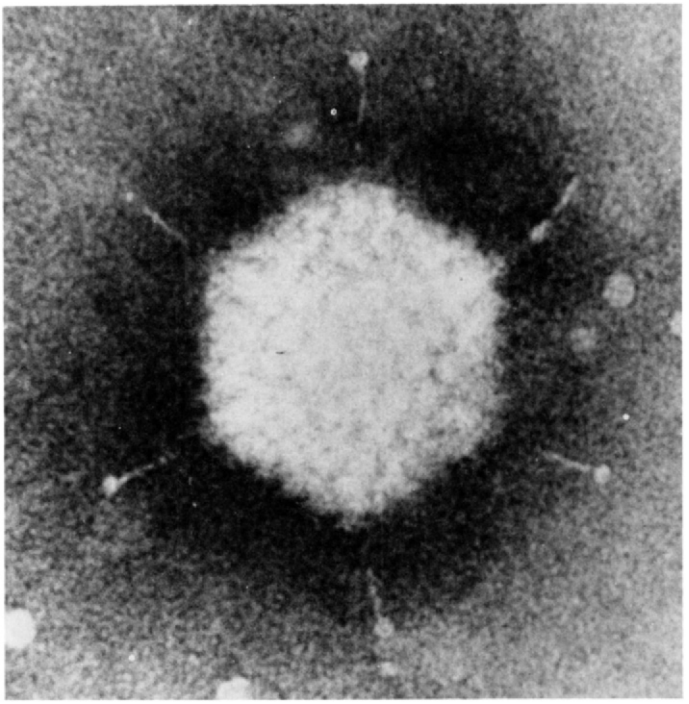

Fig. 1. Electron micrograph of adenovirus type 5, negatively stained. ×500,000. Courtesy of the late R. Valentine.

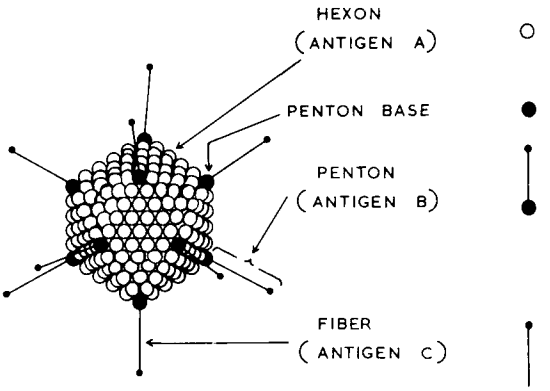

Fig. 2. Diagram of the structure of adenovirus showing the position in the virion of various components. Courtesy of H. Pereira.

capsomere (penton base) and an attached knobbed fiber. Hexons and pentons can be isolated from soluble products of infected cells or after disruption of virions. Originally, three antigens were described: a complement-fixing hexon group antigen (A antigen) common to all adenoviruses except the avian GAL virus, a penton group antigen (B antigen) toxic to cells, and a type-specific fiber antigen (C antigen).

With the awareness that antigenic sites on the intact virion may differ from some of those determined by immunization with spontaneously occurring soluble components, and that there are subpopulations of hexons of particular serotypes (Wadell, 1972), detailed analyses of adenovirus antigenic structure have been carried out using purified capsid subunits and specific antisera in absorption and HA studies. The subunits have varying capacities for HA: hexons and vertex capsomeres show no such capacity whereas all pentons and the fibers of subgroups II and III show partial HA. The latter is due to the univalency of these particles since dimers of pentons and fibers (at least in the case of subgroup III) show complete HA. Heterotypic sera, which can react with group or subgroup antigens on the fibers or vertex capsomeres can enhance HA by aggregating fibers or pentons into polyvalent complexes; this is termed the hemagglutination enhancement (HE) test. Vertex capsomeres can be identified by their ability to absorb HE antibody and thus decrease the HA normally found when a standard preparation of pentons is subsequently added; this is termed the HE antibody consumption (HEC) test.

Hexons appear to contain several antigens. The α (A) antigen is group-specific and detected in CF tests. The hexon α antigen appears to be located on the inner aspect of the capsid since antibody to it lacks HI activity and cannot be seen on ultrastructural examination (Norrby *et al.*, 1969); moreover, processes that disrupt the virion result in increased CF activity (Smith, 1965). The presence of a type-specific antigen, ϵ, was suggested by the surprising finding that only anti-hexon sera contain neutralizing antibody (Wilcox and Ginsberg, 1963; Kjellen and Pereira, 1968). Antibody to α antigen can be absorbed with heterotypic virus or hexons, leaving homotypic (anti-ϵ) antibody which is active in hemagglutination inhibition and neutralization assays. Using negative staining, this antibody has been shown to attach to the outer surface of hexons in intact virions (Norrby *et al.*, 1969). Agglutination of virions was prominent and might account for the HI activity of this antibody. Another possible mechanism for this HI activity is steric hindrance of the fiber hemagglutinin by antibody attached to paravertex hexons; this latter mechanism is supported by

the finding that the HI activity of different anti-hexon sera was inversely related to the fiber length of the viruses tested (Norrby and Wadell, 1969). Serotypes of subgroup III, with the exception of type 4 virus, differ from those of subgroups I and II in that hexons of the latter are considerably more efficient in the induction of neutralizing antibody. Antibodies to subgroup III hexons do attach to the hexons as shown by their ability to sensitize the virus to neutralization by antiglobulin antisera (Wadell, 1972). Absorption tests suggest that hexons possess minor intrasubgroup antigens as well as α and ϵ (Norrby and Wadell, 1969).

Haase and Pereira (1972) have recently demonstrated neutralizing capacity of antibody directed against the hexon ϵ antigen of adenovirus type 2. Crystallized hexons of adenovirus type 5 were coupled to Sepharose and an immunoadsorbent column prepared. Antisera to purified hexons of adenovirus type 2 were then passed over the column to adsorb group-specific (anti-α) antibody. The unbound material possessed neutralizing ability against type 2 virus but not type 5 virus. The subsequently eluted antibody neutralized neither virus. This elegant experiment demonstrates that the type-specific (ϵ) antigen is the crucial hexon antigen involved in neutralization.

Pentons can be separated into their fibers and vertex capsomeres by guanidine treatment. Vertex capsomeres have a group-specific β (B) antigen. Toxin activity is associated with these capsomeres and its neutralization is group-specific. Absorption experiments using HEC test suggested subgroup and some intersubgroup specificity (Wadell and Norrby, 1969). Recently, studies using antiglobulin to enhance neutralization of virions sensitized with antisera to vertex capsomeres confirmed this and indicated that vertex capsomeres in intact virions have subgroup specificities not found in the monomeric soluble subunits (Norrby and Wadell, 1972).

All fibers contain a type-specific γ (C) antigen which on ultrastructural examination is located at the distal (knobbed) end (Norrby *et al.*, 1969) and can participate in CF and HI reactions. Antibody to the fibers of all serotypes are able to sensitize the virus to neutralization by antiglobulin and perhaps to neutralize directly. Fibers of subgroups II and III also contain an intrasubgroup specific antigen, δ, located proximally; it cannot react with antibody if the vertex capsomere is attached. It is this antigen which is active in HE tests. Some intersubgroup specificity has also been detected (Wadell and Norrby, 1969).

Wigand and Fliedner (1968) observed that some adenovirus strains react differently in HI and neutralization tests, suggesting they might

be intermediate strains. The two type-specific antigens, γ and ϵ, can be differentiated in HI tests using soluble and virion-associated hemagglutinin, respectively. Such analyses have been reported for two strains, and the HI antigen specificity was related to the fibers and the neutralization specificity to the hexons (Norrby, 1969b). The latter relationship was not complete, suggesting mutational changes in the hexon after a previous recombination between prototypes. Of practical importance, infection with such mosaic viruses might result in HI tests indicating one prototype but neutralizing antibody and protection would be against another type.

D. Orthomyxoviruses

The structures of influenza virus and the related fowl plague virus have been reviewed recently (Laver, 1973; Schulze, 1973). Orthomyxoviruses consist of a helical nucleocapsid which contains the nucleic acid (RNA), surrounded by a pleiomorphic lipid envelope with projecting spikes of glycoprotein (Figs. 3 and 4). The nucleocapsid of influenza virus is unique among myxoviruses in that it is fragmented into at least three nucleoprotein pieces which contain segments of the viral RNA. During virus infection a nonglycosylated viral protein, termed the "membrane protein" or M protein, aligns itself under the plasma membrane and viral proteins accumulate in the cell membrane above it to the exclusion of host proteins. These viral proteins thus form discrete domains on the cell membrane before budding of the virus occurs (see review by Choppin *et al.*, 1972).

Influenza virions are 900–1100 Å in diameter and covered with two types of projecting spikes arranged in hexagonal fashion. After disruption of virus particles with lipid solvents, these spikes can be separated and the subunits of hemagglutinin and neuraminidase isolated. The hemagglutinin spikes (Fig. 5) are 40 by 140 Å rods which consist of trimers of a glycoprotein with MW 75,000–80,000 daltons. This molecule can under reducing conditions be dissociated to yield two distinct glycoproteins (HA_1 and HA_2) with molecular weights varying with the virus strain. Hemagglutinin appears to be synthesized as a single protein molecule associated with endoplasmic reticulum membranes where it is rapidly glycosylated (Taylor *et al.*, 1969; Lazarowitz *et al.*, 1971; Stanley and Haslam, 1971; Compans, 1973). Posttranslational cleavage of hemagglutinin differs for various virus strains, is host-dependent, and occurs in the plasma membrane (Lazarowitz *et al.*, 1971, 1973; Klenk *et al.*, 1972b;

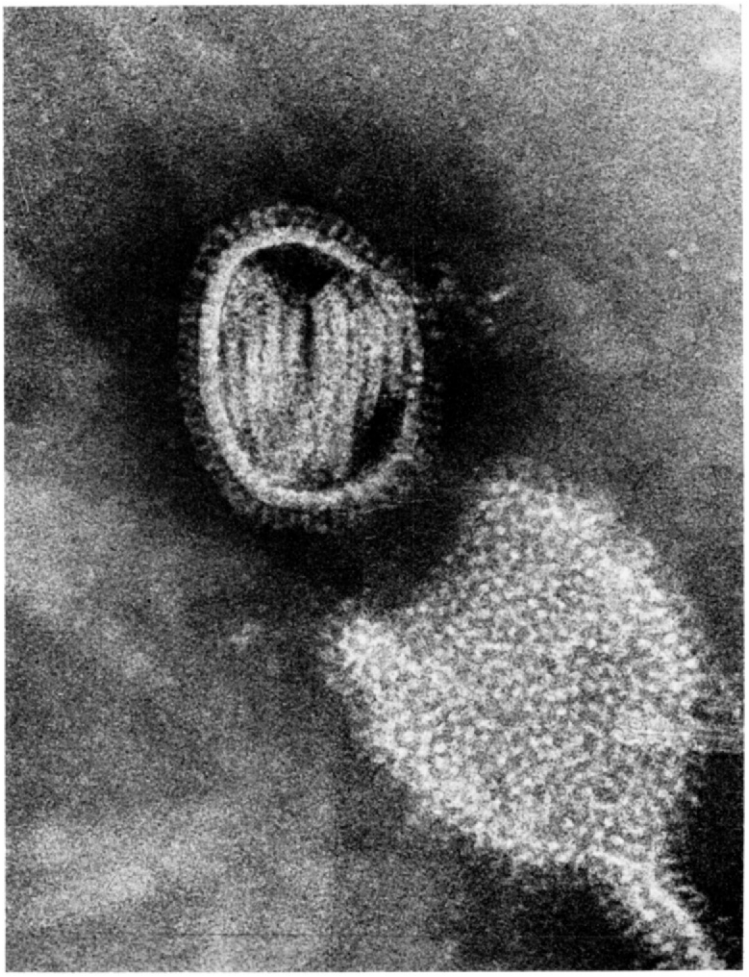

Fig. 3. Morphology of purified influenza A_2/Singapore virus particles negatively stained, showing spikes, membrane, and ribonucleoprotein. ×240,000. Courtesy of M. Nermut.

Skehel, 1972; Compans, 1973). Cleavage does not appear to affect virus infectivity or hemagglutinin titer (Stanley *et al.*, 1973). The neuraminidase spikes, shorter and distinct from the hemagglutinin spikes, consist of tetramers of subunit glycoproteins having MW 55,000–70,000 daltons (Wrigley *et al.*, 1973). The subunits of neuraminidase of some strains may consist of two polypeptides.

In contrast to the virion proteins, almost all of which are coded by

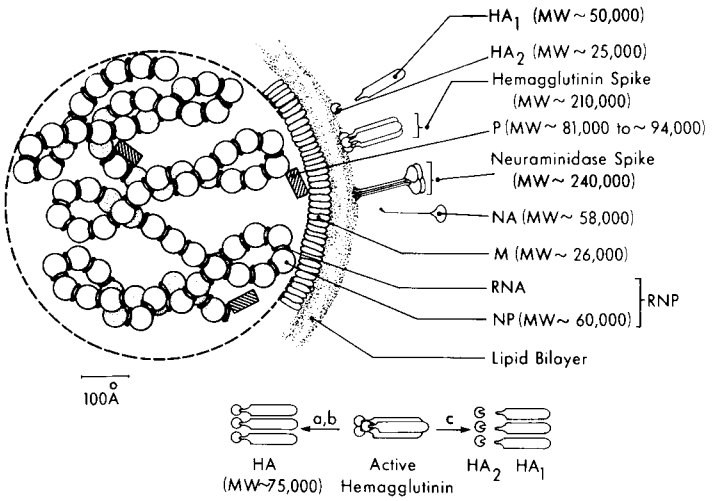

Fig. 4. Proposed model for influenza virus particle. From Schulze (1973).

the virus (Holland and Kiehn, 1970), the lipids (Kates *et al.*, 1961) and carbohydrates (Howe *et al.*, 1967) of the virion are host specific and the latter can alter virus antigenicity. The serologic specificities of protein-bound and lipid-bound carbohydrates are different. Treatment of virus with the proteolytic enzyme bromelin removes the spikes, leaving noninfectious, smooth-surfaced particles containing all of the virion lipid (Fig. 6) (Compans *et al.*, 1970). Concanavalin A, a phytagglutinin from jack beans, was found to react only with intact virions whereas the phytagglutinin from *Dolichos biflorus*, which reacts with the terminal sugar of blood group A (*N*-acetylgalactosamine), reacted only with the spikeless particles, the glycolipid moieties of which were exposed by bromelin treatment (Klenk *et al.*, 1972a). Likewise, antibody to blood group A could be absorbed with the spikeless particles but not with intact virions.

Early reports, using viral preparations probably contaminated with host cell membrane fragments, indicated that blood group antigens A and B and Forssman antigen could be detected as surface antigens on the virions if grown in cells possessing these antigens (Springer and Schuster, 1964). However, recent studies indicate that with progressive purification of the virus, Forssman antigen (a glycolipid) cannot be detected on the virion surface (Haheim and Haukenes, 1973a), but is found in the viral lipids and is accessible to antibody only after removal of the virion spikes with bromelin (Haheim and Haukenes, 1973b). A sulfated glycopeptide antigen found in the

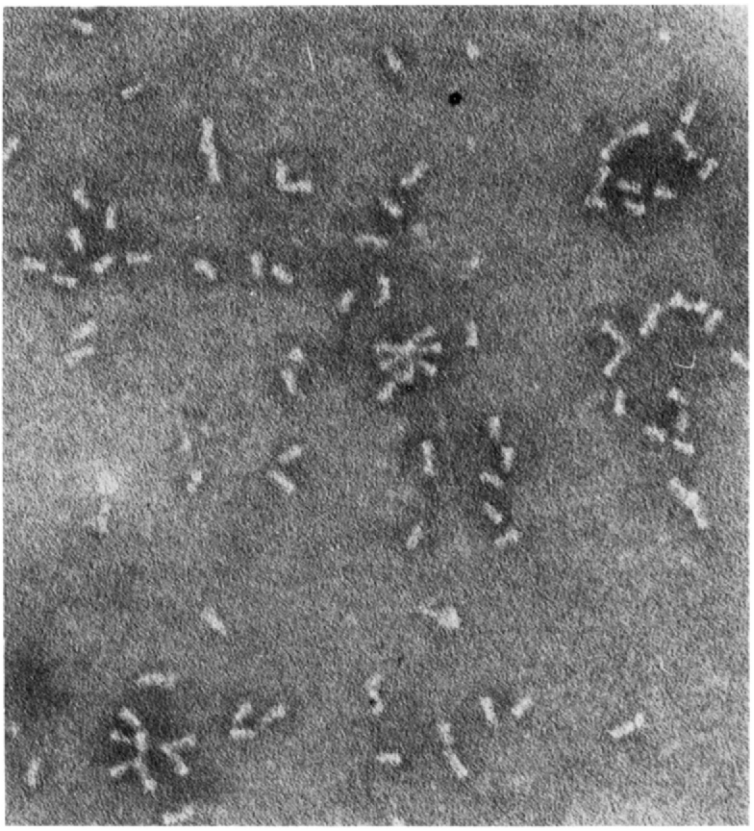

Fig. 5. Electron micrograph of negatively stained influenza virus hemagglutinin. ×250,000. Courtesy of J. Heather.

chick allantoic cavity (Haukenes *et al.*, 1966; Howe *et al.*, 1967; Lee *et al.*, 1969) and found in liver and bile of adult chickens (Harboe and Haukenes, 1966) is acquired by virus growing in the endodermal cells lining the allantoic cavity (Harboe *et al.*, 1966). Recent studies reveal this host antigen to be associated with spikes of both hemagglutinin and neuraminidase (Haheim and Haukenes, 1973b) and antibody to this host antigen reacts with the carbohydrate moieties rather than the peptide backbone of the molecule (Higginbotham *et al.*, 1971).

The virus genome determines four major antigens: hemagglutinin, neuraminidase, the membrane or M protein, and nucleoprotein. Differences in the nucleoprotein, termed the "S" (soluble) antigen,

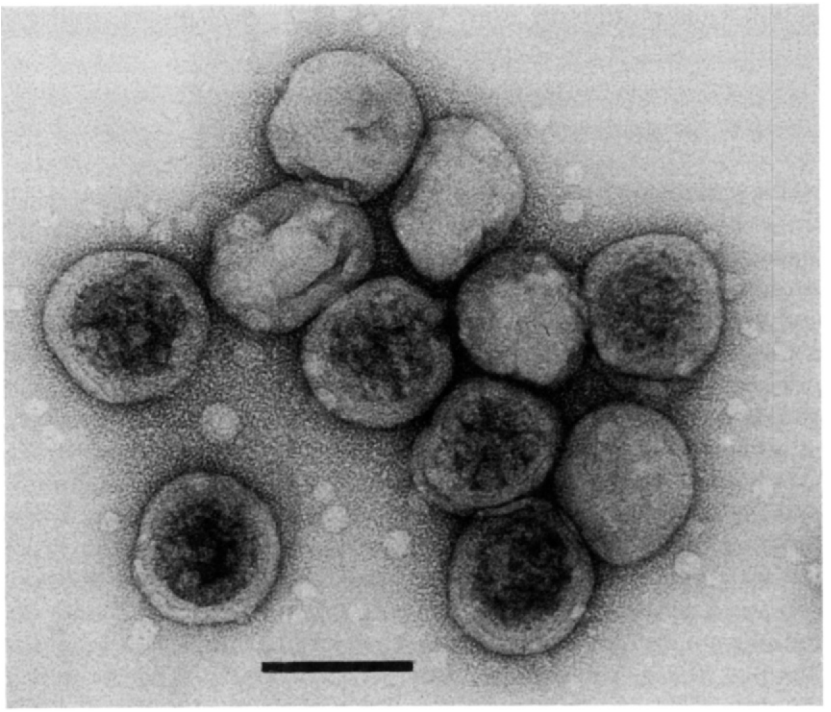

Fig. 6. Influenza A_0/WSN virions from which all glycoproteins have been removed by proteolytic treatment. Particles were negatively stained with uranyl acetate after glutaraldehyde fixation. Morphology suggestive of a double-layered wall and an internal strand is seen in some particles. ×210,000; marker = 1000 Å. From Schulze (1973).

divide influenza into three major types (A, B, and C). Although antibodies to this internal antigen and to the M protein fix complement, they have no neutralizing or hemagglutinating-inhibition (HI) activities and are probably unimportant in immunity. The hemagglutinin or "V" antigen determines strain specificity and reacts with neutralizing, CF, and HI antibody. There is great genetic stability of the internal antigens while the V antigen, exposed to immunologic selection, undergoes frequent minor changes that result in influenza epidemics. Major antigenic shifts in hemagglutinin that result in pandemics are the result of extensive changes in amino acid sequences in HA_1 and HA_2 as revealed by tryptic peptide analyses, and may involve recombination of preexisting human strains with animal strains (Laver and Webster, 1973). Neuraminidase, associated with glyco-

protein spikes different from the hemagglutinin spikes, undergoes antigenic changes independently of the V antigen.

E. Paramyxoviruses

The paramyxovirus genus includes a number of common viruses which have similarities to the orthomyxoviruses as well as some interesting differences. As is true for orthomyxoviruses, host factors determine the composition of the virion lipid coat and the glycosylation of protruding protein spikes. Host glycolipid antigens (e.g., blood group antigens) may be incorporated into the virion (Isacson and Koch, 1965). However, nucleocapsids are composed of a single protein species and nonsegmented RNA, and viral nucleic acid replication occurs in the cytoplasm rather than in the nucleus.

Within the genus there are at least three distinct virus groups. The parainfluenza viruses—including Sendai virus, simian virus 5 (SV5), mumps virus, and Newcastle disease virus (NDV)—have considerable antigenic cross-reactivity and possess hemagglutinin, neuraminidase, and hemolytic and cell-fusion activities. The HA and neuraminidase activities of SV5 (Scheid *et al.*, 1972), NDV (Scheid and Choppin, 1973), and Sendai virus (Tozawa *et al.*, 1973; Scheid and Choppin, 1974) reside in a single glycoprotein of MW 65,000–70,000. The hemolytic and cell-fusion activities of Sendai virus have long been thought to be associated with virus protein and recent studies have confirmed this. Hosaka and Shimiza (1972) showed that isolated virus glycoprotein and virus membrane lipids were inactive separately but reconstitution of hemolytic and cell-fusion activities occurred when they were recombined. Scheid and Choppin (1974) recently demonstrated a Sendai virus glycoprotein (MW 53,000), derived from a larger precursor by natural proteolytic activity *in vivo* or by trypsinization *in vitro*, which is responsible for the hemolytic and cell-fusion activities of the virion and also influences its infectivity. Antibodies to both the hemagglutinin/neuraminidase glycoprotein and to the fusion/hemolytic glycoprotein are probably important in virus neutralization, while antibodies to the nucleocapsid protein or to the internal "M" protein are not.

The measles-rinderpest-canine distemper group of paramyxoviruses are antigenically closely related to one another and lack neuraminidase. Recent studies indicate that the measles virion contains six polypeptides, two being glycoproteins that form the projecting spikes (Hall and Martin, 1973, 1974). Norrby and co-workers have correlated certain serologic tests with structural components of

measles virus (Norrby and Hammarskjold, 1972). Antibodies to nucleocapsids fix complement and antibodies that inhibit hemagglutination (HI) or hemolysis (HLI) must react with surface components. The finding that HI antibodies can block hemolysis while HLI antibodies have only slight HI activity suggests that the hemolysin represents a separate entity in the virus envelope. Following natural measles infections nucleocapsid CF antibodies predominate and neutralizing antibodies correlate better with HLI than with HI antibodies (Norrby and Gollmar, 1972). In subacute sclerosing panencephalitis (SSPE), oligoclonal IgG antibodies to the various virus components were found, especially in the CSF (Vandvik and Norrby, 1973). In one case, a population of antibodies with HLI and neutralizing but no HI activities was identified. Patients with multiple sclerosis have increased amounts of antibodies to all measles virion components in sera and CSF (Link *et al.*, 1973; Salmi *et al.*, 1973; Norrby *et al.*, 1974). The pathogenetic importance of these findings in SSPE and multiple sclerosis is uncertain.

Respiratory syncitial virus has been classed temporarily as a paramyxovirus. Although it possesses envelope spikes, it lacks hemagglutinin, neuraminidase, and hemolytic and cell-fusion activities.

F. Togaviruses

This large family of viruses includes what were formerly known as group A arboviruses (now alphaviruses) and group B arboviruses (now flaviviruses after its best known member, yellow fever virus), plus a small number of unclassified but structurally similar viruses, e.g., rubella virus and lactate dehydrogenase virus. There are about 20 alphaviruses and 40 flaviviruses grouped on the basis of cross-reactivity in HI tests (Casals, 1957). Complement-fixation, neutralization, and cross-protection tests are more specific but show some cross-reactions within each group. The ungrouped togaviruses are not serologically related.

Togaviruses consist of single-stranded RNA enclosed in a coat of core protein rich in lysine and arginine to form a nucleocapsid. A distinctive lipid bilayer containing some carbohydrate is applied to the nucleocapsid much as the M protein in influenza virions (Fig. 7). The peplomers, composed of one or two protein species and rich in hydrophobic amino acids to facilitate attachment, project outward from the lipid coat. The lipid and carbohydrate compositions of the virion are host-determined (Pfefferkorn and Hunter, 1963; Strauss *et al.*, 1970; Renkonen *et al.*, 1971). The peplomers can be removed

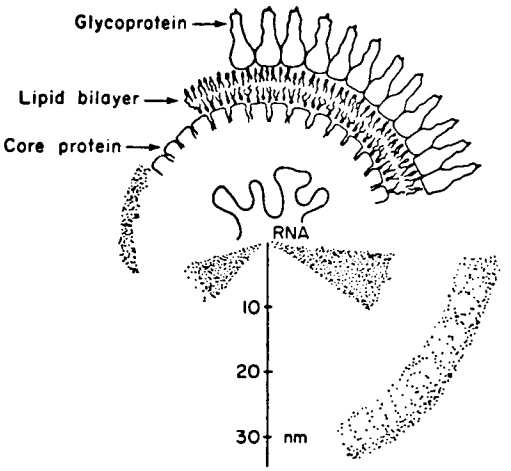

Fig. 7. Proposed structure of Sindbis virus, an alphavirus, showing the elongated glycoprotein peplomers attached to the lipid bilayer membrane and the core protein and RNA. From Harrison *et al.* (1971).

with proteases leaving the unchanged lipid-coated nucleocapsids (Compans, 1971). Treatment of virions with detergents yields nucleocapsids free of the lipid-glycoprotein envelope (Strauss *et al.*, 1968). Purified peplomer glycoproteins capable of eliciting neutralizing antibodies and of blocking the neutralizing activity of convalescent sera have been isolated from Chikungunya virus (Igarashi *et al.*, 1970, 1971), Semliki Forest virus (Appleyard *et al.*, 1970; Kennedy, 1974), Sindbis virus (Faulkner and Dobos, 1968; Bose and Sagik, 1970), and Venezuelan equine encephalitis virus (Pedersen *et al.*, 1973). Two distinct envelope glycoproteins isolated from Semliki Forest virus possess a common antigenic determinant (Kennedy, 1974). Excision of the carbohydrate moieties with various sugar hydrolases did not remove the antigen, which must therefore be part of the polypeptides.

G. Rhabdoviruses

Rhabdoviruses, of which vesicular stomatitis virus (VSV) and rabies virus have been particularly well studied, are bullet-shaped enveloped RNA viruses about 1700 Å long and 700 Å in diameter (Fig. 8). The single-stranded RNA of MW 4×10^6 daltons is associated with approximately 1000 capsomers of the "N" (nucleo-

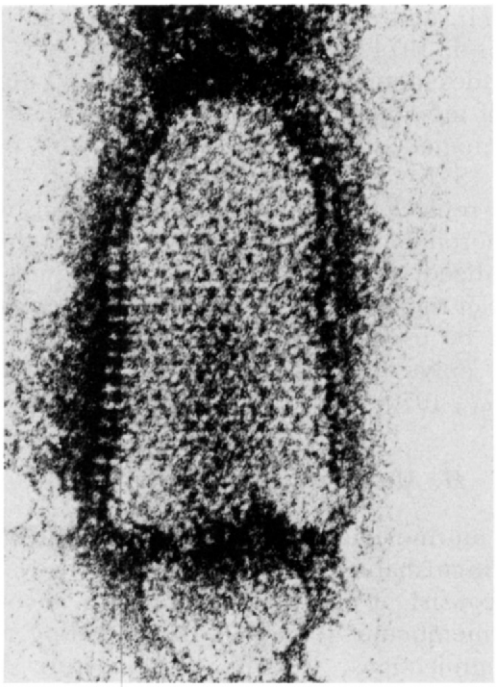

Fig. 8. Morphology of negatively stained rabies virus. On the left are well-resolved surface projections (peplomers) 60–70 Å long. ×300,000. From Hummeler *et al.* (1967).

capsid) protein and arranged in a helix of 34 turns; two other proteins, "NS" and "L," are associated with the nucleocapsid. As with influenza virus, a nonglycosylated membrane or "M" protein is closely applied to the nucleocapsid, and this protein coat is in turn surrounded by a host-derived lipid coat. The glycolipid of the virion is host-determined and thus may possess antigens of the host cell. Antisera against uninfected cells will react with highly purified virus grown in those cells, and in one study the antigen appeared to be a host hematoside incorporated into the glycolipid portion of the virion (Cartwright and Brown, 1972).

Glycoprotein peplomers (the "G" protein) of MW 69,000 daltons protrude from this lipid coat and can be removed selectively by proteases; it is the hemagglutinin and neutralizing antigen of the virion (Cartwright *et al.*, 1970; McSharry *et al.*, 1971; Kelley *et al.*, 1972). The carbohydrate portion of this glycoprotein is largely host-specified (Burge and Huang, 1970; Burge and Strauss, 1970; Grimes

and Burge, 1971), and may reflect the antigenicity of the host cell membrane (Ansel, 1974). The virus glycoprotein is easily purified and thus provides a tool with which to study alterations in the glycosylation of membrane proteins induced by cell transformation (Moyer and Summers, 1974). In mixed infections of VSV and the paramyxovirus SV5, phenotypic mixing occurs and the SV5 peplomers may replace those of VSV on VSV virions without altering their overall morphology (McSharry *et al.*, 1971); these virions can then be neutralized with antibody to SV5. It is not known if host glycoproteins can replace virus glycoprotein as peplomers. Nonionic detergents can be used sequentially to dissect the VSV or rabies virions until only the infectious ribonucleoprotein remains (Cartwright *et al.*, 1970).

H. Murine Leukemogenic Viruses

The various murine leukemogenic viruses (MuLV) are morphologically indistinguishable from one another. They are 1200 Å in diameter and consist of a nucleoid core with an outer shell surrounded by a membrane derived during budding and possessing small surface projections. Initially, rats bearing MuLV-induced tumors were found to produce CF and precipitating antibodies against species or group-specific (gs) antigen. The first such antigen defined was named gs-1, and another shown to be an interspecies antigen was named gs-3. Both these antigens, as well as type-specific antigens for strains of MuLV from the same species, reside on the same protein, p30. This protein (MW 30,000 daltons) comprises 30% of the total virion protein and is an internal protein since neutralizing antibodies do not react with it. The gs-1 antigen is widely distributed in reticular tissues of normal mice but does not always correlate with the presence of infectious MuLV.

Virus neutralization tests detect virus envelope antigens (VEA's). Two closely related envelope glycopeptides, gp69/71, have molecular weights of 69,000 and 71,000 daltons and contain prominent group-specific (common to many or all MuLV) and type-specific (limited to a particular virus) antigens, and less easily detected interspecies determinants. Neutralization studies have led preliminarily to the classification of laboratory MuLV isolates into 5 categories (Table II). High incidence leukemia mouse strains (AKR, C58) produce MuLV serologically identical to Gross virus (with GVEA) (Hartley *et al.*, 1969).

TABLE II

Classification of Murine Leukemogenic Viruses[a]

Subgroup	Type	Strains
1		Gross (radiation leukemia)
2	a	Friend, Graffi, Tennant, Rowson-Parr, SimLV
	b	Moloney, Abelson
	c	Rauscher, Rich, Breyere-Moloney
	d	Buffett (334C)

[a] From Lilly and Steeves, 1974.

Cell surface antigens (CSA's) of MuLV-infected cells were first defined in tumor transplantation studies. Animals rejecting such tumor grafts possess specifically cytotoxic antibodies which can (in the presence of complement) lyse tumors induced by the same virus. Most antibodies to the CSA's are not neutralizing antibodies since adsorption of virus-neutralizing activity with purified virus leaves the cytotoxic activity of the serum undiminished (Pasternak, 1967; Steeves, 1968). Also, immunoelectron microscopy studies demonstrate VEA's and CSA's in topographically separate regions of the cell surface (Aoki *et al.*, 1970). Cross-reactive patterns with cytotoxic antibodies define two major subgroups on the basis of CSA's: Gross and Friend–Moloney–Rauscher (FMR). Type-specific CSA determinants have not been analyzed. The Gross CSA is found on many normal and preleukemic cells of high-leukemic mouse strains (AKR). In contrast, the FMR determinant is not found on normal cells. The FMR antigen appears to be an internal virion protein that differs from p30 (Friedman *et al.*, 1974). Although they are not the principal CSA's of MuLV-infected cells, both p30 and gp69/71 are found on the surface of infected cells (Ikeda *et al.*, 1974; Yoshiki *et al.*, 1974a). Expression of the various genes coding for structural proteins of MuLV is regulated by many genes (Lilly and Pincus, 1973; Rowe, 1973). Multiple copies of some genes coding for virus proteins may be present in each cell and not coordinately linked (Strand *et al.*, 1974).

Reverse transcriptase in the core of MuLV virions carries interspecies and species-specific determinants (Parks *et al.*, 1972). The interspecies antigen of MuLV reverse transcriptase cross-reacts with that of other small mammals but not with that of primates or chickens.

III. Humoral Responses

A. Sequence of IgM and IgG Responses

The sequence of IgM and IgG responses to viral antigens is usually like that observed with nonviral antigens. Complicating factors in the humoral responses to viruses include a persisting antigenic load while infection continues, cell and tissue destruction by the virus, and influences of the virus on the immune system.

The responses of rabbits to nonreplicating viral antigens have been studied in detail with poliomyelitis type 1 (PV) (Svehag and Mandel, 1964a,b; Svehag, 1964a,b) and influenza virus (Webster, 1965, 1968a,b). In both cases with adequate antigen dose after a short inductive phase (8–12 hours in the case of PV) IgM antibodies appeared, followed later by IgG antibodies (Fig. 9). Small doses of PV produced only a transient IgM response, which after 4 days declined at a rate consistent with the metabolic decay rate of this immunoglobulin. Larger doses of PV elicited both IgM and IgG antibody responses; IgM synthesis continued for 10–14 days, whereas IgG was first detected on the third day and increased until the third week. Thereafter IgG levels remained constant for about 30 weeks and persisted at moderate titers for 2 years. This was not due to chronic infection since poliovirus does not grow in rabbit tissue and UV-irradiated virus gave the same result. Similar findings with antibodies against the bacteriophage ϕX174 in guinea pigs had been reported by Uhr and Finkelstein (1963), although the rate of an-

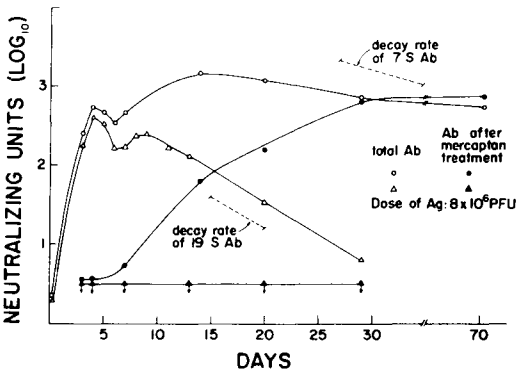

Fig. 9. Production of mercaptoethanol-sensitive (19 S) and mercaptoethanol-resistant (7 S) antibody in rabbits immunized with a large dose of poliovirus. From Svehag and Mandel (1964b).

tibody formation against the bacteriophage was less antigen-dependent than in the case of PV.

Interesting information on immunologic memory has come from the experiments with PV. With low doses of virus eliciting only IgM responses there was no detectable memory; repeated small doses of PV at monthly intervals elicited only transient and identical IgM responses (Fig. 10). However, if a second small dose of PV was administered 6 days after the first (2 days after the 19 S antibody had begun to decline), a large secondary response consisting exclusively of 19 S antibody occurred and the titer rose 100-fold. Again, this secondary response began the day after inoculation of PV and persisted for only 4 days. These results suggest that an immunologic memory of IgM antibody production does exist but is very short-lived. The early and large secondary response shows that immunocompetent cells in the primary response do not die after 4 days of antibody release but require further antigenic stimulation for continued antibody production. Similar results have been obtained with the ϕX–guinea pig system.

Studies of natural and experimental poliovirus infections of humans (using attenuated virus) have largely confirmed the findings

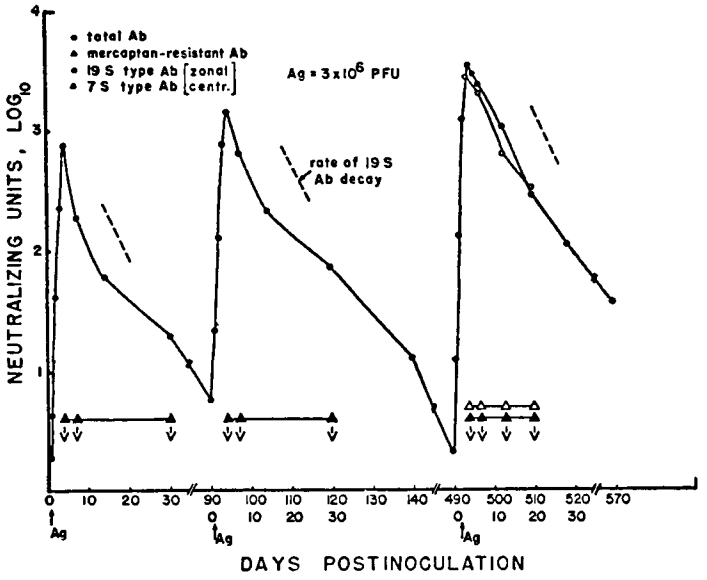

Fig. 10. Production of antibody in rabbits after repeated immunizations with small doses of poliovirus. Only 19 S antibody was produced and the responses were transient and almost identical. From Svehag and Mandel (1964b).

in rabbits previously discussed. The level of neutralizing IgM antibody rises rapidly after natural infection, reaches maximum titers in 3–4 weeks, and declines to undetectable levels by 3 months (Svehag and Mandel, 1964b; Ogra *et al.*, 1968). IgG titers rise over a prolonged period and may not attain peak levels until after 3 months. IgA antibody is not detectable until 4–6 weeks after infection and rises for at least the ensuing 8 weeks. The early neutralizing antibody is of low avidity (Sabin, 1957). Although CF antibody (against D and C antigens) attains maximum levels after about 2 months and persists for 1–5 years, neutralizing antibody decreases to one-fourth its peak titer by 2 years and persists near that level for decades.

Ogra *et al.* (1968) have compared the humoral responses to live and inactivated vaccines in infants (see Figs. 11 and 12). Beginning at 2 months of age, 3 monthly doses of trivalent inactivated virus (subcutaneously), or live virus type 1 and types 2 and 3 on successive months (orally) were administered; a booster was given at 12 months of age. Poliovirus-binding antibody titers were determined by radioimmunodiffusion and neutralizing antibody assays for type 1 virus were performed on sera and secretions. Maternally derived IgG an-

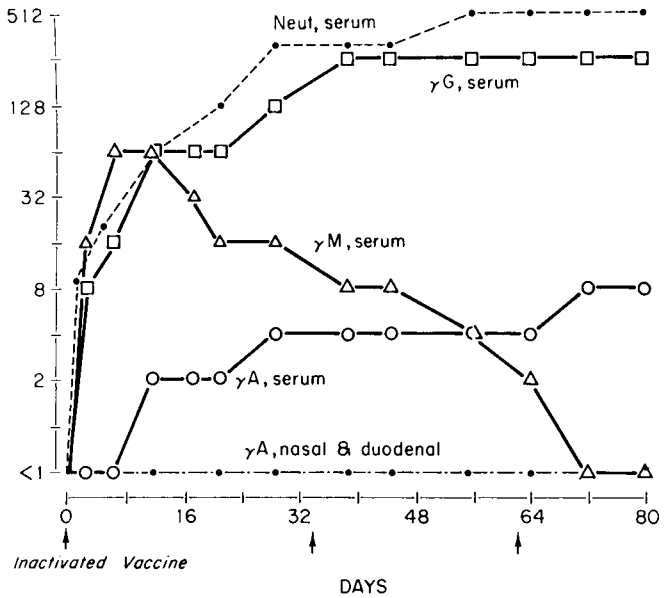

Fig. 11. Production of IgM, IgG, and IgA antibody against poliovirus type 1 in serum and secretions after immunization with three doses of trivalent inactivated virus at monthly intervals, beginning at 2 months of age. From Ogra *et al.* (1968).

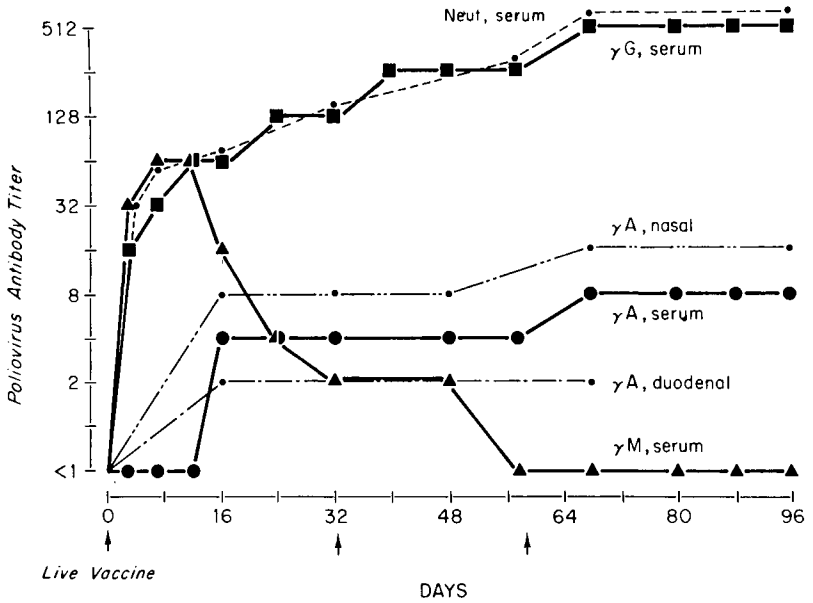

Fig. 12. Production of IgM, IgG, and IgA antibody against poliovirus type 1 in serum and secretions after immunization with live attenuated poliovirus types 1, 2, and 3, given sequentially at monthly intervals, beginning at 2 months of age. From Ogra *et al.* (1968).

tibody was detected in low titers in one-fourth of the infants aged 2 months; no sera contained IgM. IgM and IgG were detected 3 days after the first vaccine dose. The IgM titers rose more rapidly than those of IgG and became maximal during the second week before declining to undetectable levels by 8–10 weeks. IgM declined more slowly in recipients of inactivated virus as they had repeated antigenic stimulation (3 doses). IgG titers in both groups continued to rise for 7–10 weeks before leveling off. Serum IgA titers were not detected until 2 weeks after initial immunization; they rose slowly over a 3-month period. After the booster at 12 months, IgM titers showed a transient rise to previous levels, thus duplicating the findings in rabbits (Fig. 13). Recipients of live virus had neutralizing IgA in their nasal and duodenal secretions after 16–21 days, which persisted for at least 90 days (duodenal) and 300 days (nasal); recipients of inactivated virus had no secreted IgA antibody to poliovirus.

Studies of guinea pigs infected with arboviruses (Bellanti *et al.*, 1965) and of humans infected with mumps virus (Brown *et al.*, 1970; Daugharty *et al.*, 1973), Coxsackie virus (Schmidt *et al.*, 1968), influ-

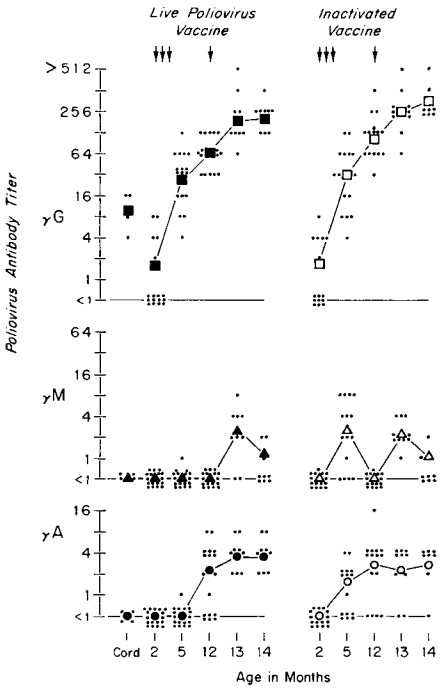

Fig. 13. Serum poliovirus type 1 immunoglobulin responses after immunization with live attenuated or inactivated virus; individual and geometric mean titers are shown. Note the IgM responses after booster immunization. From Ogra *et al.* (1968).

enza virus (Brown and O'Leary, 1971), or inoculated with soluble adenovirus antigens (Lehrich *et al.*, 1966) have demonstrated IgM and IgG patterns similar to those following poliovirus infection and suggest that this pattern of antibody response is a general phenomenon. The IgM response following natural influenza infection or immunization appears to be unique in one study in that the IgM was exclusively of a 7 S form (Brown and O'Leary, 1973).

B. Elicitation of Specific Immunoglobulin Classes

Particular antigens of a virus appear to be capable of eliciting a response of a specific class of immunoglobulin, as do nonviral antigens (Schirrmacher and Rajewsky, 1970). Cowan (1970) and Brown and Smale (1970) have shown that IgM and IgG produced in guinea pigs infected with foot-and-mouth disease virus are specific for distinctive antigenic determinants on the virus particle, and Miyamoto *et al.* (1971) have described differential reactions of 7 S and 19 S an-

tibodies with HSV antigens in infected cells. Most patients with warts produce only IgM antibodies to the human wart virus (Goffe *et al.*, 1966; Pyrhonen and Penttinen, 1972). Recently, Lee *et al.* (1974) reported that normal $B6C3F_1$ mouse sera contains 19 S antibodies that react with antigenic determinants on 3 structural proteins of MuLV while antibodies in the 7 S fraction react with only one of these proteins, p15.

Several explanations can be offered for the preferential elicitation of particular immunoglobulin classes by these viral antigens. A trivial explanation for not finding IgM against a particular antigen is that the IgM response is transient unless there is continued antigenic stimulation. An exclusively IgM response may occur with small immunogenic stimuli, as discussed above. Thus, wart virus, growing only in the epidermis, does not have ready access to the bloodstream or lymphatics to immunize the host. This has led to the suggestion that susceptibility to recurrent attacks of warts may be related to failure of very small doses of antigen to promote an IgG response, the presence of the latter being a good indicator of healing (Pyrhonen and Penttinen, 1972).

C. Genetic Control of Antiviral Antibody Responses

The genetic control of immune responses to certain antigens has been demonstrated in guinea pigs and mice and appears to be under the control of the Ir genes in the H-2 complex (see Benacerraf and McDevitt, 1972; McDevitt and Landy, 1973). Nonresponders produce only IgM antibody to the specific antigen (Grumet, 1972) unless certain conditions such as an allogeneic stimulus are imposed (Ordal and Grumet, 1972). Similar situations with viral antigens may also occur, but have not yet been described. However, an increased humoral response to measles virus has been associated with HL-A3 in man (Arnason *et al.*, 1974). An increased incidence of multiple sclerosis is associated with HL-A3 but there is no such association with optic neuritis, which often evolves clinically into multiple sclerosis. Also, patients with optic neuritis have normal levels of antibody to measles virus. The association of high titers of antibody to measles virus with multiple sclerosis may thus reflect the prevalence of high titers to the virus in people with the HL-A3 genotype.

D. Genetic Control of Virus-Induced Diseases

Many examples of genetically controlled resistance to infection within a species are known (Allison, 1965). Thus, several major

genes confer resistance to chickens and chick cells against infection by particular strains of avian leukemogenic viruses or Rous viruses coated by them. The control of murine leukemogenesis is multigenic, with different genes controlling viral expression (Akv-1 and Akv-2 genes), viral replication (Fv-1 gene), and resistance to Gross virus and virus-transformed cells (the Rgv-1 gene) (Lilly and Pincus, 1973; Rowe, 1973). The Rgv-1 gene is linked to H-2 and influences late disease patterns. It is only by inference, however, that its mechanism is thought to involve immune responses. The immunopathological disease caused by LCM virus infection of adult mice has been reported associated with H-2 (Oldstone *et al.*, 1973). This finding is not universally found in all laboratories (possibly because of virus strain differences), and awaits detailed immunologic analysis.

IV. Secretory Antibody Responses

Viruses infecting seromucous surfaces such as the respiratory or alimentary tracts must pass a first line of defense consisting of secretory antibody and inhibitory mucoprotein secretions. Francis (1942) first noted the presence of anti-influenzal antibody in human nasal secretions and for years it has been thought that virus infections of the mucous membranes could confer specific local immunity. It is now clear that the main antibody in seromucous secretions is IgA, although a compensatory increase in the levels of other immunoglobulins, especially IgM, occurs in the secretions of people with IgA deficiency (see Tomasi and Bienenstock, 1969). Thus, antibody in nasal secretions against rhinoviruses (Rossen *et al.*, 1966), parainfluenza 1 virus (Smith *et al.*, 1966), and influenza virus (Rossen *et al.*, 1966; Alford *et al.*, 1967) is predominantly IgA. However, the role of IgA antibodies in recovery from virus infections and prevention of reinfection is less clear.

Fazekas de St. Groth and Graham (1954) reported that the immunity of mice to reinfection with influenza virus is more closely correlated with the presence of antibody in nasal secretions than with antibody in serum. Several groups of investigators have reported that parenteral administration of inactivated viruses increases levels of serum IgG and IgM antibodies, but not levels of IgA antibodies in nasal secretions, whereas natural infection results in the formation of IgA secretory antibodies and better resistance to reinfection (Rossen *et al.*, 1966; Smith *et al.*, 1966; Alford *et al.*, 1967). This does not necessarily imply that IgA is responsible for the resistance to rein-

fection because the natural infection could have other effects, including local stimulation of T lymphocytes. Claims that local IgA production and protection follow aerosol administration of inactivated influenza virus (Waldman *et al.*, 1970) have not been confirmed in other laboratories (Shore *et al.*, 1973) and further work on the subject is required.

One of the arguments of Sabin (1959) in support of live poliovirus immunization is that it would stimulate local secretory antibody production, thereby limiting excretion of the virus and transmission in the population. Ogra (1973) has presented evidence that production of IgA antibody is a local reaction. Immunization of selected segments of the large intestine in subjects with double-barreled colostomies induced an IgA antibody response against poliovirus in the immunized segment with little or no response elsewhere. However, subcutaneous immunization with killed poliovirus has not only eliminated paralytic disease but also the transmission of the virus in the Swedish population, as shown by large scale examinations of feces of persons admitted to hospital for any reason (Perkins, 1974).

Deficiency of secretory IgA is not rare, occurring in about 1 in 500 persons in Western Europe and North America. Most such individuals remain healthy, although some have recurrent sinopulmonary and gastrointestinal infections (Schwartz and Buckley, 1971). Thus, in some individuals IgG and IgM antibodies appear able to substitute for IgA antibodies in secretions.

V. Thymus Dependence of Viral Antigens

After the exposure of animals to most antigens the formation of antibodies by cells of the B lymphocyte lineage requires helper effects of T lymphocytes (Miller *et al.*, 1971). This has given rise to the concept of the "thymus dependence" of antigens, some antigens being more dependent than others on T lymphocytes for a normal humoral response. Originally, thymus dependence was defined in terms of the amount of serum antibody formed to an antigen in a T lymphocyte-deprived animal. Later, it was found that IgM responses are less dependent on T lymphocytes than those of other immunoglobulin classes and that humoral responses to certain "thymus independent" antigens (pneumococcal polysaccharide type III) are regulated by T lymphocytes. "Thymus dependence" is thus a relative term and depends on animal species and strain as well as mode of antigen presentation. Generally, "thymus-independent" antigens

are large, slowly metabolized molecules with repeating antigenic determinants (epitopes). It might be expected that some viruses, many of which possess antigenic polyvalency inherent in their structures, could have some epitopes in the proper repeating spatial relationship to be immunogenically thymus-independent.

Until the recent availability of the athymic nude mouse, neonatal thymectomy or treatment with ALS was used to deplete animals of T lymphocytes and ascertain the thymus dependence of virus antigens. Although these methods of T lymphocyte depletion are not effective by present standards, the results obtained will be reviewed. Thus, neonatal thymectomy did not abolish the production of HI antibodies to polyoma virus in mice (Miller *et al.*, 1964; Mori *et al.*, 1966) or rats (Allison and Taylor, 1967), or to influenza virus (Svet-Moldavsky *et al.*, 1964) or Coxsackie B5 virus (cited in Mori *et al.*, 1967) in mice. Titers of complement-dependent neutralizing antibodies (probably IgM) to HSV in CF-1 mice neonatally thymectomized were similar to those of control mice (Mori *et al.*, 1970). The other method of T lymphocyte depletion, pretreatment of mice with ALS, did not diminish the production of HI antibodies to vaccinia virus (Hirsch *et al.*, 1968b), CF antibodies to LCM virus (Hirsch *et al.*, 1968a), or HI antibodies to influenza virus (Hirsch and Murphy, 1968).

In addition to the ineffective methods of T lymphocyte deprivation in these studies, the continuing antigen presentation that occurs in virus infections must be considered. Svet-Moldavsky *et al.* (1964) have demonstrated that the abrogation of antibody response to sheep erythrocytes observed in neonatally thymectomized mice can be overcome by repeated immunizations. Mori *et al.* (1970) reported that neonatally thymectomized mice inoculated with Japanese encephalitis virus had diminished HI titers which, however, rose to normal levels after repeated inoculations.

A few studies have suggested thymus dependence for some virus antigens. Thind and Price (1971) reported markedly depressed levels of neutralizing antibodies to Langat virus (a togavirus) in mice neonatally thymectomized and infected 6 weeks later. Virelizier *et al.* (1974b) have recently analyzed the role of T cell helper effects in the formation of antibodies against influenza virus hemagglutinin. Two hemagglutinins, each containing a cross-reactive determinant(s) and a strain-specific determinant, were used and antibody formation against each of these could be quantitated independently by immunodiffusion. Immunization of thymectomized, irradiated, and bone-marrow reconstituted (TXBM) mice showed a strong thymus

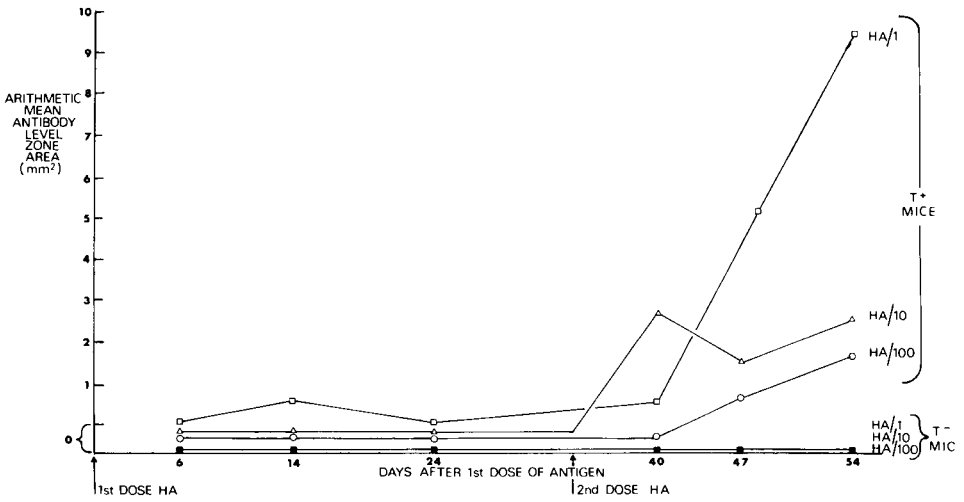

Fig. 14. Thymus dependence of influenza virus hemagglutinin (HA). Levels of antibody to HA were determined by radial immunodiffusion after immunization with various dilutions of HA of normal (T+) or TXBM (T−) mice. From Virelizier *et al.* (1974b).

dependence of antibody formation against both determinants (Fig. 14). Haller and Lindenmann (1974) and W. H. Burns (unpublished observations) have confirmed the thymus dependence of HI antibody formation to influenza virus in nude mice. B. Rager-Zisman and A. C. Allison (unpublished observations, 1974) observed that, in neonatally thymectomized mice infected as adults with HSV, levels of serum neutralizing antibodies were much lower than in intact animals; A. C. Allison and W. H. Burns have confirmed this finding in TXBM mice and nude mice (unpublished observations). Discrepancies between these findings and previous reports concerning HSV and influenza virus probably stem from the use of mice inadequately depleted of T lymphocytes in the earlier reports.

Since mice sham thymectomized, irradiated, and bone-marrow reconstituted make poor responses to some antigens months after reconstitution (Howard *et al.*, 1971; Mitchell and Humphrey, 1973), lesions in addition to T lymphocyte depletion must be present in TXBM mice. Although nude mice may possess abnormalities in addition to those presently recognized, they are probably the best animals for studying thymus dependence. W. H. Burns (unpublished observations) has compared the humoral responses of nude mice and their normal (nu/+, +/+) littermates to infections with a number of

viruses. Insignificant or very low (probably IgM) levels of neutralizing antibodies were found in nude mice infected with Coxsackie B1 virus, encephalomyocarditis virus, West Nile virus, VSV, or HSV. No HI antibodies were detected after infections with influenza or Sendai viruses and no CF antibodies were detected after infection with mouse adenovirus. However, infection with Sindbis virus resulted in high titers (1:1000) of neutralizing antibody, solely of the IgM class; this titer was comparable to that in control littermates and was sustained for 2 weeks. The antigens of Sindbis virus that react with neutralizing antibody are two membrane glycoproteins. Using rabbit antisera, Kennedy (1974) has demonstrated that the antigenic determinants of Semliki Forest virus (an alphavirus similar to Sindbis virus) are in the polypeptide portions rather than the carbohydrate moieties of the envelope glycoproteins. It will be of interest to determine if the "thymus-independent" antigenic determinants of Sindbis virus similarly reside in the protein part of the molecules. Recently, "natural" antibody in sera of nude mice has been detected that neutralizes a xenotropic virus isolated from these mice (S. Cross, H. M. Morse, and J. Hartley, personal communication, 1974). Presumably the antibody (predominantly IgM) is directed against viral envelope proteins and/or glycoproteins.

VI. B Lymphocyte Memory and "Original Antigenic Sin"

On theoretical grounds it might be supposed that vaccination with a series of antigenically related viral vaccines would produce a broad immunity against all the viruses of a group, e.g., type A influenza viruses or flaviviruses. However, this aim is difficult to achieve because of a property of immunologic memory that Francis (1955) has called "original antigenic sin." The response of various age groups of the human population to vaccinations with different strains of influenza A virus showed that the serologic response of an individual is dominated throughout his life by the type of antibody produced as a result of his first exposure to an influenza A virus (Francis, 1953, 1955; Davenport and Hennessy, 1956). This type of response is characteristic of infections of long-lived animals with any virus of which there are several cross-reacting antigenic types, e.g., flaviviruses—group B arthropod-bone viruses (Hearn and Rainey, 1963), paramyxoviruses (van der Veen and Sonderkamp, 1965), and enteroviruses (Mietens *et al.*, 1964). It can be reproduced in laboratory animals either by successive infections with cross-reacting viruses or successive vaccination with inactive vaccines made from such cross-

reacting viruses. The phenomenon is also observed when mice are immunized with purified cross-reactive influenza virus hemagglutinins (Virelizer *et al.*, 1974a). Analogous responses have been reported in animals immunized with cross-reactive serum albumins, HL-A antigens, and haptens. Westaway *et al.* (1974) recently reported that, after sequential infections of rabbits with related togaviruses, the cross-reactive antibodies produced after rechallenge were of the IgG class while IgM antibodies were specific for the most recent immunizing virus. In geographic areas where flavivirus infections are common and serologic diagnosis difficult because of cross-reacting antibodies and the recall phenomenon, determination of IgM specificity can permit diagnosis (Edelman *et al.*, 1973; Edelman and Pariyanonda, 1973).

Thus, the original antigenic sin phenomenon is not only of general interest from the theoretical point of view but of practical importance in immunization programs and in serologic epidemiology. It is paradoxical, since it implies a qualitative failure in the specificity of immunologic memory. The cellular basis of the phenomenon is unknown, and investigations in detail have begun using two purified influenza hemagglutinins, H_0 and H_1, possessing specific as well as cross-reactive determinants (Virelizier *et al.*, 1974a,b). As mentioned above, formation of antibody against both these determinants is thymus-dependent. However, the thymus dependence of antibody formation against the cross-reactive determinant could be overcome by repeated inoculations of hemagglutinin in TXBM mice, indicating the presence of memory in these animals. Strong, secondary type responses were observed in primed, thymus-deprived mice after reconstitution with syngeneic virgin thymus cells, showing that specific immunologic memory is elicited by both determinants despite the absence of detectable antibody secretion. These observations are interpreted as examples of immunologic recognition and memory mediated by B lymphocytes. They suggest that the helper effect of T lymphocytes is exerted at a late stage in the differentiation of specific populations of B cells into antibody-secreting cells.

These findings are similar to those of Roelants and Askonas (1972) and I. M. Zitron (personal communication) for nonviral antigens. A similar interpretation might explain the results of Yamanouchi *et al.* (1974b) who reported that acute infection with rinderpest virus depressed the primary response of rabbits to sheep erythrocytes, but left intact the secondary response after reimmunization 3 weeks later. Rinderpest virus, like measles virus which it closely resembles, may transiently impair T cell helper effects yet allow B lymphocyte proliferation.

Several experiments have shown that B lymphocyte memory is responsible for original antigenic sin. Thus, when spleen cells from mice primed with H_0 are transferred to normal or irradiated recipients and the latter are challenged with H_1, secondary responses to specific H_0 determinants, as well as cross-reactive determinants, are obtained. Treatment of the spleen cells with anti-θ sera and complement does not affect this type of recall. However, similar experiments using irradiated recipients show that, in addition to the memory which is responsible for the special type of recall, there is a T lymphocyte memory that increases specifically the response to the determinant on the first hemagglutinin (H_0) which is not cross-reactive.

VII. Neutralization

A. Mechanisms of Neutralization

The decrease in infectious titer after mixing antibody and virus is termed neutralization and has been reviewed by Svehag (1968). Most studies have been carried out in the fluid phase *in vitro* and each system of virus–cell–antibody–complement components presents unique features. The influence of the host cell was shown by Kjellen and Schlesinger (1959), who found different levels of residual infectivity of VSV when the same virus–antibody complexes were assayed on chick and human cells. Complexes of virus and mammalian antibody were more efficiently neutralized when propagated on mammalian cells than chicken cells, whereas chicken antibody was more effective with chicken cells. Philipson (1966) reported analogous findings with poliovirus when assayed on monkey and human cells, and others have made similar observations with various virus–host cell systems (Lafferty, 1963a; Hawkes, 1964). Thus neutralization is a complex process involving three components: virus, antibody, and host cell. Neutralization *in vivo* where leukocytes and accessory factors are present may differ in important respects from that observed in cell cultures.

The initial interaction between virus and antibody has been extensively studied. Some workers found the interaction to be reversible, e.g., with influenza virus (Burnet *et al.*, 1937; Fazekas de St. Groth, 1962; Lafferty, 1963a), while others felt it was essentially irreversible, e.g., with poliovirus (Dulbecco *et al.*, 1956; Mandel, 1961; Philipson, 1966), adenovirus (Kjellen, 1962), NDV (Granoff, 1965),

and HSV (Yoshino and Taniguchi, 1965b). Philipson (1966), using a two-phase aqueous polymer system that distributes free and antibody-bound virus in different phases, examined the interaction of poliovirus with various immunoglobulins. After a short lag, IgM antibody bound virus irreversibly while IgG antibody, following first-order kinetics, bound virus rapidly and irreversibly. Using a countercurrent distribution procedure with a total dilution factor of 10^{18}, only a small fraction of poliovirus–antibody complexes could be dissociated. Mandel (1961) showed that such complexes were stable for 50 days when resuspended in media without antibody. However, dissociation can occur and infectious poliovirus recovered after exposure of the complexes to acid or alkali (Mandel, 1961), treatment with fluorocarbon (Ketler *et al.*, 1961), sonication (Keller, 1965), or exposure to proteolytic enzymes (Keller, 1968).

The Fc part of the immunoglobulin molecule determines many of its biologic properties (complement fixation, placental transfer, binding to certain cells) and its influence on virus neutralization has been examined indirectly by observing neutralization with Fab fragments. The purity of Fab fragments varied in different experiments, but generally Fab was found to neutralize influenza virus (Lafferty, 1963b), poliovirus (Vogt *et al.*, 1964), HSV (Ashe *et al.*, 1968, and LDV (Notkins *et al.*, 1968), but not adenovirus type 5 (Kjellen, 1964, 1965).

Antibody in proper amounts can agglutinate or coat virions and prevent their adsorption, as has been shown for NDV (Rubin and Franklin, 1957) and poliovirus (Mandel, 1962; Keller, 1966). However, virus–antibody complexes can sometimes adsorb to host cells, although complexes containing the larger IgM do so more slowly than those containing IgG perhaps because of greater steric hindrance (Mandel, 1967). Adsorbed complexes may penetrate more slowly and be more susceptible to further neutralization by added antisera than nonadsorbed free virus (Rubin and Franklin, 1957; Rubin, 1957; Mandel, 1962).

Correlations between antigenic structure and neutralization have been made for several viruses, and a few examples will now be considered. The neutralization of adenovirus takes place in two steps, with firm initial antibody–virus binding followed by increasing inactivation (Kjellen, 1962). Although either univalent or bivalent Fab fragments bind to adenovirus and block whole antibody HI activity, neither fragment blocks the neutralizing activity of whole antibody. Since HA is mediated by the fiber γ antigen while neutralization requires interaction with the hexon ϵ antigen, this could be ex-

plained if Fab fragments bind to γ but not to ϵ. Binding of Fab fragments of antisera prepared against capsid subunits would clarify this. Since antisera against the hexon ϵ antigens neutralize (Wilcox and Ginsberg, 1963; Kjellen and Pereira, 1968; Philipson, 1969) while those against fibers usually do not (Kjellen and Pereira, 1968; Pettersson *et al.*, 1968), neutralizing antibody may not prevent adsorption to host cells but may alter a later step such as uncoating. Intrasubgroup heterotypic antisera neutralize adenovirus type 5 at low pH, and addition of these heterotypic sera to homotypic sera at normal pH enhances neutralization (Kjellen and Pereira, 1968). It was suggested that low pH or the interaction of homotypic antibody with the outer aspect of hexons might expose inner sites with subgroup specificity which then could react with heterotypic antibody and enhance neutralization. According to this view, conformational changes of the hexons are important in adenovirus neutralization, possibly leading to faulty uncoating and degradation in heterophagic vacuoles, as has been described for antibody-complexed NDV and vaccinia (Dales and Kajioka, 1964; Silverstein and Marcus, 1964; Dales, 1969).

Foot-and-mouth disease virus (FMDV) is an icosohedral particle which has been shown to have three classes of antibody-binding sites (Brown and Smale, 1970). One is a 12 S protein subunit on the faces of the particles which can be separated from the virus by heat or acid treatment and reacts only with IgG. Both sites at the vertices bind IgG and one, which is trypsin sensitive, is the only site of attachment of IgM antibody. Neutralization studies with antisera against whole virus and adsorbed with trypsin-treated virus or 12 S subunits indicate that both vertex sites are involved in neutralization while the face site is not (Rowlands *et al.*, 1971).

Virion glycoproteins appear to be important antigens involved in the neutralization of many viruses. The G protein of VSV is a glycoprotein forming the spikes that protrude from the virus coat and can be solubilized by detergents and purified. Antisera produced against this protein have good neutralizing activity (Kelley *et al.*, 1972). The envelope glycoprotein of another rhabdovirus, rabies, can also be extracted and purified; it can induce neutralizing antibody and protects animals against later challenge with rabies virus (Wiktor *et al.*, 1973) and offers a new form of vaccine. Cohen *et al.* (1972) have reported purification from HSV-infected cell of a virion glycoprotein that is capable of stimulating in rabbits monoprecipitin antisera that neutralize HSV. Other workers have isolated envelope glycoproteins from HSV types 1 and 2 which react with type-specific neutralizing antibodies (Powell *et al.*, 1974).

Neutralization of influenza virus results primariily from the interaction of antibody with hemagglutinin although high concentrations of antibody to neuraminidase can neutralize some virus strains (Kilbourne *et al.*, 1968). Antibodies to hemagglutinin can prevent virion attachment to cells while antibodies to neuraminidase do not. Both antibodies prevent viropexis and in their presence virions remain at the cell surface (Dourmashkin and Tyrrell, 1974). Antineuraminidase may also interfere with egress of virus from infected cells.

B. Complement-Dependent Neutralization

The capacity of "natural antibody" (thought to be IgM) and early immune IgM antibody to neutralize viruses is increased by reaction with the complement system. Normal human sera and rabbit early immune sera require complement to neutralize T coliphages whereas late immune sera of humans and rabbits do not (Muschel and Toussaint, 1962). After inoculation of rabbits with HSV, early (8-day) sera contained both IgM and IgG antibodies which required complement for neutralization (Yoshino and Taniguchi, 1964, 1965a). Late sera contained both IgM and IgG which could neutralize HSV without complement, but complement increased the IgM neutralizing capacity and raised the neutralization rate constant but not titer of late IgG (Hampar *et al.*, 1968). Neutralizing antibody against rubella virus, especially IgM and early IgG, also requires complement (Rawls *et al.*, 1967; Leerhoy, 1968). The inefficiency of early and late IgM and early IgG antibody against equine arteritis virus in neutralization was not affected by complement, but an exception to the above findings is the observation that late IgG antibody to this virus is strongly dependent on complement for neutralization (Hyllseth and Pettersson, 1970; Radwan and Burger, 1973). This finding was noted in sera from five species and is therefore probably characteristic of the virus rather than the source of antibody.

It is not clear how complement increases neutralization. Since the antibody in early immune sera often has low avidity for virus, it is possible that the reaction with complement stabilizes the virus–antibody complex. In the HSV system, however, the complex of virus and early sera is stable and some other mechanism must be operative (Yoshino and Taniguchi, 1965b). These complexes can be neutralized by late sera, so that the binding of early serum antibodies must leave crucial sites for infection exposed. The accumulation of complement proteins on the virion surface may cover these important sites and interfere with adsorption, penetration, or uncoating; ag-

gregation of virus particles can also occur (Oldstone *et al.*, 1974).

Virus–antibody complexes of HSV and equine arteritis virus adsorbed to cell surfaces were found to be susceptible to complement during the early period of cell–complex interaction, suggesting that complement can influence a step later than adsorption (Yoshino and Taniguchi, 1967; Radwan and Burger, 1973). Since penetration of virus–antibody complexes into cells is known to be prolonged compared to virus alone, reaction with complement at this stage may be important.

The sequence of complement component reactions with the virus–antibody complex is the same as for other antigen–antibody systems. Studies using purified components of complement and HSV or NDV complexed with early IgM have shown that C1 and C4 are required for neutralization and that the effects of suboptimal amounts of C4 can be supplemented by the addition of C2 and C3 (Daniels *et al.*, 1969, 1970; Linscott and Levinson, 1969). Similar findings for complement enhancement of HI activity of early IgM antibody bound to influenza virus have been reported (Reno and Hoffman, 1972). Oldstone *et al.* (1974) found that complement-enhanced neutralization of polyoma virus requires C3 as well as C1, C2, and C4. Enhanced neutralization occurred primarily by agglutination of virus–antibody complexes.

Electron microscopic studies have demonstrated that avian infectious bronchitis virus, a coronavirus with a host-derived lipid coat containing 200 Å projections, can undergo complement-dependent virolysis similar to complement-dependent erythrocyte lysis (Berry and Almeida, 1968; Almeida and Waterson, 1969). Antibody from infected chickens reacted only with the virus-specified projections, recognizing the virion membrane as self (host-derived). However, antibody from rabbits immunized with virus grown in chickens reacted with the virion membrane and projections, whereas rabbit antibody to uninfected chicken cells reacted only with the membrane. The neutralizing capacity of all three antisera was enhanced by complement, and electron microscopy showed complement attached to projections when antibody was bound there and 100 Å holes in the membrane after complement reacted with antibody at that site. Complement-mediated virolysis has also been described for rubella virus (Almeida and Laurence, 1969), influenza virus (Almeida and Waterson, 1969), Gross leukemogenic (AKR) virus (Oroszlan and Gilden, 1970), and equine arteritis virus (Radwan *et al.*, 1973).

The preceding discussion has considered only the classical complement activation pathway, which initially requires the conversion of C1 to C1 and subsequent interaction of C1 with C4 and C2 before

activation of C3 and the remaining components. Interest has recently turned toward an alternate pathway in which C3 is activated without interaction of the preceding three components (Osler and Sandberg, 1973). Certain immunoglobulins or immunoglobulin fragments, after binding antigen, can activate complement by the alternate pathway. Guinea pig γ2 antibodies efficiently activate complement by the classic pathway in which interaction of the Fc portion of the antibody molecule with C1 is essential. However, the $F(ab')_2$ fragments of γ2 immunoglobulins activate complement via the alternate pathway. A. L. Sandberg (personal communication) has used $F(ab')_2$ fragments of anti-HSV γ2 to determine whether complement-dependent enhancement of HSV neutralization can occur by the alternate pathway. She found that the $F(ab')_2$ fragments bound to the virus but were not as efficient in enhancing neutralization as were the parent undigested antibodies. Furthermore, there was only minimal lysis of HSV-infected rabbit kidney cells by the alternate pathway after binding of the $F(ab')_2$ fragments to the cell membrane. More viruses must be examined before conclusions can be drawn concerning the importance of the alternate pathway in virus neutralization, but there may be a specific requirement for the first complement components in the enhancement of neutralization of some viruses.

The *in vivo* consequences of complement reactions with virus–antibody complexes are often detrimental to the host, producing arteritis, glomerulonephritis, and choroiditis (Oldstone and Dixon, 1971; Oldstone, 1974). In addition, C3 bound to complexes may facilitate their attachment to macrophages, neutrophils, and B lymphocytes, which have C3 receptors. This could enhance their clearance by such cells if viral growth is restricted in them or, conversely, facilitate virus growth and spread if the cells are susceptible.

C. Infectious Complexes and Antiglobulin Neutralization

The residual infectivity of virus after exposure to excess antibody is called the persistent fraction, the presence and nature of which has been reviewed by Notkins (1971) and Majer (1972). Burnet *et al.* (1937) first noted the existence of a nonneutralizable fraction of an animal virus, and studies of many viruses *in vitro* (picornaviruses, togaviruses, myxoviruses, herpesviruses, poxviruses, and reoviruses) and *in vivo* (lactate dehydrogenase virus, H-1 virus, Visna virus, and Aleutian mink disease virus) demonstrate the generality of the phenomenon. That the virus in the persistent fraction is not a genetic variant was shown in studies demonstrating similar behavior of non-

neutralized virus progeny and the parent population in neutralization tests (Dulbecco *et al.*, 1956; Notkins *et al.*, 1966b; Hahon, 1970b; Majer and Link, 1970).

The persistent fraction has been shown to consist of virus–antibody complexes by physiochemical methods—density-gradient centrifugation (Kjellen, 1965), countercurrent distribution (Philipson, 1966), and ratezonal centrifugation (Notkins *et al.*, 1971). However, most studies have been based on the use of antiglobulins (Ashe and Notkins, 1966), when antibody directed against the antiviral immunoglobulin is reacted with the virus–antibody complexes and any increase in neutralization noted. Notkins *et al.* (1966b) found the persisting infectious virus in the plasma of mice infected with LDV to be in virus–antibody complexes which could be neutralized by treatment with antisera against mouse immunoglobulins. With rabbit antibody against HSV, Ashe and Notkins (1967) found that the persistent fraction could be markedly reduced with antisera against rabbit immunoglobulins, although such antisera had no effect on virus not "sensitized."

Using antiglobulins against various immunoglobulin classes, it has been shown that IgM, IgG, and IgA can sensitize virus. Studies of unfractionated antisera demonstrate that the sensitizing antibodies in mice infected with LDV are IgG and IgA; anti-mouse IgM did not neutralize the persistent fraction (Notkins *et al.*, 1968). Human antisera to Venezuelan equine encephalitis (VEE) virus, likewise, had sensitizing IgG and IgA but not IgM antibodies (Hahon, 1970b). Hampar *et al.* (1968) could sensitize HSV with IgM antibody from rabbit late immune sera but not from early sera.

The sites of antiglobulin interaction have been examined. Univalent antiviral Fab fragments were found to sensitize HSV (Ashe *et al.*, 1968, 1969) and poliovirus (Keller, 1968) and anti-Fab could neutralize sensitized VEE virus (Hahon, 1970b), indicating that the Fab portion of the sensitizing antibody can bind to the antiglobulin and result in neutralization. The persistent fraction of VEE virus could also be neutralized with anti-allotype antiglobulin for an Fc allotype and with anti-Fc antiglobulin, demonstrating that the Fc portion can also bind the antiglobulin.

Virus sensitization is generally thought to occur when antibodies are attached to noncritical sites on the virion, exposure of which is not required to initiate infection. This may hinder attachment of other antibodies to critical sites. If suboptimal amounts of antisera are reacted with virus, it is found after subsequent addition of antiglobulin that a large fraction of virus has been sensitized but not

neutralized by the antiviral antibody (Ashe and Notkins, 1967; Notkins *et al.*, 1968). Thus, it was found that for LDV sensitization occurs sooner than neutralization and for both LDV and HSV that as sensitization increases the neutralization constant diminishes. Presumably, antiglobulin binding to antiviral antibody at noncritical sites sterically hinders interaction of virus with cells. Accordingly, univalent antiglobulin Fab fragments neutralize sensitized virus less well than larger whole antiglobulin (Ashe *et al.*, 1968; Notkins *et al.*, 1968).

Rheumatoid factor (RF), a human IgM with specificity for the Fc portion of aggregated or complexed IgG antibodies, has also been found to react with virus–IgG antibody complexes (Ashe *et al.*, 1971). Rabbit or human anti-HSV IgG antibody was reacted with HSV and then with human RF. No reduction in the persistent fraction of these HSV–IgG–RF complexes occurred unless the preparations were allowed to react further with complement or anti-human IgM antiglobulin. Apparently the attachment of RF provides new sites for complement reaction and attachment on the complex. If Fab fragments of anti-HSV were used to sensitize the virus, no enhancement of neutralization was observed after reaction with complement or anti-IgM, presumably because of the inability of RF to attach to the Fab fragment. Hayashi *et al.* (1973) have shown that RF can bind to infected cells after they have been exposed to antiviral antibody. Whether RF plays a protective role *in vivo* by enhancing neutralization of infectious virus–antibody complexes is unknown. Alternatively, by enhancing the complement-fixing capacity of complexes it may lead to increased pathology in immune complex diseases.

VIII. Capacity of Macrophages to Support Virus Replication

Mims (1964) has summarized evidence that viruses introduced into the lungs or other sites are taken up by macrophages in the local tissues and in lymph nodes, where they are demonstrable by immunofluorescence. Likewise, viruses entering the blood from the primary site of multiplication or introduced into the bloodstream (e.g., by arthropod vectors) are removed by Kupffer cells and other macrophages lining blood sinuses. Usually, the viruses cannot multiply sufficiently well in the macrophages of adult hosts to spread to other cells which are highly susceptible, such as hepatic parenchymal cells. However, if the macrophage barrier is bypassed by exposing liver parenchymal cells directly to infection (e.g., by injecting

virus into the bile duct) a lethal infection with widespread multiplication of virus in parenchymal cells results. Similarly, the brain is a highly susceptible organ, and intracerebral injection of many viruses in adult animals will result in lethal infections.

If macrophages represent an important barrier to the spread of infection there should be a relationship between the capacity of a virus to multiply in macrophages and virulence in adult animals. Such a relationship exists, and is most strikingly demonstrated where major genetic factors determine the susceptibility to infection. Bang and Warwick (1960) found that adult mice of the Princeton (PRI) strain are susceptible to lethal infection with the Nelson strain of mouse hepatitis virus (MHV-2), whereas the C3H strain of mice is resistant. Mating experiments show that the susceptibility to infection segregates as a single Mendelian dominant genetic factor. It was found, moreover, that cultures of hepatic or peritoneal macrophages taken from susceptible hosts support multiplication of MHV with cytopathic effects, whereas macrophages from resistant mice do not. This interesting observation showed that inherited resistance is a property of individual macrophages, manifested *in vitro* in the absence of an acquired immune response. Observations on other MHV strains also emphasize the relationship between virulence and the capacity of viruses to multiply in macrophages. Thus, the avirulent strain MHV-1 does not multiply in macrophages, whereas the virulent strain MHV-3, which after intraperitoneal injection into adult mice of most strains produces a lethal hepatitis, multiplies readily in mouse peritoneal macrophages with giant cell formation (Fig. 15) (Allison and Mallucci, 1965). Only the A strain of mice resists infection with MHV-3 as adults, and cultures of peritoneal macrophages from this strain again show no cytopathic effects after exposure to MHV-3 (J. L. Virelizier and A. C. Allison, unpublished observations, 1974).

Parallelism between inherited resistance of mice to viruses and the capacity of their mononuclear phagocytes to support virus multiplication is not confined to the murine hepatitis viruses. Another system that has been analyzed in detail is the inherited resistance against arthropod-borne viruses of the B group. Sabin (1954) found that the striking difference in susceptibility of HSVS and PRI mice to yellow fever virus is determined by a single pair of genes, with resistance dominant. The PRI mice also show marked resistance to other flaviviruses. This resistance is not a general one to all types of virus; in fact, the PRI mice which are resistant to flaviviruses are highly susceptible to MHV-2, and the two resistance factors segregate in-

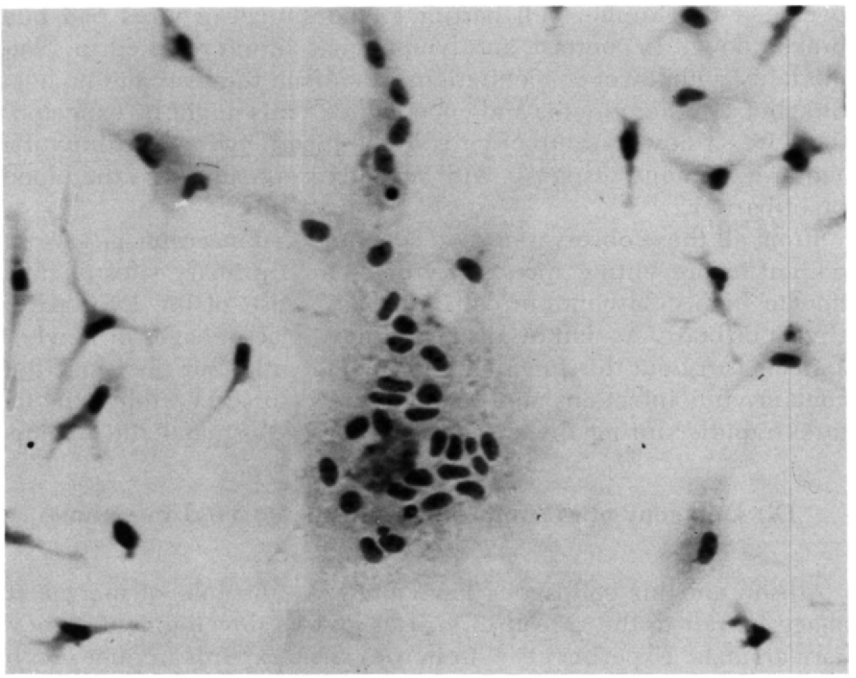

Fig. 15. Mouse peritoneal macrophages infected with mouse hepatitis virus (MHV-3), showing giant cell formation. ×200.

dependently among the offspring of hybrids (Kantoch *et al.*, 1963). It was shown by Goodman and Koprowski (1962) that macrophages from susceptible mice support multiplication of flaviviruses very efficiently, whereas macrophages from resistant mice do not. Similar observations have subsequently been made in Koprowski's laboratory on mice congenic for the resistance gene. B. Rager-Zisman and A. C. Allison (unpublished observations, 1974) have found that encephalomyocarditis virus, which is virulent in adult mice, can replicate in macrophages. Hence, there is little doubt that the capacity of a virus to multiply in macrophages is an important aspect of virulence, but not of course the only one.

It was also found (Zisman *et al.*, 1969) that the resistance of weanling mice to intraperitoneal injection of herpes simplex virus could be broken down by injections of silica particles or of rabbit anti-mouse macrophage serum. Both these treatments lead to rapid mortality beginning 5 days after infection, with high titers of virus in the liver and virus-induced hepatic necrosis. This is the result ex-

pected if the Kupffer cell barrier to the spread of virus had been broken down. In contrast, anti-lymphocytic serum resulted in a later mortality with lower concentrations of virus in the liver and no hepatitis, but a higher viremia and encephalitis. This might be expected if there was a slow buildup of virus in the blood, owing to failure of an acquired immune response, with secondary spread across the blood–brain barrier.

From all these observations we conclude that macrophages are important in preventing spread of viruses from primary sites of infection to highly susceptible cells, such as those of the liver parenchyma or brain. As will be discussed next, macrophages of newborn animals carry out this function very inefficiently, but the capacity to contain virus infections and prevent spread to susceptible cells matures rapidly during the first few weeks of postnatal life in mice.

IX. Ontogeny of Macrophage Resistance to Viral Infections

Allison and his colleagues have analyzed the role of the macrophage barrier to the spread of viruses and its development in newborn animals. Experiments with herpes simplex virus in mice can be used to illustrate the point, which probably applies also to many viruses as well as to other intracellular infections. Intraperitoneal inoculation of newborn mice with herpes simplex virus is followed by rapid spread of virus to the viscera, encephalitis, and death, whereas similar injections into weanling or adult mice result in no viral spread to the liver or brain and no morbidity or mortality. The resistance to intraperitoneal infection develops rapidly during the first few weeks of life. Transfers of peritoneal macrophages from adult CBA to syngeneic suckling mice were able to confer a large measure of protection against intraperitoneal infection with herpesvirus; similar transfers of peritoneal lymphocytes from adult animals were without any protective effect (Hirsch *et al.*, 1970). Further studies showed that the adult macrophages, particularly when stimulated by prior injections of proteose-peptone, were much more efficient at taking up and preventing the spread of virus than macrophages from suckling animals, whether stimulated or not. This was reflected in the diminished capacity of infected adult macrophages to establish infectious centers when cultured together with susceptible cells, and similar findings were reported by Johnson (1964).

In addition to this diminished capacity of adult macrophages to transfer virus to contiguous cells, which for various reasons was

thought not to be due to release of interferon and the basis of which is still not well defined, adult macrophages may replicate the virus less well than macrophages from neonates. This view is reinforced by studies of Stevens and Cook (1971a) showing that herpes simplex virus in adult mouse macrophages undergoes an abortive cycle of replication, with synthesis of virus nucleic acids and antigens, and the formation of nascent capsids lacking dense cores, in the nucleus. Hence, a late stage of herpesvirus synthesis, perhaps involving DNA metabolism or nucleocapsid formation, appears to be blocked in adult mouse macrophages.

The human counterpart to these experiments is the severe, generalized infection which herpes simplex virus produces in newborn children (Wheeler and Huffines, 1965; Nahmias *et al.*, 1969). Usually this is herpesvirus type 2 acquired during childbirth from a maternal genital infection; occasionally it is type 1 infection. The virus disseminates widely, to the central nervous system, liver and other organs, and administration of antibody has not provided any demonstrable protection. The great majority of children die. Immaturity of macrophages in the human newborn may well be an important contributory factor allowing the dissemination of the infection. That the Kupffer cell barrier is poorly developed in human fetuses and newborns is suggested by involvement of hepatic parenchymal cells in congenital rubella infections; this is not seen in adult cases of rubella (Stern and Williams, 1966). Rager-Zisman and Allison (1973b) also found that newborn mice could be protected from intraperitoneal inoculation of Coxsackie B virus by syngeneic adult peritoneal exudate cells; the presence of very small amounts of antibody increased the protective effects.

Maturation of macrophage virucidal capacity during the first few weeks of life may be linked to cell-mediated immunity and stimulation with bacterial or other antigens. The work of Mackaness (1970) and others suggests that a lymphocyte-mediated immune response increases the capacity of macrophages to kill organisms unrelated to those providing the antigenic stimulus. Allison (1965) reported that macrophages taken later from neonatally thymectomized animals support the multiplication of the avirulent virus MHV-1, whereas the virus multiplies to a very limited extent in macrophages from intact adult animals. This work has been continued using the more virulent MHV-3 strain of virus in genetically resistant A strain mice; they succumb to MHV-3 if infected soon after birth or if they are neonatally thymectomized and infected as adults (Dupuy *et al.*, 1973; Levy-Leblond and Dupuy, 1974).

The latter investigators and J. L. Virelizier and A. C. Allison (unpublished observations, 1974) have found that peritoneal macrophages taken from neonatally thymectomized mice support the multiplication of the virus, with giant cell formation, whereas those from normal mice of the same age do not. Also, TXBM mice of the resistant A strain when tested 2 weeks after thymus deprivation are resistant to MHV-3 and no virus replication was observed in cultures of peritoneal macrophages from such animals. However, when similar mice were tested 3 months after thymus deprivation, at a time when most macrophages present probably have developed in an environment deficient in T lymphocytes and putative thymic hormones, they were found to be susceptible to MHV-3 infection and peritoneal macrophages replicated the virus *in vitro*.

Macrophages from thymus-deprived mice have generally been thought to be normal. However, the above observations demonstrate that at least one property of "mature" macrophages, virucidal capacity, depends on the presence of the thymus and/or T lymphocytes during at least some of their development. Other macrophage functions, such as participation in granuloma formation, are deficient in newborn and neonatally thymectomized mice (Yang and Skinsnes, 1973). However, transfer experiments with macrophages from these animals are necessary before conclusions can be made concerning the role of T lymphocytes (which is established) and the developmental dependence of these macrophages on the thymus and T cells. Experiments demonstrating the inability of macrophages from newborn animals to participate in immune responses *in vivo* (see Hardy *et al.*, 1973) should be reexamined with this possibility in mind.

X. Surface Changes of Virus-Infected Cells

A. Virus-Coded Antigens

1. Virion Antigens

Many viruses have envelopes derived from host cell membranes and all viruses are intimately related to host cell membranes during adsorption, penetration, and replication. This can lead to profound changes in the cell membrane as well as the basic physiology and behavior of the cell and its relationship to its environment (see review by Allison, 1971). Infection with most viruses results in cell membrane alterations that can be detected by the appearance of new

antigens and sometimes as a change in the ability to bind particular lectins or other materials. Table III lists viruses now known to produce antigenic changes in host membranes as detected biologically in transplantation studies or directly by immunofluorescence, immunoradiolabeling, immunoelectron microscopy, and assays employing cytotoxic antibody or lymphoid cells. It should be noted that the antisera used in many assays were produced by active infection with the virus, and antigenic changes may represent virus-induced host cell alterations and not antigens the structures of which are encoded in the virus genome.

Most of the surface antigens appear early in infection. Thus, cells infected with influenza virus produce viral protein in 1 hour (Noll, 1962) and virus-specific cell surface antigens are detectable 4 hours after infection (Hahon and Eckert, 1972). New surface antigens appear on cells infected by NDV at 3 hours, VEE and Sindbis viruses at 4 hours, vaccinia virus at 4 hours, HSV at 6 hours, and measles, rinderpest, and canine distemper viruses at 20 to 24 hours. Studies with protein and nucleic acid synthesis inhibitors have demonstrated that new protein must be synthesized before the antigens are detectable (i.e., the antigens are not adsorbed virions) but that nucleic acid synthesis is not usually required. Thus the antigenic changes probably represent early functions of the virus genome. An apparent exception is the transplantation rejection antigen (TRA) produced by SV40 infection, which does not appear in lytic infection but appears late in the transformation of nonpermissive cells (Smith and Mora, 1972).

Infection by some viruses produces no detectable surface antigens. Picornaviruses, although associated with cytoplasmic membranes during development, do not bud through membranes and lack membrane envelopes. Perhaps significantly in this regard, they also lack carbohydrates which, in those viruses containing carbohydrates, are attached to virus proteins while the latter are embedded in host membranes. Cells infected by Coxsackie B5 virus (a picornavirus) have no surface antigens detectable by immunofluorescence or radiolabeling (Hayashi *et al.*, 1973). A strain of vaccinia virus has been reported which does not produce a major surface antigen normally found early during infection (Ito and Barron, 1972b).

Most cells transformed by the Moloney variant of murine sarcoma virus (MSC) also produce helper murine leukemogenic virus and possess surface antigens specified by the latter virus. A few MSV-transformed cell lines not producing helper virus are available and Strouk *et al.* (1972) could not detect virus-specific surface antigens

TABLE III

Demonstration of Virus-Specific Surface Antigens on Infected Cells

Virus	Assay[a]	Reference
Murine leukemogenic	CT	Slettenmark-Wahren and Klein, 1962
	CT	Geering *et al.*, 1966
	IEM	Aoki *et al.*, 1970, 1972
	CT	Shirai *et al.*, 1971
	CT	Stockert *et al.*, 1971
	LC	Proffitt *et al.*, 1973
Murine mammary tumor	IA	Nishioka *et al.*, 1969
Feline leukemia	IEM	Oshiro *et al.*, 1971
	RI	Boone *et al.*, 1973
Avian tumor	IEM	Gelderblom *et al.*, 1972
	LC	Kurth and Bauer, 1972
	IEM	Gelderblom and Bauer, 1973
	IEM	Phillips and Perdue, 1974
Orthomyxoviruses		
Influenza	IEM	Duc-Nguyen *et al.*, 1966
	CT	Brier *et al.*, 1971
	IF	Hahon and Eckert, 1972
Paramyxoviruses		
Mumps	IEM	Duc-Nguyen and Rosenblum, 1967
	LC	Speel *et al.*, 1968
Sendai	CT	Eaton and Scala, 1969
SV5	CT	Holmes *et al.*, 1969
Measles, rinderpest,	IF	Yamanouchi *et al.*, 1970
canine distemper	LC	Rustigian *et al.*, 1971
	LC	Labowskie *et al.*, 1974
Respiratory syncitial	LA	Epsmark, 1965
Togaviruses		
Chikungunya	IF	Mantani and Igarashi, 1971
VEE	IF	Hahon, 1970a
EEE	CT	Catanzaro *et al.*, 1974
Sindbis	HA	Burge and Pfefferkorn, 1967
	IF, LC	McFarland, 1974
Dengue	CT, IEM	Catanzaro *et al.*, 1974
Rhabdoviruses		
Rabies	CT	Fernandes *et al.*, 1964
	CT	Wiktor *et al.*, 1968
Arenaviruses		
LCM	LC	Lundstedt, 1969
	CT, LC	Oldstone *et al.*, 1969
	LC	Wright *et al.*, 1972
	IF, LC	Cole *et al.*, 1973
	LC	Marker and Volkert, 1973
	LC	Zinkernagel and Doherty, 1974

TABLE III (*Cont.*)

Virus	Assay[a]	Reference
Adenoviruses		
Adenovirus type 12	CT	Berman, 1967
	IF	Hollinshead and Alford, 1969
	IF	Vasconcelos-Costa, 1970
	IF	Vasconcelos-Costa *et al.*, 1973
Herpesviruses		
Herpes simplex	IF	O'Dea and Dineen, 1957
	CT	Roane and Roizman, 1964
	IA	Epsmark, 1965
	IEM	Nii *et al.*, 1968
	CT	Brier *et al.*, 1971
	IF	Lowry *et al.*, 1971
	IEM	Miyamoto *et al.*, 1971
	IF	Nahmias *et al.*, 1971
	IA	Ito and Barron, 1972a
	CT	Smith *et al.*, 1972
Varicalla-zoster	IF	Gershon *et al.*, 1974
Cytomegaloviruses	IF	Thé and Langenhuysen, 1972
Epstein–Barr	IF	Klein *et al.*, 1968
	IF	Dunkel and Zeigel, 1970
Marek's disease	IF	Chen and Purchase, 1970
Poxviruses		
Vaccinia, cowpox, monkeypox	IA, IF	Miyamoto and Kato, 1968, 1971
	IF	Ueda *et al.*, 1969
	CT	Brier *et al.*, 1971
	RI	Hayashi *et al.*, 1972
	IA	Ito and Barron, 1972b
Shope fibroma	IF, CT	Ishimoto and Ito, 1969, 1971
	IF	Tompkins *et al.*, 1970b
Papova		
SV40	CT	Smith *et al.*, 1970
	RI	Ting and Herberman, 1970
	CT	Wright and Law, 1971
	RI	Kedar *et al.*, 1972
Polyoma	IF	Irlin, 1967
	IF	Malmgren *et al.*, 1968
	RI	Ting and Herberman, 1970

[a] Key: CT, antibody + complement cytotoxicity; IA, immune adherence; IEM, immunoelectron microscopy; IF, immunofluorescence; LC, lymphocyte-mediated cytotoxicity; RI, radioimmunoassay.

on such cells using hemadsorption and lymphocyte cytotoxicity assays. Stephenson and Aaronson (1972) failed to detect transplantation antigens on similar cell lines, but Law and Ting (1970) did find transplantation antigens and the issue is not settled.

2. Nonvirion Antigens

Some virus-induced surface antigens are thought to be virus coded but are not found in the virion. Gross leukemogenic virus produces a variety of antigens in infected cells (Aoki *et al.*, 1972), among which are nonvirion surface antigens (GCSAa and GCSAb). Similarly, the transplantation antigens of adenoviruses, SV40, and polyoma virus have not been identified in virions.

B. Alteration of Host Antigens or Receptors

1. Appearance of "New" Host Antigens

Some recognizable new surface antigens probably result from unmasking of antigenic substructure already present or derepression of host genes. Cells of a hamster kidney line (BHK) which normally have no demonstrable Forssman antigen acquired it after transformation with SV40, polyoma virus, or Rous sarcoma virus (Fogel and Sachs, 1962; O'Neill, 1968; Robertson and Black, 1969).

2. Changes in Lectin Receptors

The appearance on transformed cells of accessible receptors for lectins, glycoproteins from plants and invertebrates capable of agglutinating certain cells by binding with particular carbohydrates, may also represent an unmasking phenomenon or rearranging of membrane components. Burger (1973) has reviewed the subject and it appears that lectin receptors on normal and transformed cells may be present in equal amounts. Virus transformation or mild treatment of normal cells with proteases does not lead directly to clustering of lectin-binding receptors, but does increase the mobility of lectin-binding sites which can cluster in the presence of multivalent lectins (Rosenblith *et al.*, 1973).

Even so, there are demonstrable changes in the concentrations of membrane components and related enzymes during the transformation process (Brady *et al.*, 1973). Sialic acid levels were found to be reduced in SV40 (Wu *et al.*, 1968) and polyoma virus (Ohta *et al.*, 1968) transformed cells when compared to parental cells; there is

usually a reduction in higher ganglioside homologues as well (Brady and Mora, 1970). The higher gangliosides are formed by the sequential addition of various hexose moieties and sialic acid and is mediated by transferase enzymes. An examination of one transferase involved has revealed it to be markedly reduced in most SV40 and polyoma virus transformed cells (Brady *et al.*, 1973). Viral alterations in enzymes concerned with membrane biosynthesis and structure (such as ganglioside transferases) might alter phenotypic expression in much the same way as the lysogenic conversion of *Salmonella* by ϵ phage (Robbins and Uchida, 1965).

3. Appearance of an Fc Receptor

The appearance on cells infected with HSV of a receptor that binds the Fc portion of rabbit or human IgG molecules has been reported (Watkins, 1965; Yasuda and Milgrom, 1968; Shimizu, 1971; Westmoreland and Watkins, 1974). The Fc receptor appeared on the surfaces of all infected cells tested, including fibroblasts and epithelial cells from mice, hamsters, monkeys, and humans. Receptors appeared on HeLa cells within 4 hours after infection and at 11 hours after infection an estimated 5×10^4 molecules of IgG per cell bound to the cell membrane during 30 minutes at 37°C. Experiments with actinomycin D and puromycin suggested an early need for RNA synthesis after infection and for continuing synthesis of receptors. No evidence was obtained to suggest unmasking of Fc receptors already present in a cryptic form. Antisera against uninfected host cells or HSV blocked the subsequent attachment of immunoglobulin to the infected cells. This could be due to steric hindrance or the Fc receptor might contain host and viral antigenic determinants; a viral protein glycosylated by host enzymes and functioning as an Fc receptor could have this characteristic. It is not known whether the virus codes for a protein which is the receptor itself or which can be glycosylated by cellular enzymes and resemble the Fc receptor known to be present on macrophages, neutrophils, B lymphocytes, and possibly some T lymphocytes. Alternatively, infection could result in derepression of the appropriate host gene(s) controlling synthesis of the receptor. This latter possibility is intriguing in view of recent suggestions that the Fc receptor is a product of the Ir gene complex. Since these genes control some immune responses, infection of lymphocytes by viruses that activate genes in this complex might affect immune responses or perhaps permit autoimmune responses.

4. Loss of Host Antigens

Virus infections can result not only in the appearance of "new" host antigens, but also in the diminution of normal membrane antigens. Hecht and Summers (1972) found a 70% reduction in the capacity of VSV-infected mouse cells to adsorb cytolytic antibodies directed against H-2 antigen. This was not due to inhibition of protein synthesis as treatment of cells with cycloheximide for a similar time period did not reduce the H-2 concentration, nor did infection with encephalomyocarditis virus which inhibits host protein synthesis. Whether other cell antigens, particularly tumor antigens, will behave similarly during infection merits examination. At least one tumor antigen is known to cocap and thus to be linked with H-2 antigen and conceivably may be altered by those factors influencing H-2 density (Gooding and Edidin, 1974). Recently, Hecht and Summers (1974) reported that NDV-infected L (mouse) cells lose 50% of their H-2 antigen.

C. Capping of Viral Antigens

The polar redistribution (capping) of surface immunoglobulins (Taylor *et al.*, 1971) and receptors for immune complexes (Miller *et al.*, 1973) on lymphocytes and of transplantation antigens on a variety of cell types (Kourilsky *et al.*, 1972) has been reported by many laboratories and much speculation concerning its physiological importance has arisen. Joseph and Oldstone (1974) have described capping of measles virus antigen on infected HeLa cells; it required active cell metabolism and multivalent antibody. Capping has also been observed for mumps antigen on infected cells (K. Hayashi, personal communication) and for gs-1 and gs-3 antigens on Gross virus-induced leukemic cells (Yoshiki *et al.*, 1974a) and may be a general phenomenon for virus-specific cell surface antigens.

Capping of viral antigens on the surface of infected cells could have a number of consequences. If viral nucleocapsids must align themselves beneath membrane containing viral proteins before budding (e.g., myxoviruses), capping could interfere with viral morphogenesis and release. Alteration of the density of viral antigens on the cell surface might result in ineffective interaction of the cell with lymphocytes (Cole *et al.*, 1973) or of the attached antibody with complement (Brier *et al.*, 1971). Some nonlytic infections thus protected from effective immune responses might persist.

D. Antigenic Modulation

A phenomenon similar to capping that results in reversible losses of surface antigens, termed antigenic modulation, can also occur. A host antigen, the TL (thymus-leukemia) antigen in mice, is an organ-specific allotypic determinant on thymocytes of certain mouse strains with its genetic locus near the D end of the H-2 locus (Boyse *et al.*, 1965; Boyse and Old, 1969). Leukemias induced by leukemogenic viruses in the TL-positive mouse strains result in the appearance of the TL antigen on leukemic cells. If such leukemic cells are transplanted to TL-negative animals previously immunized with TL antigen and producing antibody to it, the antigen is quickly lost from the cell surface but can reappear if the leukemic cells are transplanted back to a TL-positive host (lacking antibody to TL). Phenotypic expression of TL was found to markedly reduce the demonstrable quantity of H-2 antigens, especially those specified by the D region. With loss of TL by antigen modulation, an increase in the H-2 antigens was observed (Boyse *et al.*, 1968; Old *et al.*, 1968). Antigenic modulation for the GCSAa antigen of leukemic cells, which appears to be coded by the Gross leukemia virus rather than the host, has also been reported (Aoki *et al.*, 1972).

E. Possible Combinations of Host and Viral Antigenic Determinants

Recent experiments by Zinkernagel and Doherty (1974) raise the interesting possibility that some virus-induced surface antigens may be combinations of virus and host material. These investigators reported that lymphocytes from LCM-infected mice are capable of mediating a cytotoxic reaction only with infected target cells which share similarity at either the K or D region of the H-2 locus. The reactive lymphocytes were sensitive to anti-θ and complement and thus are T lymphocytes. (It should be mentioned that Cole *et al.* (1973) found no such H-2 requirement in a similar system.) R. V. Blanden (personal communication) has obtained similar results using ectromelia virus. One explanation for this phenomenon is that the interaction of cytotoxic T lymphocytes with somatic cells requires an intimate contact that is strongly dependent on some degree of histocompatibility, as is suggested for the interaction of helper T lymphocytes and B lymphocytes (Katz *et al.*, 1973a,b). An alternative explanation is that the immune lymphocytes recognize a composite antigen consisting of virus protein and H-2 material, or that the virus

infection results in altered H-2 antigens that are no longer recognized by the lymphocytes as "self."

XI. Evidence for Cell-Mediated Immunity to Viral Antigens

A. Introduction

Over the past couple of decades knowledge of the structure of antibodies and their protective and pathological roles in immunity have been well defined. Currently, much interest is centered on understanding lymphoid cells and cell-mediated immunity. Various methods have been used to illustrate the presence of cell-mediated immunity against virus-specific antigens. Each has its own advantages and limitations. Although delayed hypersensitivity to many viruses or viral antigens has been demonstrated, it must be interpreted with caution. For example, some reactions are elicited by antigens from the heterologous cells in which viruses are grown. Antibody-mediated reactions to replicating antigens may be maximal between 24 and 72 hours after intradermal inoculation; even the histology may be ambiguous. Of the *in vitro* tests for cell-mediated immunity, MIF production correlates better with delayed hypersensitivity than does blast transformation (David and Schlossman, 1968; Rocklin *et al.*, 1970). With peptides of tobacco mosaic virus and glucagon, delayed skin reactions correlated with the production of MIF by lymphoid cells *in vitro*, but not always with the stimulation of DNA synthesis on the exposure of host lymphocytes to the antigens (Spitler *et al.*, 1970; Senyk *et al.*, 1971). It seems that MIF production is a particularly good *in vitro* correlate of delayed hypersensitivity in guinea pigs, but attempts to obtain MIF production with cells from other species, including man, have not been easily reproducible in all laboratories.

Many studies of lymphocyte stimulation by viruses have been performed, but for the most part they have been inadequately controlled to exclude the possible involvement of host cell antigens and to characterize the participating cells. The manner of antigen presentation—whether infected cells or virus, infectious or inactivated—can determine the type and extent of response obtained. The blastogenic response primarily represents antigenic recognition by lymphocytes, and Gershon *et al.* (1973) have found that for T lymphocytes this may not correlate with the development of recognized T lymphocyte

functions. The lymphocyte cytotoxicity assays represent effector functions as well as antigen recognition (Bach *et al.,* 1973) and may be complicated by factors (such as immune complexes) that influence either of these functions.

What is considered cell-mediated immunity in the animal is a complex multicomponent phenomenon involving functionally different lymphocyte populations as well as macrophages, K cells and antibodies, soluble mediators, and other factors. Obviously, to assay one component of this process *in vitro* is rather artificial, and all *in vitro* assays must be interpreted with caution. Even so, from these investigations some useful generalizations are beginning to emerge. Sensitization of T lymphocytes in virus infections is an early event, being demonstrable 3 to 7 days after infection, reaching a peak of cytotoxic reactivity at 7 to 14 days and then falling to near background levels. A population of memory cells, not themselves cytotoxic but able to generate cytotoxic effector cells, then persists for weeks or months. The finding of stimulated DNA synthesis by lymphocytes in the presence of virus antigen does not necessarily imply T lymphocyte sensitization, especially in secondary responses where B lymphocyte proliferation may predominate.

This section can consider this large subject only in outline form. Evidence for delayed hypersensitivity (DH) to virus antigens will be briefly mentioned and the *in vitro* correlates summarized.

B. Poxviruses

1. Vaccinia Virus

In 1907, von Pirquet suggested that hypersensitivity contributes to the local inflammatory lesion of primary vaccinia, and vaccinia virus has continued to be used in studies of DH. Broom (1947) demonstrated in humans that prevaccination lesions can occur in the absence of specific antibody and Turk *et al.* (1962), by injecting virus-antiserum mixtures, produced typical DH reactions in guinea pigs without demonstrable humoral antibody. Allison (1967) could passively transfer DH against vaccinia in inbred guinea pigs with peritoneal exudate cells but not with serum. Pincus and Flick (1963) used anti-mononuclear cell antisera to inhibit local vaccinia skin lesions in guinea pigs.

In vitro studies have shown that lymphoid cells from humans immunized with vaccinia can respond specifically to antigenic chal-

lenge by generating MIF (Tompkins *et al.*, 1970a). Spleen and peripheral blood lymphocytes from vaccinia-infected rabbits, when exposed to UV-inactivated vaccinia, were found to incorporate up to thirty times as much thymidine into their DNA as appropriate controls (Rosenberg *et al.*, 1972a). This stimulation was observed from 3 to at least 120 days after infection and was at its peak during the second week of infection. Elfenbein and Rosenberg (1973) have subsequently shown that both T and B lymphocytes participate in this proliferation. Using heat-inactivated vaccinia virus, Epstein *et al.* (1972) have demonstrated transformation and interferon production by human lymphocytes from immune donors.

2. Ectromelia Virus

This poxvirus of mice, which cross-reacts with vaccinia virus, produces a severe and often fatal disease. Fenner (1949) showed that there is an allergic component as measured by swelling of the foot on challenge with live virus at various times after primary infection. The allergy was first seen on the seventh day and increased in magnitude thereafter. Since generalized infection of the skin occurs on the sixth day, Fenner considered it unlikely that allergy played an important part in localizing virus in the skin. However, more recent studies by Blanden (1970, 1971) have further defind the role of cell-mediated immunity to this virus *in vivo*.

Gardner *et al.* (1974) have used a chromium-release cell-mediated cytotoxicity assay with infected L cells as target cells to study the generation of immune lymphocytes after infection. Mouse spleen cells showed cytotoxic activity 2 days after immunization and this activity peaked at 6 days before declining to low levels by day 10. This activity was not diminished by the removal of macrophages or the addition of antisera to ectromelia or by exogenous interferon, but was abrogated by prior treatment of the lymphocytes with anti-θ antibody and complement and thus is mediated by T lymphocytes.

3. Fibroma Virus

When inoculated into the skin of adult domestic rabbits, this poxvirus induces a rapidly growing tumor that subsequently regresses. Intradermal challenge with virus on the fifth day produced DH reactions (Allison, 1966; Allison and Friedman, 1966). *In vitro* production of MIF can also be elicited from lymphocytes obtained 5 days after virus inoculation (Tompkins *et al.*, 1970a).

C. Herpesviruses

1. Herpes Simplex Virus

HSV can elicit DH reactions in a large proportion of adults and a lower proportion of children (Nagler, 1944; Rose and Molloy, 1947; Anderson and Kilbourne, 1961). DH to HSV in guinea pigs was demonstrated by Brown (1953) and later Lausch *et al.* (1966) showed that DH could be induced by a single injection of infective HSV. The skin reaction can be elicited by a soluble antigen (Anderson and Kilbourne, 1961; Jawetz *et al.*, 1951, 1955), and recently Rogers *et al.* (1972) demonstrated that DH can be induced in guinea pigs with a soluble antigen. It will be interesting to determine if DH can be induced and/or elicited with the glycoprotein recently shown to be a neutralizing antigen for HSV (Cohen *et al.*, 1972; Powell *et al.*, 1974).

In vitro studies demonstrated that inactivated HSV can stimulate DNA synthesis in sensitized lymphocytes (Rosenberg *et al.*, 1972a) and differentiate type 1 and type 2 virus (Rosenberg *et al.*, 1972b).

2. Epstein–Barr Virus

Gerber and Lucas (1972) have shown that peripheral blood lymphocytes from previously infected subjects are stimulated to incorporate thymidine by UV-inactivated EB virus whereas lymphocytes from seronegative subjects are not stimulated.

3. Cytomegaloviruses

It has already been noted that the various *in vitro* tests purporting to demonstrate cell-mediated immunity may depend crucially on the manner of antigen presentation and may reflect different functions of lymphocytes. The former point is well demonstrated in the report of Thurman *et al.* (1973) concerning MIF production and transformation of lymphocytes from CMV-infected patients after exposure of the lymphocytes to virus or to virus-infected cells. The lymphocytes from CMV-infected patients responded by production of MIF when cultured with purified virus but were transformed only by culture with CMV-infected cell lines. Virus-specific antigens on the virion surface and the membranes of infected cells probably differ quantitatively and qualitatively, and may elicit different lymphocyte responses or stimulate different lymphocyte populations.

D. Myxoviruses

Beveridge and Burnet (1944) found skin hypersensitivity of the DH type with influenza virus. Interestingly, they stated that live attenuated virus administered intranasally was more effective in inducing skin hypersensitivity than killed virus vaccine injected subcutaneously. Mice inoculated with inactivated influenza virus developed cell-mediated immunity as shown by *in vitro* tests (Feinstone *et al.*, 1969). Hellman *et al.* (1972) reported the stimulation by PR8 influenza virus of thymidine incorporation into spleen cells of sensitized mice, beginning 7 days after infection and peaking at 14 days before declining. MIF assays have been employed by Wetherbee (1973) to demonstrate systemic cell-mediated responses in guinea pigs infected intranasally with influenza virus. Gadol *et al.* (1974) compared MIF production by lymphocytes from bronchial washes or spleens of guinea pigs infected intranasally or by the footpad route. Intranasal infection led to MIF production predominantly by lung lymphocytes whereas parenteral inoculation resulted mainly in systemic immunity.

E. Paramyxoviruses

1. Mumps Virus

Enders *et al.* (1945) demonstrated dermal hypersensitivity to mumps virus and positive reactions were related to immunity. DH has been produced in guinea pigs with mumps virus (Glasgow and Morgan, 1957) and Speel *et al.* (1968) showed that spleen cells from mice immunized with mumps virus are toxic to Chang cells persistently infected with mumps. Lymphocytes from mice infected with mumps virus have been shown to produce MIF *in vitro* (Feinstone *et al.*, 1969). Using inactivated mumps virus, Smith *et al.* (1972) have demonstrated stimulation of DNA synthesis in human lymphocytes after primary exposure and for up to 40 years after primary exposure and correlated this with delayed skin sensitivity.

2. Measles Virus and SSPE Agent

Skin testing with measles virus has been complicated by contaminating antigens derived from cells used to culture the virus (Isacson, 1968). A number of *in vitro* assays have been employed to assess cell-mediated immunity to measles virus and the closely related agent of subacute sclerosing panencephalitis (SSPE). A colony-

inhibition assay used lymphocytes from monkeys infected with measles virus and BSC-1 cells persistently infected with the virus (Rustigian *et al.*, 1971). Labowskie *et al.* (1974) reported that lymphocytes from seropositive individuals were capable of morphological destruction and lysis of cell lines persistently infected with measles. This reaction could be blocked by pretreatment of the target cells with antisera to measles. Thurman *et al.* (1973) and Ahmed *et al.* (1974) reported that lymphocytes from seropositive individuals produced MIF and lymphotoxin on exposure to purified measles virus, but interestingly, blastogenic responses did not occur on exposure of the lymphocytes to virus preparations but, as with CMV, were elicited when the lymphocytes were cultured with virus-infected cells.

There has been much controversy concerning the nature of cell-mediated immunity in general and to measles antigens or virus in particular in patients with subacute sclerosing panencephalitis. There are reports of diminished lymphocyte responses to PHA (Kolar, 1968) and lack of normal dermal hypersensitivity reactions to common test antigens as well as delayed rejection of skin allografts (Gerson and Haslam, 1971). However, Jabbour *et al.* (1969) found all of eight patients tested to have normal skin responses to *Candida* and two of three patients could be sensitized to dichloronitrobenzene. J. F. Soothill (private communication) has found normal lymphocyte responses to PHA in eight cases. Normal *in vitro* cell-mediated immunity to antigens other than measles was reported for twenty cases (Moulias *et al.*, 1971). A generalized defect in cell-mediated immunity is therefore not established.

Two reports on five patients with SSPE confirm normal reactivity of lymphocytes from SSPE patients in MIF and lymphotoxin-release assays using virus preparations of measles or the SSPE agent as the antigen, or in transformation assays using SSPE-infected cells as the stimulant (Thurman *et al.*, 1973; Ahmed *et al.*, 1974). A blocking factor found in high concentration in the cerebrospinal fluids of SSPE patients and in lesser concentration in their sera was found to block the reactions of lymphocytes from SSPE patients and normal seropositive individuals in the MIF, lymphotoxin-release, and transformation assays specifically for the SSPE agent and not against other antigens (Sell *et al.*, 1973; Ahmed *et al.*, 1974). This factor is heat-labile, larger than MW 150,000, sensitive to the actions of trypsin and neuraminidase, neutralized by rheumatoid factor, and may be an antigen–antibody complex.

Using the lymphocyte-mediated cytotoxicity assay of Labowskie

et al. (1974), J. A. Bellanti and his colleagues (personal communication) have confirmed the normal reactivity of lymphocytes from seven SSPE patients against measles-infected cells. These workers also found specific blocking factors in the sera of these patients. In conclusion it appears that SSPE patients have the capacity to mount cell-mediated immune responses in general and to measles-SSPE virus antigens in particular, but reaction to the latter are thwarted by blocking factors, possibly immune complexes, that are found predominantly in the CSF.

F. Murine Leukemogenic Viruses

Cell-mediated responses to murine leukemias have been recognized for years. However, congenital infections by the murine leukemia viruses formerly were thought to result in tolerance to the virus antigens and only recently has circulating antibody to these viruses been demonstrated. The concept of "split tolerance" in which humoral responses are not accompanied by cell-mediated responses was then suggested. Recently, Proffitt *et al.* (1973) presented evidence for cell-mediated immunity in carrier C3H mice for Moloney leukemia virus. Lymphocytes from such animals mediated cytotoxic reactions against syngeneic cells infected with Moloney leukemia virus but not against uninfected cells. Wahren and Metcalf (1970) had reported similar results using C3H carrier mice (for Moloney MuLV) and embryo cell cultures from these mice as target cells; lymphocytes from normal C3H mice were not cytotoxic for these target cells. The latter investigators also reported cytotoxic reactions of lymphocytes from preleukemic AKR mice for cell monolayers of AKR embryos or of AKR thymic epithelial cells. Many tissues of AKR mice are known to possess Gross MuLV antigens and these target cells probably possessed viral antigens since the cytotoxic reaction was prevented by pretreatment of the target cells with antisera to Gross cell surface antigens. At variance with the above studies, Chieco-Bianchi *et al.* (1974) have reported no cytotoxic reactions between spleen lymphocytes from adult C57BL/6 mice infected neonatally with Moloney MuLV and syngeneic Moloney MuLV-infected target cells. Differences in the production of the virus carrier state and in the cytotoxic assays employed may account for this discrepancy. In this last study a chromium-release assay was used while in the previous studies a microcytotoxicity assay (similar to colony inhibition assays) was used and important differences in these assays are known (Biesecker *et al.*, 1973; Bloom *et al.*, 1973).

G. Togaviruses

1. Sindbis Virus

Beginning a few days after footpad inoculation of mice with Sindbis virus, lymphocytes from draining nodes and spleen underwent blastogenesis after exposure to the virus (Griffin and Johnson, 1973). This sensitivity peaked 6 days after infection and returned to control levels by 16 days. Lysis of infected syngeneic mouse embryo cells by immune lymphocytes followed a similar but slightly shorter time course (McFarland, 1974). This latter function was sensitive to treatment with anti-θ antisera and complement and thus probably represents a T lymphocyte activity.

2. Venezuelan Equine Encephalitis Virus

Stimulation of spleen lymphocytes from immune mice by Venezuelan equine encephalitis virus (VEE) was reported by Adler and Rabinowitz (1973). The reactivity of lymphocytes from mice receiving one immunizing dose of virus was abolished by treatment with anti-θ sera and complement, while that of lymphocytes from mice receiving two immunizing doses was not. Reactivity of the latter lymphocytes was sensitive to treatment with antisera against mouse immunoglobulin plus complement. These results suggest that after primary immunization antigen recognition and proliferation in the immune spleen cell population are due to T lymphocytes while after secondary immunization the reactive cells are predominantly B lymphocytes. These findings are in agreement with those of Elfenbein and Rosenberg (1973) for vaccinia virus.

3. Rubella Virus

A chromium-release assay using persistently infected BHK cells has been described and only peripheral blood lymphocytes from seropositive subjects were cytotoxic in this assay (Steele *et al.*, 1973). Smith *et al.* (1973) have described an assay to measure the blastogenic responses of human lymphocytes after exposure to rubella antigen. Using a similar assay Lee and Sigel (1974) examined the influence of immune complexes on the blastogenic responses of blood lymphocytes from immune rabbits after exposure to inactivated rubella virus. The response was not altered by the presence of complexes of virus and anti-rubella IgG but virus complexes with anti-rubella IgM were markedly inhibitory. Normal IgM mixed with rubella virus or anti-rubella IgM mixed with poliovirus or influenza

virus had no effect. In a study with HSV (Rosenberg *et al.*, 1972a) hyperimmune sera mixed with HSV did not inhibit blastogenesis; anti-HSV antibodies in this sera probably were of the IgG class. Examination of the effects on immune responses of virus–antibody complexes with attention to antibody class will be of especial interest with measles virus and the SSPE agent.

H. Arenaviruses

There is considerable evidence for *in vivo* cell-mediated responses after infection of adult rodents with LCM virus (Lehmann-Grube, 1971; Cole, 1974). For example, Tosolini and Mims (1971) described the time course of the development of DH reactions in acutely infected mice by using the footpad test. *In vitro* assays have employed persistently infected L cells as target cells for immune lymphocytes. Benson (1962) first showed that immune spleen lymphocytes inhibited the adherence and growth of infected L cells, and this phenomenon was studied in more detail by Lundstedt (1969). Similar studies demonstrating cytotoxic effects of immune lymphocytes have employed the Hellstrom method (Wright *et al.*, 1972) and chromium-release assays (Oldstone *et al.*, 1969; Cole *et al.*, 1973; Marker and Volkert, 1973; Zinkernagel and Doherty, 1974). Cole *et al.* (1973) have demonstrated that the immune lymphocytes responsible for this specific cytotoxicity are sensitive to anti-θ antisera and complement. Effector activity appears a few days after infection, peaks at 10 days, and then falls sharply off—a pattern similar to that after infection with ectromelia virus or Sindbis virus.

Recent reports from three laboratories indicate that the immune spleen cells obtained early during infection differ from late immune spleen cells (Mims and Blanden, 1972; Johnson *et al.*, 1974; Volkert *et al.*, 1974). The former cells, in addition to being cytotoxic in *in vitro* assays, possessed antiviral activities and protected mice during acute lethal infections. Late immune cells lacked these properties *in re* acutely infected mice, but had strong antiviral activity when transplanted to chronically infected mice—a situation in which early immune cells had little effect. Also, the cytotoxic and antiviral activities of the early immune cells were relatively resistant to X-irradiation, while the antiviral activity (on transfer to chronically infected mice) of late immune cells was radiation sensitive. Functions of both populations of cells were sensitive to treatment with anti-θ antisera and complement, and thus T lymphocytes are involved in both populations. Perhaps the late immune cells comprise relatively few actively

cytotoxic T lymphocytes but many "memory" T lymphocytes capable, over a period of time longer than that involved in acute infection, of producing a large population of cytotoxic cells.

XII. Persistence of Immunity to Viruses

A lifelong immunity against reinfection is produced by many viruses, including smallpox, poliomyelitis, yellow fever, and the acute childhood exanthemas. In 1847 Panum showed that in the Faroe islands, where successive measles epidemics were separated by intervals of 65 and 31 years, each epidemic infected all those who had not been previously exposed but spared those who had been exposed. Reports of antibodies persisting in the absence of reinfection include those against yellow fever virus for 75 years (Sawyer, 1931), poliomyelitis among the Eskimos for 40 years (Paul *et al.*, 1951), and Rift Valley fever for 12 years (Sabin and Blumberg, 1947). Reexposure to virus may increase the duration of immunity. Thus, Krugman *et al.* (1966) found rises in neutralizing antibody titers in immune subjects exposed to measles, and similar observations have been made on vaccinated subjects exposed to smallpox (Downie and McCarthy, 1958).

Two explanations have been put forward for prolonged anti-viral immunity in the absence of reinfection; according to one there is persistence of specific clones of immunocompetent cells (Burnet, 1959) and according to the other persistence of virus (Olitsky and Long, 1929a,b). Observations of antibody in rabbits 2 years after inoculation with inactivated poliovirus (Svehag, 1964b) and similar data in man after administration of killed virus vaccines show that immunologic memory exists, but whether it can explain a lifetime of antibody production in the absence of further antigenic stimulation is uncertain.

Many viruses are known to persist after infection, and in 1929 Olitsky and Long (1929a) suggested "that the immunity in virus diseases may be linked with the persistence in the body of the living virus." De Koch (1924) found the blood of a horse recovered from an attack of equine pernicious anemia to be infectious 7 years later. The salivary gland virus of guinea pigs could be recovered any time after infection and in the presence of antibody (Cole and Kuttner, 1926). Vaccinia virus was isolated from rabbits 4 months after inoculation (Olitsky and Long, 1929a,b). Measles virus has been isolated from brain and lymph nodes of patients with subacute sclerosing panen-

cephalitis (Horta-Barbosa *et al.*, 1969; Payne *et al.*, 1969) and from the lymph nodes and spleens of normal subjects some years after infection (Enders-Ruckle, 1965). Adenoviruses are commonly isolated from human tonsillar and adenoidal tissues (Israel, 1962; Strohl and Schlesinger, 1965), and in rabbits they persist for long periods in splenic and lymph node cells (Pereira and Kelly, 1957; Reddick and Lefkowitz, 1969; Allison, 1970; Faucon *et al.*, 1974). Mouse cytomegaloviruses can be isolated from the spleen and lymph nodes of chronically infected mice (Henson *et al.*, 1972). Some viruses such as lymphocytic choriomeningitis virus and lactate dehydrogenase virus often persist for the lifetime of host animals even in the presence of antibody.

The human herpesviruses are well known for their latency and tendency to establish recurring infections. Human CMV is often isolated from leukocytes of normal persons (Diosi *et al.*, 1969; Perham *et al.*, 1971; Lang, 1972). EB virus may be induced in lymphocytes years after recovery from infectious mononucleosis (Diehl *et al.*, 1968). Varicella-zoster virus remains dormant for years after chicken pox and reappears clinically as zoster. Initial HSV infection presents as a childhood stomatitis with subsequent positive titers for CF and neutralizing antibody (Yoshino *et al.*, 1962). HSV can later be isolated from asymptomatic subjects as well as those with herpetic lesions (Buddingh *et al.*, 1953; Kaufman *et al.*, 1967). Over 40 years ago Goodpasture (1929) suggested that the virus remains in a dormant state between recurrences in the sensory ganglia. Direct proof for this concept was lacking until recently, when Stevens and Cook (1971b) isolated HSV from the spinal ganglia of mice weeks after recovery from posterior paralysis following virus inoculation of hind footpads, and from rabbit trigeminal ganglia months after recovery from corneal infection (Nesburn *et al.*, 1972; Stevens *et al.*, 1972). Virus was recovered only from the ganglia and not from proximal or distal nerve structures except during acute infection. Virus could not be detected in the ganglia by electron microscopy or by infectious virus assay, and only after the ganglia were explanted and cultured for a period of time *in vitro* could virus be recovered. Recovery of HSV from *in vitro* cultures of human trigeminal ganglia obtained at autopsy from patients without active herpes infection has now been reported (Bastian *et al.*, 1972; Baringer and Swoveland, 1973). J. R. Baringer (1974) has recently isolated type 2 (genital) herpes from the sacral ganglia of humans. It appears that the virus is activated by the explantation and culture, probably due to metabolic changes in the neurons subsequent to injury rather than to the re-

moval of the tissue to an environment free of immune responses. This interpretation is consistent with the finding that HSV can be activated in dorsal ganglia after sciatic nerve injury (Walz *et al.*, 1974) and with the well-known observation that proximal rhizotomy of the human trigeminal ganglion results in activation of HSV (Cushing, 1905; Carton and Kilbourne, 1952).

XIII. Tolerance to Viral Antigens

The work of Traube in the 1930's showed that in mouse colonies vertical transmission from mothers to offspring of lymphocytic choriomeningitis (LCM) virus establishes a symptomless lifelong infection in which no antiviral antibody is demonstrable in the circulating blood. Infection of adult mice with LCM leads to an immunopathological disease and antibody formation. The lack of antibody after congenital LCM virus infection was one of the observations that led Burnet and Fenner (1949) to postulate the existence of immunologic tolerance. More recently, Oldstone and Dixon (1967) and Benson and Hotchin (1969) have demonstrated in mice congenitally infected with LCM virus the presence of antiviral antibody and the accumulation in the kidneys of immune complexes containing viral antigen and antibody (Oldstone and Dixon, 1971). Hence, the congenitally infected mice are able to make some antibody against viral antigens. Volkert and Hannover-Larsen (1965) found that, if spleen cells from LCM-immune mice (infected as adults) are transferred to syngeneic congenitally infected carrier animals, very high levels of antibody are formed, but no immunopathological disease results.

The simplest interpretation of these findings appears to be that LCM viral antigens produce a tolerance resembling that occurring with autoantigens in low dose, namely that T lymphocytes specific for viral antigens become unresponsive while specific B lymphocytes remain able to respond to antigen (Allison, 1973). In the absence of specific helper cells, only a small amount of antibody is produced, and this combines with antigen liberated into the circulation to form immune complexes which accumulate in the kidney, leading eventually to immunopathological glomerulonephritis. However, when virus-specific T cells are supplied by adoptive immunization, a helper effect greatly increases antibody formation in the recipients. A helper role of T cells in antibody formation against LCM viral antigens is demonstrated by the experiments of Cole *et al.* (1972).

Adoptive immunization of congenitally infected LCM virus carrier mice with spleen cells from syngeneic immune donors resulted in high antibody levels, as already described, but treatment of the spleen cells with anti-θ serum before transfer virtually abolished this effect. Thus, even in the presence of B cells from immune donors, little antibody is formed unless sensitized T cells are also present. The interpretation of these experiments is complicated by the fact that in the recipients the level of virus is reduced, so that the antigenic load is less and the chances of finding free antibody are increased. However, the levels of antibody in recipients are so high that this is unlikely to be the whole explanation.

For a long time it was thought that mice congenitally infected with leukemogenic viruses would likewise be tolerant to virus-specific antigens. However, Aoki *et al.* (1966) detected antibody to Gross leukemogenic virus cell surface antigens (GCSA's) in the sera of old C57BL/6 mice. Mellors *et al.* (1969) found that Gross soluble antigens, a mixture of virus-specific antigens (Aoki *et al.*, 1972), increased in the sera of NZB mice until 9 months of age, after which increasing titers of antibody to these antigens could be detected and immune elimination of the viral antigen occurred while glomerulonephritis progressed; renal eluates contained 7 S antibody to Gross soluble antigen (Mellors *et al.*, 1971). Previously preparation in mice of antisera to Gross virus had resulted in the production of antibodies against GCSAs but not of neutralizing antibodies directed against virion envelope antigens (GVEA's). This led to the hypothesis that, due to occult infections early in life, mice might be tolerant to VEA's. However, old NZB mice were found to make "natural antibody" to GVEA's (Aoki *et al.*, 1970). Recently, Yoshiki *et al.* (1974b) reported that NZB, NZW, and their F_1 hybrid mice contain remarkably high concentrations of the viral envelope glycoprotein gp69/71, and that this protein is deposited as immune complexes in the glomeruli of these mice in much greater concentration than the major structural protein of the virion, p30.

Studies of the high-leukemic AKR mouse strain have also provided evidence for antibody responses to endogenous infections of Gross leukemogenic virus. Oldstone *et al.* (1972) reported glomerulonephritis developed spontaneously in AKR mice and renal eluates contained antibodies to GCSA's and complement-fixing antibodies to internal viral components. Using immunofluorescence, Markham *et al.* (1972) found IgG antibody and complement along with the viral antigens gs-1 and gs-3 in the kidneys of 8- to 10-month-old AKR mice. Hollis *et al.* (1974) eluted from AKR mouse kidneys IgM and IgG antibodies directed against the virion reverse transcriptase; as

expected, these antibodies reacted with mouse and feline but not avian reverse transcriptase. Much of the antibody against AKR virus antigen found in the glomeruli may be directed to viral antigens produced *in situ* by infected cells, especially mesangial cells (Pascal *et al.*, 1973; Yoshiki *et al.*, 1974b).

Other strains of mice also produce natural antibody to Gross viral antigens. Thus low-leukemic RF mice (Hanna *et al.*, 1972) and C57BL/6 mice (Porter *et al.*, 1973) produce antiviral antibody and develop chronic glomerulonephritis; over half of the antibody eluted from kidneys of the latter mice at 1 year of age reacted with GCSA's. Predominantly IgM antibodies to GVEA's were also deteced in sera and glomeruli of C57BL/6 mice as well as in $B6C3F_1$, BALB/c, and AKR mice (Ihle *et al.*, 1973; Batzing *et al.*, 1974). Using a sensitive radioimmune precipitation assay in which serum is reacted with isotopically labeled virus and the complexes formed then precipitated with appropiate antiglobulins, antibodies to GVEA's (mainly IgG) have been detected in the sera of most inbred mouse strains studied (Nowinski and Kaehler, 1974), and some of these antigens have been characterized as virion envelope glycoproteins (Ihle *et al.*, 1974). Recently, Lee *et al.* (1974) examined the sera of normal $B6C3F_1$ mice and found 19 S antibody which reacted with 3 antigenic components of Gross MuLV—gp69/71, a second viral envelope glycoprotein with MW 43,000 daltons, and the small virus core protein p15. Antibody in the 7 S fraction reacted only with the nonenvelope protein, p15. Only 19 S antibody neutralized the xenotropic BALB:virus-2, which cross-reacts with Gross virus (*vide infra*).

Humoral responses are also made to other vertically transmitted murine oncornaviruses. Hirsch *et al.* (1969) and Branca *et al.* (1972) found that mice congenitally infected with Moloney leukemogenic and sarcoma viruses have complexes of viral antigen and antibody in their glomeruli. Recently, endogenous murine oncornaviruses, released spontaneously from cells of BALB/c and NZB mice and unable to grow in mouse cells but able to grow in cells of other species and thus called xenotropic viruses, have been isolated and designated BALB:virus-2 and NZB-MuLV, respectively. Neutralizing antibodies to these two viruses are cross-reactive and widespread, being present in high titers in 2-month-old mice of all strains tested except the NIH strain (Aaronson and Stephenson, 1974). These investigators suggest that previous studies detecting "natural" antibody to the Gross viral antigens may have been primarily directed against the BALB:virus-2 class of endogenous viruses but cross-reactive at a lower level with Gross-type virus. Some cross-reactivity between BALB:virus-2 and Gross leukemogenic virus does

exist since antisera against the latter virus neutralized the former, but normal BALB/c sera which neutralized BALB:virus-2 did not neutralize viruses of the Gross or FMR subgroups and the antigenic relationships of these viruses awaits clarification. Another xenotropic virus has been isolated from athymic nude mice and neutralizing antibody to this virus, mainly IgM, has been detected in high titers in the nude mice from which the virus was isolated; this sera also neutralized BALB:virus-2 and NZB-MuLV (S. Cross, H. M. Morse, and J. Hartley, personal communication, 1974).

Mice infected *in utero* or neonatally or inheriting viral genomes in a Mendelian, chromosomal fashion can make antibody responses to the viral antigens and thus are not tolerant to these antigens in the classic sense. Whether they can also mount cell-mediated immune responses to these viral antigens is less clear. Recently, Proffitt *et al.* (1973) have found that lymphocytes from C3H carrier mice (carrying Moloney leukemogenic virus) are cytotoxic to cells bearing virus-specific antigens. If these reactions are mediated by T lymphocytes, a lack of tolerance is suggested. Similar observations for Gross leukemogenic virus (MuLV-G) have been published by Wahren and Metcalf (1970). Absence of tolerance in mice neonatally infected with polyoma virus has been found by Allison (1970): lymphocytes from such animals were able to protect immunosuppressed recipients from polyoma tumor formation. Many investigators have described T lymphocyte-mediated cytotoxicity of cell cultures in which LCM virus is replicating (*vide supra*). It is observed in mice infected with LCM virus as adults but not in carrier mice infected as newborns. This supports the view that the carrier mice do not have T lymphocytes reactive against virus-specific antigens, not necessarily the same antigens that enter the circulating blood. These studies suggest that it is more difficult to induce tolerance to virus-specific antigens than was formerly supposed, although some examples of selective tolerance (e.g., specifically induced unresponsiveness of T lymphocytes after congenital LCM infection) appear to exist. It is not yet known whether this is due to deletion or inactivation of T lymphocytes or temporary inhibition of their responses by antigen or immune complexes.

XIV. Cooperative Effects of Viral Antigens on Immunogenicity

Examples of a response to a strong immunogen increasing the response to a second, weaker immunogen coupled to it have been extensively studied, especially carrier effects in which a hapten has

been coupled to different carrier proteins (Katz and Benacerraf, 1972; Leskowitz, 1972). In an analogous way the presence of the highly immunogenic chicken isoantigen B along with the weaker isoantigen A on the same cells will elicit a stronger than normal immune response to the latter (Schierman and McBride, 1967). Conceivably, highly immunogenic viral coat proteins may increase the response to less immunogenic components. Tumor transplantation antigens are often weakly immunogenic, and the immunogenicity of various tumors has been improved by association of the tumor antigens with stronger immunogens by forming somatic hybrids with more antigenic cells (Watkins and Chen, 1969), or by virus infection (reviewed by Lindenmann, 1974). Thus, Lindenmann and Klein (1967) prepared viral oncolysates by infecting Ehrlich ascites tumor cells with influenza virus and showed that immunization of mice with such preparations would protect them against 100 lethal doses of the uninfected tumor cells eleven days later. Even if inactivated, partially purified virus from oncolysates was protective, indicating direct viral lysis of the tumor *in vivo* was not involved. Antisera to the virus (prepared against influenza virus grown in eggs) eliminated the protective effect and simple mixtures of virus and tumor cells were not effective, indicating that close coupling of viral and tumor antigens was required. These findings have been confirmed (Hakkinen and Halonen, 1971). Since the virions were not rigorously purified after oncolysis, it cannot be decided from these experiments whether the immunogen was fragments of cell membrane containing virus antigens or virions containing host material. The latter interpretation is favored in similar experiments with VSV and fowl-plague virus in which the viral oncolysates were immunogenic only when prepared following the appearance of progeny virus, long after the incorporation of virus proteins into cell membranes (Lindenmann, 1970).

Immunizing hamsters with highly purified and inactivated VSV grown in SV40-transformed hamster cells, Ansel (1974) induced transplantation immunity to the same and another SV40-transformed cell line. Although similar immunization with VSV grown in a spontaneously transformed hamster cell line conferred no such immunity, more cell lines transformed by SV40 and other viruses must be studied before specificity for the SV40 transplantation rejection antigen (TRA) is established in this system. Also, it is unclear whether the SV40 TRA was incorporated into the virion structure or adsorbed onto its surface as a contaminant; some host material is incorporated into VSV virions (*vide supra*), but cell surface antigens can be adsorbed onto the virions (Hecht and Summers, 1972).

In other studies virus was not purified from tumor lysates and

tumor cell membranes containing virus antigens may be the immunogen. Thus, Eaton *et al.* (1973) reported increased immunogenicity of a Gross virus lymphoma when syngeneic mice were immunized with lysates of tumor cells infected with NDV or Sendai virus, and membrane fractions were more effective than concentrated virus. Kobayashi *et al.* (1970) have shown that infection of rat tumors with Friend virus increases their immunogenicity and similar findings in the hamster for SV40-transformed human cells have been reported using oncolysates prepared by infection with NDV (Axler and Girardi, 1970). A syngeneic system using SV40-transformed 3T3 cells transplanted to BALB/c mice and oncolysates produced by influenza virus or VSV yielded similar results (Boone *et al.*, 1971, 1974; Boone and Blackman, 1972). Boone *et al.* (1974) found that tumor immunity cannot be induced by viral oncolysates if mice are first made tolerant to the virus by cyclophosphamide treatment. This was considered evidence for a carrier–hapten relationship between the virus and tumor antigens, but interpretation is difficult because of the complexities of cyclophosphamide treatment (Aisenberg, 1973; Lagrange *et al.*, 1974).

The use of viral oncolysates from human tumors to improve the immunizing efficiency of patients to their own tumors following surgery, radiotherapy, or chemotherapy is potentially important, especially if inactivation of the virus does not diminish tumor immunogenicity. Infection of cancer patients with various viruses has sometimes been thought to have beneficial effects (Webb and Smith, 1970). However, critical evaluation of this approach is required. The cooperative effect in virus infections may also prove useful in the preparation of antisera to cellular isoantigens in allogeneic systems (Lindenmann and Klein, 1964) and of potent antilymphocyte sera in xenogeneic systems (Bandlow *et al.*, 1972, 1973).

Cooperative phenomena may also help explain the broadening reactivity of late viral antisera, since the immune response to minor viral determinants may be stimulated by response to stronger ones. Certain types of autoimmunity may involve cooperative effects if self-antigens are coupled to strongly immunogenic viral antigens during infection. It has been shown that immunization of chickens with egg-grown influenza virus results in antibody production against an autologous mucopolysaccharide antigen (*vide supra*) present embryologically in endodermal cells lining the allantoic cavity and in the liver and bile of adults (Harboe and Haukenes, 1966; Harboe *et al.*, 1966; Schoyen *et al.*, 1966); no pathological lesions were reported. Virus particles have been found in NZB mice, which

manifest many autoimmune phenomena (Mellors *et al.*, 1969), in dogs with a lupus syndrome (Lewis, 1974), and possibly in patients with systemic lupus erythematosus (reviewed by Andres *et al.*, 1972), but the role of viruses in these autoimmune phenomena has yet to be clearly defined.

XV. Influence of Virus Infections on Immune Responses

Several viruses are known to influence the activity of the immune system (see review by Notkins *et al.*, 1970). Many virus infections result in nonspecific immunoglobulin elevation (LDV, VEE, Rauscher leukemia, Aleutian mink disease, equine infectious anemia) or depression (most leukomogenic viruses). Some infections, as with Aleutian mink disease virus, result in elevated immunoglobulins but a diminished humoral response to unrelated antigens (Porter *et al.*, 1965; Kenyon, 1966; Lodmell *et al.*, 1970). Mice infected with murine leukemogenic viruses have diminished circulating antibody to sheep erythrocytes and bovine serum albumin. The Jerne plaque technique has been used to demonstrate at a cellular level a diminished response to sheep erythrocytes in mice infected with mouse CMV (Osborn *et al.*, 1968), Rowson–Parr virus (Bendinelli and Nardini, 1973), Friend virus (Salaman and Wedderburn, 1966; Chan *et al.*, 1968), Rauscher leukemogenic virus (Siegel *et al.*, 1969; Bennett and Steeves, 1970), and Moloney leukemogenic virus (Salaman and Wedderburn, 1966).

Cell-mediated immunity may also be affected, as shown by prolonged skin-graft rejection in mice following infections with LDV (Dent *et al.*, 1965; Howard *et al.*, 1969), Marek's disease virus (Purchase *et al.*, 1968), and NDV (Woodruff and Woodruff, 1974). Von Pirquet (1908) noted that measles infection resulted in a decreased dermal hypersensitivity to tuberculin, and this phenomenon has been observed during natural measles infection (Nablant, 1937; Beck, 1962) and shortly after vaccination with attenuated virus (Mellman and Wetton, 1963; Brody and McAlister, 1964; Starr and Berkovich, 1964). Transient diminished skin test responses to antigens of *Candida* and vaccinia virus, dinitrochlorobenzene, and poison ivy have also been observed following attenuated measles virus vaccination (Blumhardt *et al.*, 1968; Fireman *et al.*, 1969). Lymphocytes from subjects recently vaccinated with the attenuated virus showed transient diminished blastogenesis and DNA synthesis *in vitro* when exposed to measles antigen, tuberculin, and *Candida*

antigens, but not to appropriate concentrations of PHA (Smithwick and Berkovich, 1966; Fireman *et al.*, 1969; Finkel and Dent, 1973). Yamanouchi *et al.* (1974b) reported marked suppression of dermal hypersensitivity to PPD in sensitized rabbits for 2 weeks following infection with rinderpest virus.

Transient suppression of tuberculin sensitivity has also been reported during a few other viral infections. Suppression was observed during the acute phase of paralytic poliomyelitis (Carnevale and Iovino, 1959) and after immunization of tuberculous children with Sabin 1 vaccine (Berkovich and Starr, 1966). The latter authors reported one-third of the tuberculous children studied became tuberculin negative 4 to 6 weeks after Sabin immunization, and this state persisted for as long as 2 months. The long delay between vaccination and suppression was unexplained. The same authors (Starr and Berkovich, 1964) reported that 8 of 17 tuberculous children with varicella infection became tuberculin negative near the end of the 14-day incubation period and during the exanthem; reactivity returned to normal in a few days.

Nonspecific functions such as clearance by the reticuloendothelial system (RES) may be depressed (LDV, ectromelia, LCM, dengue, Sandfly fever) or elevated (VEE, murine leukemia viruses) by virus infections. Infection by LDV presents a special example of impaired clearance. Following infection, phagocytic activity of the RES as reflected by carbon particle clearance is only transiently impaired (Mahy, 1964) while there is permanent impairment of the clearance of certain enzymes (LDH, isocitrate dehydrogenase, malate dehydrogenase, aspartate transaminase) but not others (alkaline phosphatase, alanine transaminase). Little consideration has been given to the clearance of endogenous enzymes (those enzymes that are useful intracellularly but which, after becoming extracellular due to cell leakage or cell death, have no known extracellular functions). It has long been held that the RES plays a role in clearing proteins generally (Hyman and Paldino, 1960) and, based on their studies showing decreased clearance of endogenous enzymes after RES blockade, Wakim and Fleisher (1963) suggested a similar role for the RES in clearing endogenous proteins. Infection by LDV, which replicates almost exclusively in macrophages *in vitro*, appears specifically to eliminate or impair the function of a subpopulation of macrophages or cells of the RES responsible for clearance of certain endogenous enzymes. The specificity of the clearance cells for certain enzymes is presumably on a nonimmunological basis since there is no evidence to suggest that the extracellular enzymes are structurally (an-

tigenically) altered and therefore the animal should be tolerant to them. One can postulate that specific membrane receptors for the enzymes cleared exist on a particular population of cells infected by LDV. Because of its selective tropism, LDV may prove to be a useful tool to define and explore the functions of subpopulations of macrophages, especially in regard to their clearance functions.

There are many ways in which virus infections influence the immune system. Lytic infections may affect the thymus. Rowe and Capps (1961) described a virus, recently characterized as a herpesvirus (Parker *et al.*, 1973) which causes transient massive destruction of thymus cortex and medulla in newborn mice. Studies by S. Cross and H. M. Morse (personal communication) demonstrate that this virus (called the thymic agent) replicates in thymocytes of newborn mice and later can be found only in the salivary glands. Several weeks after infection, lymphocytes from thymus, lymph node, and spleen have minimal reactivity in the graft-versus-host reaction; lymph node cells show normal reactivity at 8 weeks postinfection. Mitogenic responses to T lymphocyte mitogens but not to lipopolysaccharide are also depressed for weeks (G. H. Cohen, personal communication). A human herpesvirus, varicella-zoster, can also grow in the thymus. Cheathem *et al.* (1956) observed evidence of virus growth in thymuses of children dying of fatal varicella infections. Many intranuclear inclusion bodies were seen in thymus reticular cells and Hassall's corpuscles were necrotic.

Measles infection of humans can result in thymus changes. White and Boyd (1973) studied autopsy material from eight children less than 3 years of age who died during or shortly after measles infection. Beginning 4 days after the onset of illness, there was severe aggregation and syncytial formation of thymocytes in the thymus cortex and subsequent total loss of cortex in some cases. No recovery of cortex was discernible until 3 or 4 months after infection. Examination of other lymphoid tissues, including spleen and mesenteric lymph nodes, did not reveal similar changes. In tissue cultures a prominent cytopathic effect of measles infection is syncytial formation (Enders and Peebles, 1954). White and Boyd felt the syncytial cell formation in the thymus differed from the Warthin–Finkeldey "giant cells" (Warthin, 1931) found in peripheral lymphoid tissue during the prodromal stage of measles. It is difficult to relate these thymus observations to the previously noted suppression of delayed hypersensitivity reactions by measles infection since, in mice at least, the effects of thymectomy on cell-mediated immunity are not seen for weeks. More likely, immunosuppression occurs because of

Fig. 16. Effect of infection with lactate dehydrogenase virus (LDV) on thymus-dependent area of mouse spleen. (A) Section of normal BALB/c mouse spleen showing white pulp. (B) Section of BALB/c mouse spleen 48 hours after infection with LDV. Note depletion of lymphocytes in periarteriolar (thymus-dependent) area of white pulp.

direct effects of measles virus on lymphocytes as demonstrated *in vitro*. Infections by other viruses of the medipest subgroup of paramyoviruses also produce lesions in the thymus as well as in other lymphoid tissues. Canine distemper virus infections of dogs produced thymus necrosis (McCullough *et al.*, 1973), as did rinderpest virus infections of rabbits (Yamanouchi *et al.*, 1974a).

Infection of mice with LDV causes transient depletion of thymus-dependent areas of lymph nodes and spleen (Fig. 16) (Proffitt *et al.*, 1972; Snodgrass *et al.*, 1972). Although LDV can grow in macrophage cultures, it does not replicate in lymphocyte cultures and cannot be demonstrated by electron microscopy within the lympho-

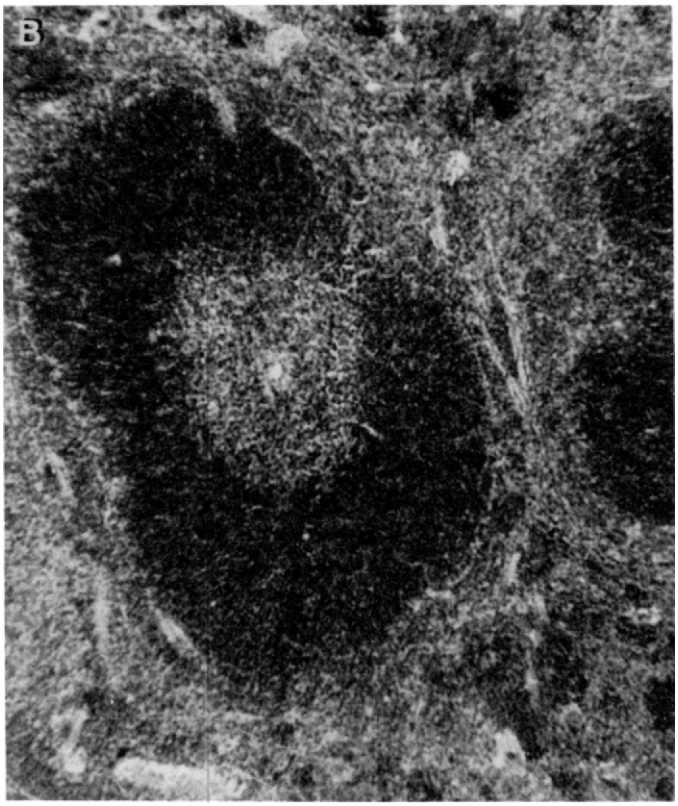

Fig. 16B.

cytes of infected spleens (Snodgrass *et al.*, 1972). It is postulated that its toxic effect on lymphocytes is mediated by viral growth in dendritic macrophages near the lymphocytes with disruption of tropic influences or the release of toxic materials. Alterations in T lymphocyte migrations could also result. Peripheral lymphoid necrosis is a common feature of infections by mouse hepatitis virus (Hirano and Ruebner, 1965; Biggart and Ruebner, 1970) as well as the medipest viruses already mentioned. The bursal infectious agent, an avian virus that causes lymphoid necrosis in young chickens resulting in a syndrome termed Gumboro disease, replicates in thymus, lymph nodes, spleen, and the bursa of Fabricius (Cheville, 1967). A severe inflammatory reaction occurs in the bursa, which is the only lymphoid organ that does not repopulate with lymphocytes after infec-

tion. During infection, viral antigen and virus particles can be detected in macrophages in the bursa but not in the affected lymphocytes. Damage to the lymphocytes may therefore be indirect and possibly of an autoimmune nature.

Many viruses are known to replicate in lymphocytes (Mims, 1964; Gresser and Lang, 1966; Edelman and Wheelock, 1967; Wheelock and Toy, 1973) and infections may result in chromosomal abnormalities (Nichols, 1966) and cell death. Persistent infections may result in more subtle disturbances of cell function. It has been hypothesized that infected lymphocytes may have alterations in protein synthesis, responses to hormones, interactions with other lymphoid cells, macrophages, or antigens, and in migration characteristics. Migration of lymphocytes *in vivo* is partly determined by cell membrane characteristics such as sialic acid content (Woodruff and Gesner, 1969; Berney and Gesner, 1970). The surfaces of infected cells are often altered (*vide supra*) and the appearance on cell membranes of viral neuraminidase after infection with SV5 can result in release of cell membrane sialic acid (Klenk and Choppin, 1970, 1971; Klenk *et al.*, 1970). Woodruff and Woodruff (1974) have reported that inoculation of NDV into rats causes a transient diversion of homing thoracic duct lymphocytes from lymph nodes and spleen to liver with resulting depletion of lymphocytes in thymus-dependent areas of nodes and spleen. This appeared to be due to viral neuraminidase acting on the lymphocyte surface. The importance of this phenomenon in natural infections is not clear.

Theoretically, latent infections in lymphocytes might convert to productive or lytic infections when the lymphocytes are stimulated to divide. Virus activation occurs in graft-versus-host reactions and in mixed lymphocyte reactions (Hirsch *et al.*, 1970, 1972), and the induced viruses (endogenous leukemogenic viruses) were found primarily in blastogenic lymphocytes (André-Schwartz *et al.*, 1973). Presumably, the viral genome is present in the phenotypically normal cells as postulated by the oncogene theory (Todaro and Huebner, 1972) and is activated by processes triggered by an allogeneic stimulus.

The importance of immunologic activation of viruses to human disease is suggested by the high incidence of malignancies of the RES found in renal allograft patients (Penn and Starzl, 1972). Relevant to this, Hirsch *et al.* (1973) reported that mice receiving ALS treatment as well as allografts had an increased incidence of activated viruses in their spleens compared to those receiving allografts without ALS treatment; no animals receiving ALS alone pro-

duced virus. Thus, host-versus-graft reactions can activate oncogenic viruses, especially if the host is receiving ALS therapy. Human lymphocytes stimulated with ALS replicate VSV (Edelman and Wheelock, 1968b) and HSV (Kleinman *et al.*, 1972) to higher titers than untreated cells.

The activation of nononcogenic viruses from lymphocytes by allogeneic stimulation is suggested by the cytomegalovirus posttransfusion syndrome. Isolation of CMV from donors or recipients prior to blood transfusion is rare (Mirkovic *et al.*, 1971) and lymphocyte–lymphocyte interactions may be required. A similar situation may exist for EB virus activation after transfusion (Henle *et al.*, 1970). Whether severe or chronic infections may also activate viruses is not known.

Some viruses, such as VSV (Edelman and Wheelock, 1967, 1968a), yellow fever (Wheelock and Edelman, 1969), mumps (Duc-Nguyen and Henle, 1966), and HSV (Nahmias *et al.*, 1964), grow little—if at all—in lymphocytes unless they are stimulated with PHA or antilymphocyte sera (Edelman and Wheelock, 1968b). VSV appears to grow predominantly in blastogenic T lymphocytes and perhaps preferentially in those with cytotoxic potential (Kano *et al.*, 1973). Concanavalin A and pokeweed mitogen stimulation of spleen lymphocytes resulted in 33-fold and 17-fold increases in virus plaque-forming cells, respectively, while lipopolysaccharide stimulation resulted in only a two-fold increase above background. Prior treatment of cells with anti-θ sera and complement or the use of athymic nude mouse spleen cells resulted in no virus plaque-forming cells. Mixed cultures of DBA/2 and CBA lymphocytes produced many cytotoxic lymphocytes and large numbers of virus plaque-forming cells were observed while cultures of BALB/c and DBA/2 lymphocytes resulted in even more blastogenesis but less cytotoxicity, and few virus plaque-forming cells were observed.

Infections by viruses requiring lymphocyte proliferation for their own replication, occurring fortuitously or activated from a latent state at a time of antigenic stimulation, might eliminate a rapidly dividing clone of immunocompetent cells specific for the stimulating antigen. Conceivably, a virus lytically infecting lymphoid cells at a time when they are responding to the antigenic stimulus provided by the virus itself might eliminate clones of immune cells specific for those virus antigens. Thus, specific states of tolerance similar to those produced by cyclophosphamide treatment of animals after antigenic exposure or virus infection (Nathanson and Cole, 1970) might arise.

Several approaches have been taken to establish which population of lymphocytes is primarily affected by virus infections. The ability of infected cells to participate in graft-versus-host reactions (Purchase *et al.*, 1968; Howard *et al.*, 1969; Bennett and Steeves, 1970), reject skin grafts (Howard *et al.*, 1969), and be stimulated by PHA or an *in vitro* allogeneic exposure (Hagry *et al.*, 1970) has been used to determine the immunocompetence of infected T lymphocytes, and transfer-reconstitution experiments with infected bone marrow cells have been used to ascertain B lymphocyte competence (Bennett and Steeves, 1970; Shearer *et al.*, 1973). Interpretation of these experiments is difficult since transfers of infected cells often result in transfer of infectious virus as well. Experiments with Friend virus are particularly complicated by contamination with Rowson–Parr virus, lymphatic leukemia virus, and LDV (Bennett and Steeves, 1970). In a recent transfer-reconstitution experiment, Shearer *et al.* (1973) demonstrated that the antibody response to a D-amino acid polypeptide (a thymus-independent antigen) was significantly reduced after infection of bone marrow cells with the SJL/J leukemogenic virus, regardless of whether thymocytes were infected or not. Hence, infection with this virus may result in a decrease in the response of B lymphocytes, but the experiments provide no information on possible effects on T lymphocytes. Peled and Haran-Ghera (1974) found that infection of mice with the radiation leukemia (Gross) virus resulted in depressed responses of spleen cells to sheep erythrocytes but normal responses to the thymus-independent antigens polyvinylpyrrolidine and pneumococcal polysaccharide SIII. They concluded that the immunosuppressive effect of this virus is on T lymphocytes.

The direct effect of measles virus infection on lymphocytes has been investigated by H. McFarland (personal communication). A mouse-adapted strain of virus was used to infect mice and measles antigen was present in spleen for 6 to 12 days after inoculation, but T lymphocytes were not killed as demonstrated by normal numbers of lymphocytes sensitive to anti-θ and complement. Mice were primed with ovalbumin-DNP (OA-DNP), a carrier–hapten conjugate, or with chicken γ-globulin (CGG), a secondary carrier, and their spleen cells used in combination to adoptively immunize irradiated recipient mice; secondary anti-hapten responses were measured after challenge with the appropriate conjugate. The effect of measles virus infection was ascertained by infecting either the CGG primed donor or the OA-DNP primed donors, and it was found that infection of CGG primed donors resulted in diminished anti-hapten responses while

infection of OA-DNP donors had no effect on the response. Sindbis virus infection of the carrier donor did not affect the response. Although these studies demonstrate an effect of measles virus infection on the helper cell population of T lymphocytes, impairment of other T lymphocyte activity may be responsible for the observed impairment of delayed hypersensitivity.

Finally, virus infections can abrogate tolerance. Inoculation of BALB/c mice with subimmunogenic amounts of pneumococcal polysaccharide (SIII), a relatively thymus-independent antigen, results in unresponsiveness to subsequent immunogenic amounts of antigen; this state lasts for months and is specific for SIII (Baker *et al.*, 1974). Tolerance in this system appears to be mediated by suppressor T cells since it is broken by the administration of ALS along with SIII; also, tolerance to SIII cannot be induced in athymic nude mice. P. J. Baker and W. H. Burns (unpublished observations) have found that infection with LDV will abrogate tolerance, although to a lesser extent than ALS administration. This is not due to an adjuvant effect since well-known adjuvants like complete Freund's adjuvant, poly I-C, and lipopolysaccharide do not abrogate tolerance in this system. Figure 16 shows that 48 hours after infection the thymus-dependent areas of the spleen (and lymph nodes) are depleted of lymphocytes and have the appearance of lymphoid tissue following administration of ALS. Whether LDV alters T cell migration or is directly or indirectly toxic to T cells is not clear. It is doubtful that the virus selectively impairs the functions of suppressor T cells, but rather impairs the functions of all or most T cells. In this system with SIII as antigen, the predominant influence of T cells on specific B cells is a suppressive one and destruction or impairment of T cell functions results in a diminution of their suppressive effect. Thus, as demonstrated here, virus infections can unbalance the normal regulatory mechanisms of the immune system. The frequency of virus infections in all animals, the presence of "endogenous viruses" in most or all cells, and the persistent and latent infections by "exogenous viruses" in many cells require an awareness by immunologists of the influences of these viruses on immune responses.

XVI. Concluding Remarks

During the past two decades the science of immunology has advanced rapidly. The various classes and subclasses of immuno-

globulins have been recognized and their structures and properties elucidated. Interactions of T and B lymphocytes and macrophages in the induction of immune responses have been extensively documented. Reactions of receptor immunoglobulins on B lymphocytes and the mechanism of synthesis of immunoglobulins in their progeny have been analyzed. Products of T lymphocytes activated by antigens and mitogens have been defined, and their effects on macrophages and other cells studied. Components of the complement system have been isolated and characterized, and the alternate path of activation of the complement system has been recognized.

At the same time considerable advances have been made in molecular virology and genetics. The structures of viruses, their nucleic acids, the specific polymerases involved in their replication, and the mechanisms of synthesis of the protein and glycoprotein components of their capsids and envelopes have all been studied in detail. The genetics of animal viruses and the genetic control of immune responses have received increasing attention. Temperature-sensitive mutants of viruses have been used to study their mode of replication and the mechanisms of malignant transformation. Human patients and experimental animals with inherited deficiencies of immunoglobulin or complement component formation and thymus deficiencies have already provided valuable information about differential susceptibility to infections.

For the most part the developments in immunology, virology, and genetics have been parallel but independent. In the future, the accumulated information and powerful analytical methods developed in these disciplines should be pooled for a combined approach to problems that have so far seemed intractable.

Some benefits of this sort of approach are already apparent. Many remarkable features of humoral immune responses came to light during the course of studies of antibodies against viruses. These include some of the earliest analyses of secretory antibodies, later shown to be mainly of IgA specificity, and features of immunologic memory such as "original antigenic sin" and IgM memory following repeated exposure to small doses of antigen. The cellular basis of these responses is still imperfectly understood, and with the high sensitivity of the plaque neutralization system, viral antigens offer interesting material for further study. Current studies analyzing at the cellular level the formation of B lymphocyte memory, the response of such B cells to the same or cross-reactive antigens and the influence of T lymphocytes on that recall should provide basic knowledge concerning T and B lymphocyte interactions.

Studies have just begun on the thymus dependence of viral

antigens, most of which resemble other proteins in being thymus-dependent. However, one (Sindbis envelope glycoprotein) is relatively thymus-independent and biochemical analysis of this molecule may provide insights concerning this interesting property.

The science of immunology began with observations of Jenner and later von Pirquet on the body's responses, beneficial and pathological, to virus infections. The devastating effects on patients with thymus deficiencies of virus infections usually mild in normal humans was the first indication of the biologic importance of cell-mediated immunity. Virus infections (as with ectromelia virus) of experimental animals have provided model systems for analyzing *in vivo* the roles of various lymphoid cells. The recent development of *in vitro* assays for various lymphocyte functions make feasible correlative studies with *in vivo* activities of different lymphocyte populations. Interesting information from such studies, particularly those using LCM virus, has already been discussed.

Mechanisms of immunity mediated by T lymphocytes and the part they play in protection against some virus infections are still imperfectly understood, but still less is known about the biologic importance of the cytotoxic system in which antibody sensitizes virus-infected target cells for destruction by nonspecific effector (K) lymphoid cells. This has recently been shown to occur *in vitro* with herpesvirus-infected cells and could play a role in immunity and immunopathology (Rager-Zisman and Bloom, 1974; Shore *et al.*, 1974). Model systems of viral infections should be useful in defining the importance of this phenomenon.

Finally, although first noted by von Pirquet almost 70 years ago, the effects of virus infections on immune responses has only recently received much attention. As already discussed, the presence of viral antigens with less immunogenic antigens may enhance responses to the latter. Viruses that infect or affect particular populations of lymphoid cells, e.g., the thymic agent and LDV, have been reported and the latter can abrogate tolerance to certain antigens. The tropism of viruses may prove to be a useful property for selectively depleting animals of certain lymphocyte populations. The causative role of such viruses in diseases and autoimmune phenomena is still relatively unexplored.

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