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Antibody-Mediated Destruction of Virus-Infected Cells

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I. Introduction

The nature of immune responses elicited by viral infections in animal hosts and the role such responses play in eliminating infection,

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producing tissue injury, or encouraging virus persistence have attracted extensive interest in recent years. These interactions are inevitably intricate. Added to the fundamental complexity of the immune response itself is the complicated nature of viruses as antigens, with their intracellular site, ability to replicate, multiple separate antigenic molecules, and, in some cases, ability to infect and replicate in lymphocytes, macrophages, and their precursor cells.

A number of immunologic mechanisms have been defined by which virus-infected cells can be killed *in vitro*. Antibody-independent cell-mediated cytotoxicity against virus infected target cells has received great attention. This is especially so in the last 5 years, since the demonstration that killing of virus-infected cells by specifically sensitized cytotoxic T cells occurs and is H-2 restricted in the mouse. This subject has recently been reviewed in this series by Zinkernagel and Doherty (1979). Even more recently the "spontaneous" cytotoxicity of murine and human lymphocytes for virus-infected targets (and for uninfected targets and tumor cell lines) has become the subject of intensive investigation, with the demonstration that the activity of natural killer cells can apparently be augmented by interferon. This topic, as it pertains to virus-infected cells, has again been reviewed for the murine (Welsh, 1978) and human (Santoli and Koprowski, 1979) systems.

Serum antibody, produced in response to virus infections, is of major importance in preventing the spread of infection by virtue of neutralizing free virus in extracellular fluids (reviewed by Daniels, 1975; Burns and Allison, 1975). Virus neutralization by antibody is enhanced by complement; antibody and complement can lyse enveloped virions, and complement may lyse or neutralize some viruses in the absence of antibody (reviewed by Oldstone, 1975; Cooper, 1979; Cooper and Welsh, 1979).

Antibody can also act in a number of ways on virus-infected cells, and this review is concerned with those effects of the immune response on virus-infected cells that are mediated by antibody. We deal with the actions of antibody alone, of antibody and complement, and of antibody interacting with cytotoxic cells, on virus-infected target cells. It should become apparent that, despite the foregoing emphasis on the role of antibody in neutralizing free virus and of cytotoxic T cells in killing virus-infected cells, antibody can profoundly affect virus-infected cells *in vitro;* there is little reason for thinking these actions may be less important *in vivo* than other cytotoxic mechanisms demonstrated *in vitro*. Furthermore, as with cytotoxic T cells, antibody-mediated effects on virus-infected cells can occur prior to re-

lease of progeny virus, eliminating a source of continuing virus production.

In surveying this subject, emphasis is given to the effect of antibody on virus-infected cells in homologous human systems and, particularly in Sections III and IV, to work from this laboratory.

II. Viral Antigen Expression and the Antibody Response

A. Viral Antigen Expression on the Surfaces of Infected Cells

Some aspects of the mechanism by which viral proteins are expressed on the surfaces of infected cells merit discussion, as these are the target antigens whose recognition initiates immune lysis. All enveloped RNA viruses, which acquire an outer lipid envelope by budding from the host cell plasma membrane, express viral glycoproteins on the cell surface. This group comprises, in part, togaviruses, negative strand RNA viruses (arena-, rhabdo-, orthomyxo-, and paramyxoviruses) and retroviruses. DNA viruses, such as herpes viruses, which bud from the nuclear membrane, and poxviruses, which manufacture their own envelope, also express structural viral glycoproteins on the cell surface. In addition, virus infection may cause expression of nonstructural viral proteins (e.g., tumor-specific transplantation antigens) or alter expression of host membrane proteins.

It is worthwhile briefly to summarize the mechanism for assembly of enveloped viruses. After viral entry into a cell and uncoating, transcription of viral mRNA and its translation into viral proteins on host cell ribosomes follow. Enveloped viruses have three main classes of structural proteins: nucleocapsid proteins, matrix (M) proteins, and envelope glycoproteins. The nucleocapsid protein is associated with the viral nucleic acid, the M protein (not present in togaviruses) is a peripheral membrane protein associated with the cytoplasmic surface of the cell membrane (see Choppin and Compans, 1975). The glycoproteins are inserted into the lipid bilayer of the plasma membrane, probably as transmembrane proteins, with a hydrophobic region in the bilayer and a hydrophilic glycosylated portion projecting on the outer surface (reviewed in Compans and Kemp, 1978).

The evidence suggests that viral glycoproteins are synthesized, glycosylated, and transported to the plasma membrane by the same mechanism as host cell integral membrane and secretory glycoproteins. The work of Lodish and colleagues on vesicular stomatitis virus

(VSV) glycoprotein provides a model for this process (Rothman and Lodish, 1977). Briefly, the nascent polypeptide chain synthesized on membrane-associated polyribosomes, is inserted through the membrane of the endoplasmic reticulum, insertion apparently being mediated by a specific "signal" peptide sequence in the chain in accordance with the "signal hypothesis"(Blobel and Dobberstein, 1975). Glycosylation then occurs on the noncytoplasmic side of the membrane utilizing the host cell glycosylating enzymes, and the protein is transported to the plasma membrane in a vesicle that fuses with the membrane. Viral glycoproteins appear to be mobile in the lipid bilayer, as are host cell integral membrane proteins (see Section III), and become distributed at random over the surface of the cell. Prior to the process of budding, glycoproteins locate in the area of budding; M protein aligns under the glycoproteins on the cytoplasmic side of the membrane, the nucleocapsids also align under the M protein, and the whole complex is then enveloped in a bud of plasma membrane. In the virion, as in the cell, the glycoproteins are thus the exposed viral proteins. The lipid envelope reflects the composition of the cell plasma membrane from which it is derived (Lenard and Compans, 1974). Most evidence indicates that host cell membrane proteins are almost totally excluded from the viral envelopes during budding (see Holland and Kiehn, 1970). There is some circumstantial evidence that H-2 antigens can be incorporated into Friend leukemia virus (FLV) (Bubbers and Lilly, 1977). This was based on adsorption of antisera to H-2 by FLV purified from viremic mice. However, virus had to be disrupted to demonstrate this adsorption, and there is as yet no firm confirmatory biochemical evidence demonstrating incorporation of gene products of the major histocompatibility complex (MHC) into enveloped viruses. It does seem definite that cellular actin, a peripheral membrane protein, can be incorporated into some enveloped viruses, e.g., measles virus, VSV, and influenza virus, as demonstrated by its presence in purified virions. However, this is probably a passive association, as exposure of infected cells to cytochalasin B does not prevent incorporation of actin into VSV or influenza virions, or inhibit budding (Griffin and Compans, 1979). Hence, contractile protein function is apparently not required for the budding process.

Viruses may in some circumstances bud from particular domains of the plasma membrane. Studying two different cultured lines of epithelial cells grown as monolayers, Boulan and Sabatini (1978) found that influenza virus, simian virus 5, and Sendai virus budded exclusively from the apical (free) surface whereas VSV budded only from the basolateral plasma membrane. This implies that certain viruses

might be less susceptible to immune recognition at the cell surface because they bud from areas of the cell removed from direct contact with blood.

A point worth making is that these viruses must express virus-specific proteins on the cell membrane before release of free virions occurs. It is also apparent that lysis of a cell infected with a budding virus would be unlikely to result in release of infectious virus, as the nucleocapsids would be devoid of the envelope glycoproteins that mediate virion attachment to the cell membrane prior to entry.

The herpes simplex viruses (HSV), which have been widely used in *in vitro* cytotoxicity studies, are structurally much more complex, coding for some 50 viral polypeptides (Roizman and Furlong, 1974). Viral proteins are inserted in cytoplasmic and plasma membranes, and the viral cores can gain envelopes by budding from any of these membranes. An intriguing feature of HSV is their ability to induce the appearance of an Fc receptor for human or rabbit IgG on the membrane of infected cells (Watkins, 1964; Westmoreland and Watkins, 1974). Although the structural relationship of this receptor to the "endogenous" Fc receptors on lymphocytes and phagocytic cells is unknown, it seems possible that this is a virus-encoded gene product, and it can reportedly be blocked by a $F(ab')_2$ antibody to HSV (McTaggart *et al.*, 1978; Adler *et al.*, 1978). It has also been reported that cytomegalovirus can induce Fc receptors on infected cells (Rahman *et al.*, 1976; Westmoreland *et al.*, 1976). It remains to be seen whether this novel surface structure participates in any definite way in immunologic reactions. Some preliminary evidence indicating that this may be so is summarized in Section III.

Unlike other DNA viruses, poxviruses replicate in the cytoplasm rather than the nucleus and, unlike budding viruses, synthesize their own lipid membranes, but nevertheless express viral antigens on the cell surface, which can appear as early as 1 hour after infection (Ada *et al.*, 1976).

B. The Antibody Response to Viral Infection

Studies with poliovirus in rabbits (Svehag and Mandel, 1964) and humans (Ogra *et al.*, 1968) show a similar sequence in the development of antibody responses to that described for other viral and nonviral antigens. After immunization the level of neutralizing IgM antibody attains maximal titers in 3–4 weeks and is undetectable by 3 months. IgG titers rise in parallel but continue to increase for several months and persist at lower levels for years. Serum IgA antibody is first detectable several weeks after immunization, and titers rise for

several months. Secretory IgA responses occur at local sites of infection of mucosal surfaces with live virus. Subsequent challenge with virus elicits a further transient IgM, in addition to a continuing IgG, response. Studies with a number of other viruses show a similar pattern of response. Many viral antigens appear to be thymus dependent, as indicated by their eliciting no antibody or only small amounts of IgM antibody in nude mice (Burns *et al.*, 1975; reviewed in Oldstone, 1979). Although in all these studies of the immune response antibody is measured as neutralizing antibody, it is very likely that the same antibodies also recognize antigens on infected cells; neutralizing antibodies are directed against external proteins on the virion, which are likely to be those expressed also on infected cell membranes.

Much of the work on the relative importance of responses to individual viral antigens has been done with influenza virus. This is largely because the biochemistry of influenza virus, including detailed knowledge of the structure of the glycoproteins, is better understood than for other viruses. Passive transfer of antibody to the M or nucleoprotein (both internal) polypeptides did not protect infected mice, whereas transfer of antibody to the hemagglutinin (HA) or, to a lesser extent, the neuraminidase, was protective (see Virelizier *et al.*, 1979). Direct visualization of the bromelain-extracted influenza HA and its reaction with IgG antibody, by electron microscopy, shows that antibody reacts with the tip of the molecule—the end of the spike farthest from the membrane of the virion or cell membrane (Wrigley *et al.*, 1977), and the only site on the HA likely to be sterically accessible to antibody on the virion.

In paramyxoviruses the relative response to the two envelope proteins may be important in effective immunity. Norrby and associates (Norrby *et al.*, 1975; Norrby and Penttinen, 1979) showed that formalin-inactivated mumps vaccine or Tween 80–ether-inactivated measles vaccine induce antibodies only to the viral HA, not to the fusion (F) protein (or hemolysin), compared to immunization with live attenuated viruses, which induces antibodies against the F protein. In the case of measles virus, exposure to wild-type virus after immunization with the inactivated virus sometimes produces the clinical syndrome of "atypical measles" with prominent pulmonary infiltrates and severe rash, and the inactivated vaccines generally produce a poor level of immunity.

The extensive literature on the subject of humoral immune responses and viral antigens is reviewed by Ogra *et al.* (1975) and Burns and Allison (1975).

III. Effect of Antibody Alone on Virus-Infected Cells

In the absence of any effector system, such as complement or antibody-dependent cytotoxic K cells, antibody itself can act on virus-infected cells to affect the release of progeny virus and expression of viral antigens. These actions appear to be at least partially reversible and, in some circumstances, capable of protecting the infected cell from subsequent immunologic attack.

A. Antibody-Induced Redistribution of Surface Viral Antigens

The phenomenon of capping of integral membrane proteins by antibodies or lectins is well described. Binding of antibody initially causes patching of the antigen, followed by polar redistribution of the cross-linked membrane proteins as a cap. The initial patching is energy independent, whereas capping is temperature and energy dependent and involves the participation of the cell contractile proteins. Divalent antibody is required to produce capping. Binding of antibody appears to induce transmembrane linkages of integral membrane proteins to intracellular actomyosin-containing filaments at the patching stage for cells in suspension (Bourgignon and Singer, 1977) and in monolayers (Ash *et al.*, 1977). Most, if not all, integral membrane proteins can behave in this way. The subject as it applies to B lymphocytes has been reviewed in this series by Schreiner and Unanue (1976).

Viral glycoproteins expressed on cell membranes can also be capped by antibody as shown independently for measles (Joseph and Oldstone, 1974; Lampert *et al.*, 1975; Ehrnst and Sundquist, 1975) and influenza (Rutter and Mannweiler, 1976) and subsequently for other viruses. Capping of measles viral glycoproteins required active cell metabolism, a functioning cytoskeleton, membrane ATP, and divalent antibody (Joseph and Oldstone, 1974) (see Table I). These glycoproteins are thus capable of lateral diffusion in the membrane and behave in this respect as do host cell integral membrane proteins (Fig. 1). Electron microscopy studies of measles virus-infected cells showed that the nucleocapsids move in concert with the glycoproteins on the cytoplasmic side of the membrane as the latter cap (Lampert *et al.*, 1975), indicating that a transmembrane connection exists between the surface and internal polypeptides. In studies using fluoresceinated monospecific antisera, low concentrations of cytochalasin B are reported to prevent this associated movement of nucleocapsids (Tyrell and Ehrnst, 1979). More-detailed studies are needed to deter-

TABLE I
INHIBITION OF CAP FORMATION IN HELA CELLS INFECTED WITH MEASLES VIRUS[a]

Reagent	Final concentration of sample	Percentage Inhibition of capping
Sodium azide	10^{-2} *M*	57
Sodium azide	10^{-3} *M*	42
2,4-Dinitrophenol	10^{-5} *M*	50
2,4-Dinitrophenol	10^{-6} *M*	30
Oligomycin D	10^{-7} *M*	70
Oligomycin D	10^{-8} *M*	20
N,N'-Dicyclohexyl carbodiimide	10^{-4} *M*	0
N,N'-Dicyclohexyl carbodiimide	10^{-5} *M*	0
1-Ethyl-3(3-dimethylaminopropyl) carbodiimide	10^{-2} *M*	0
1-Ethyl-3(3-dimethylaminopropyl) carbodiimide	10^{-3} *M*	0
Colchicine	10^{-2} *M*	84
Colchicine	10^{-3} *M*	31
Cytochalasin B	10 μg/ml	92
Cytochalasin B	1 μg/ml	38
Vinblastine sulfate	10^{-5} *M*	69
Vinblastine sulfate	10^{-6} *M*	31
Ethylenedinitrile tetraacetate	10^{-1} *M*	0
Ethylenedinitrile tetraacetate	10^{-2} *M*	0

[a] Effect of various inhibitors on antibody-induced cap formation on measles virus-infected HeLa cells (from Joseph and Oldstone, 1974). Cells were preincubated with these inhibitors for 15 minutes at 37°C and then with FITC conjugated antibody to measles for 1 hour at 37°C.

mine whether separate viral envelope glycoprotein species expressed on the membrane cocap, or whether they can behave as independent molecules.

The evidence on whether host cell membrane proteins, particularly products of the MHC genes, cocap with viral proteins is somewhat contradictory, partly owing to studies with different systems and antisera. Rauscher virus gp70 was initially reported to cocap with H-2 on tumor cells (Schrader *et al.*, 1975). Later, using well defined antisera, it was found that retroviral gp69/71 cocapped with H-2 and with TL on a mouse thymoma line (Bourgignon *et al.*, 1978). However, in this same study, capping of T200, which is a host cell surface glycoprotein, also induced cocapping of H-2, and of the Thy 1 and TL antigens. This suggested that H-2 antigens may cocap with a variety of independent cell surface molecules, possibly, as postulated in this report, because they are all linked to the actomyosin filaments (Bourgignon *et al.*,

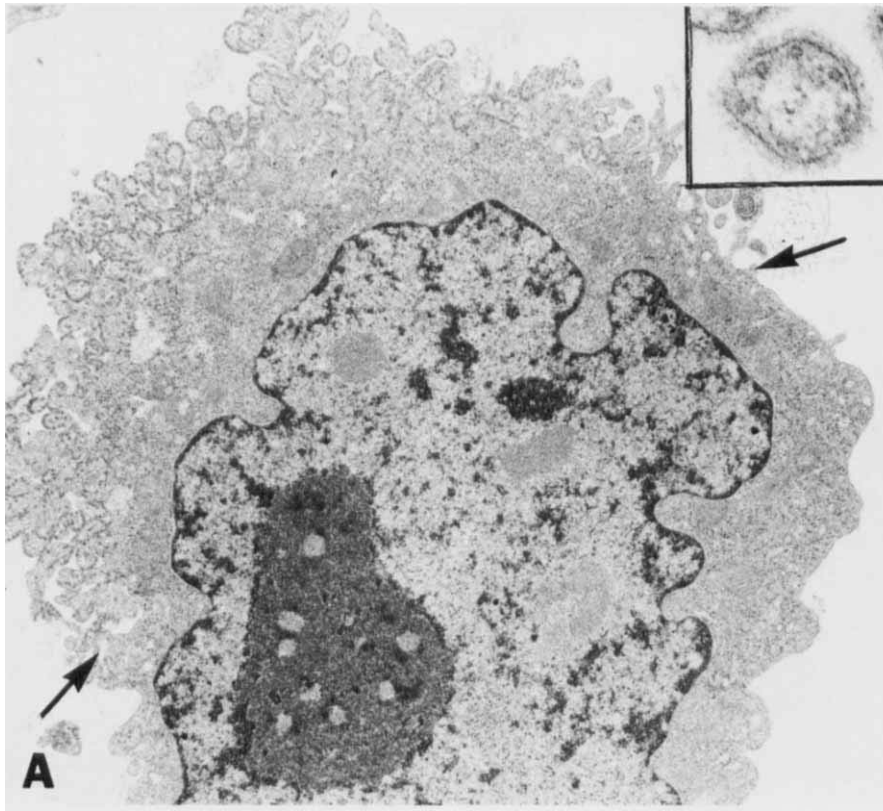
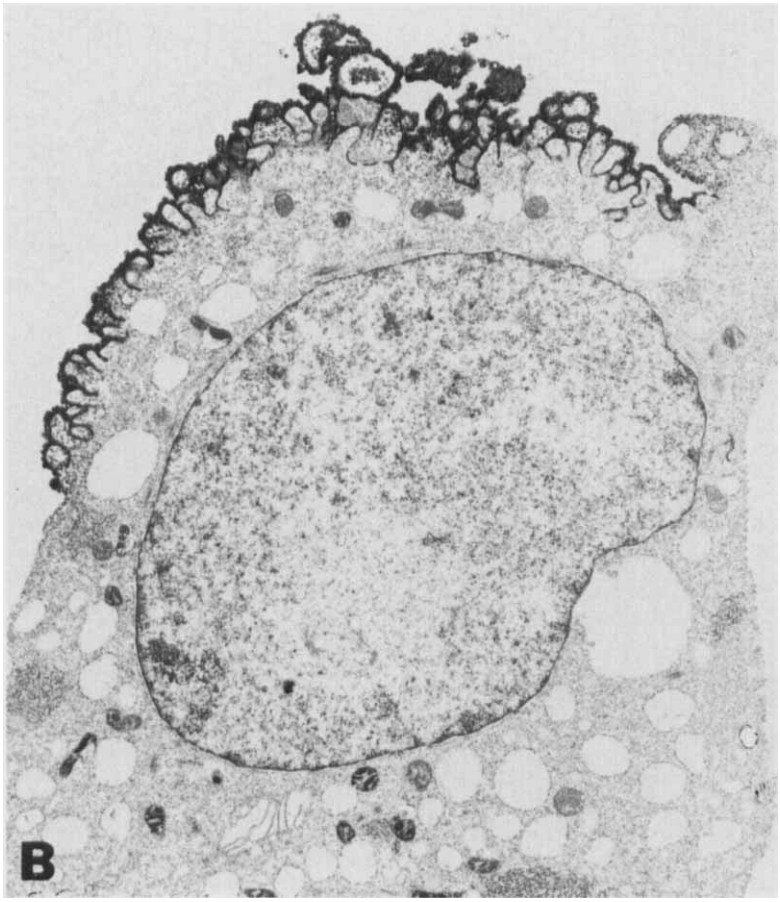
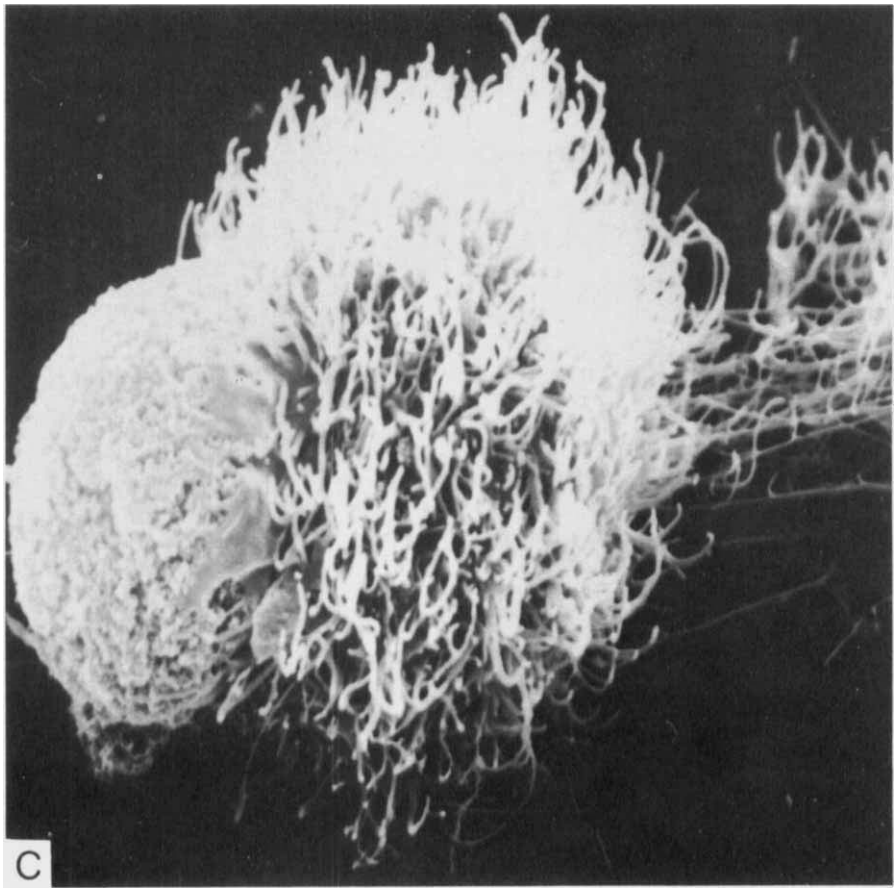


FIG. 1. (A) Electron micrograph of a measles virus-infected HeLa cell after incubation with IgG antibody to measles virus for 1 hour at 37°C. The budding virions have moved to one pole (between arrows) of the cell. Inset shows higher magnification of measles virion. (B) Measles virus-infected HeLa cell exposed to antimeasles virus antibody and peroxidase-labeled antihuman IgG antibody. Capping of the viral antigen –antibody complex has occurred. Minimal endocytosis of the peroxidase label was observed. (C) Scanning electron microscopy of measles virus-infected HeLa cell after capping with antibody to measles, showing movement of the microvilli into a polar position (Lampert *et al.*, 1975).

1978). In contrast to those reports with retroviruses, measles viral antigens expressed on the plasma membrane do not cocap with HLA antigens (Haspel *et al.*, 1977). Lymphocytic choriomeningitis virus (LCMV) expresses one surface glycoprotein on infected cells, and this viral polypeptide does not cocap with either H-2K or H-2D determinants (M. B. A. Oldstone, unpublished observations). Furthermore, in a recent careful biochemical study using immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-



PAGE), no evidence for molecular association of MHC molecules and Moloney virus gp70 could be found on a Moloney virus lymphoma line (Fox and Weissman, 1979). One major interest of these observations is their relevance to the debate on whether cytotoxic T cells recognize MHC products and viral antigen as a complex, or as two independent molecules (see Zinkernagel and Doherty, 1979). In this context additional evidence arguing against a direct molecular association of H-2 antigens and viral glycoproteins, at least occurring during transcription or translation, is provided by experiments mixing independently synthesized proteins. Thus, secondary *in vitro* cytotoxic T cell responses can be generated by liposomes containing isolated



H-2 antigens and Sendai viral glycoproteins (Finberg *et al.*, 1977); and heterokaryons formed by fusing cells bearing SV40 antigens, but inappropriate H-2 antigens, with uninfected cells of appropriate H-2 haplotype, are reported to act as targets for H-2 restricted cytotoxic T cells (Watt and Gooding, 1980).

The actual binding of antibody to determinants at the cell surface could be affected by a number of local factors. As an example, Sissons *et al.* (1979b) investigated the binding site of measles virus antibodies to the surface of radioiodinated measles virus-infected HeLa cells. After exposure of intact cells to IgG containing measles antibody at 4°C to inhibit subsequent capping, the cells were washed and deter-

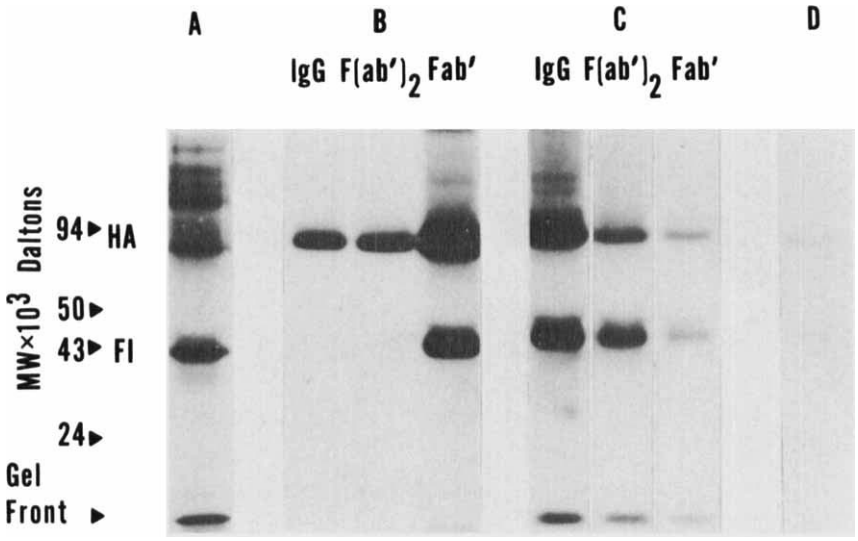


FIG. 2. Binding sites of IgG antibody and its fragments on surface of measles virus-infected HeLa cells. Autoradiogram depicts reduced SDS-PAGE analysis of immunoprecipitates from surface radioiodinated HeLa cells acutely infected with measles virus. (A) Detergent-solubilized cells, not immunoprecipitated. (B) IgG, $F(ab')_2$, or Fab' added in equal amounts to intact cells, followed by washing, detergent solubilization, and addition of protein A. (C) IgG, $F(ab')_2$, or Fab' added after detergent solubilization of cells. (D) Control. HA, measles virus hemagglutinin; F1, the major reduction fragment of the measles virus fusion (F) protein. Note that IgG and $F(ab')_2$ added to intact cells bind only to HA, whereas the univalent Fab' binds to both HA and F1 (panel B). This could be explained by divalent antibody binding to HA sterically hindering the access of antibody to F. (From Sissons *et al.*, 1979b.)

gent solubilized, after which the material binding to staphylococcal protein A was analyzed by SDS-PAGE. IgG antibody bound to both viral glycoproteins on cells infected 24 hours, but at later stages of infection antiviral IgG or $F(ab')_2$ bound only to the HA glycoprotein whereas the same amount of Fab' bound to both HA and F glycoproteins (Fig. 2). These findings were interpreted as showing that, at high densities of antigen, divalent antibody to HA could sterically block the access of antibody to F at the cell surface. Studies of actual binding sites of antibody at the infected cell surface would be of interest in regard to other viruses.

B. Antibody-Induced Antigenic Modulation of Virus-Infected Cells

Antigenic modulation is a term originally introduced by Boyse *et al.* (1963, 1967) to describe the reversible loss of the TL^+ (thymic leukemia antigen) phenotype from TL^+ leukemia cells passaged in immunized mice. These cells lost their sensitivity to lysis when a new source of anti-TL antibody and guinea pig complement was added, but the TL^+ phenotype and susceptibility to lysis returned after culture in the absence of antibody or passage in unimmunized mice. Antigenic modulation of TL, and in other tumor systems, is reviewed in detail by Stackpole and Jacobson (1978). It now seems that a similar phenomenon can occur when virus-infected cells are exposed to antibody, although the mechanism may not necessarily be precisely identical to the TL system.

1. Antigenic Modulation of Measles-Infected Cells

Joseph and Oldstone (1975) found that culture of measles virus-infected HeLa cells in the presence of antibody to measles virus rendered them resistant to lysis when exposed to a fresh source of human antibody and human complement. The authors termed this "antibody-induced viral antigenic modulation." Antibody-induced measles virus antigenic modulation has subsequently been studied in detail. HeLa cells persistently or acutely infected with measles virus were incubated in heat-inactivated human serum containing antibody to measles virus and then maintained in suspension culture with 20% of the same heat-inactivated human serum. At timed points cells were removed from culture and washed. The infected cells' susceptibility to lysis by human antiviral antibody and complement underwent a progressive decline, which was maximal by 12 hours. This correlated with loss of accessible viral antigens from the cell surface as detected by surface staining with fluoresceinated anti-measles IgG or by binding of radiolabeled antibody (see Fig. 3). After the initial few hours of culture in the presence of antibody, only minimal degrees of cap formation were evident on the measles-infected cells. Acutely infected cells maintained in culture regained their susceptibility to lysis by 48 hours, whereas persistently infected HeLa cells regained susceptibility only if washed and recultured in the absence of antibody. This difference probably reflects the differing rate and density of viral glycoprotein synthesis between the two cell types, the acutely infected cell synthesizing enough viral antigen to eventually bind all the antibody in the experimental system used and escape modulation. Under these

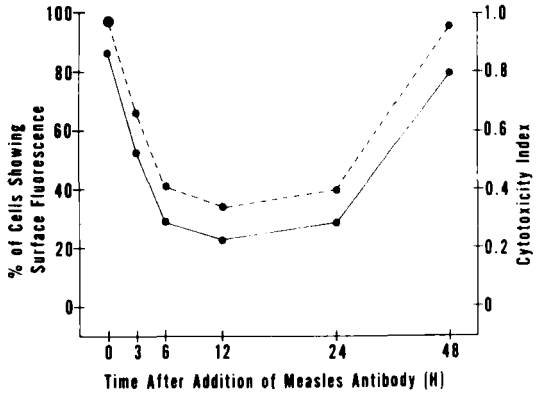


FIG. 3. Antigenic modulation of measles viral antigens expressed on the surface of acutely infected HeLa cells. Alteration of surface expression of measles viral antigens (●----●) and antibody-mediated complement (C)-dependent lysis (●——●) after incubation of acutely infected HeLa cells with measles virus antibodies. HeLa cells were infected with measles virus at a multiplicity of infection of 0.2, and on day 4 of infection they were cultured in suspension with antiviral antibody. Surface expression of measles virus antigens and susceptibility to antibody-mediated C-dependent lysis parallel each other. (From Joseph and Oldstone, 1975.)

same conditions, cells persistently infected with measles virus completely lost their susceptibility to lysis by peripheral blood lymphocytes from immune subjects, within 3 days in culture (Oldstone and Tishon, 1978).

Culture of cells infected with measles virus for longer periods in the presence of antibody can result in the selection of attenuated virus (Rustigian, 1966; Gould and Almeida, 1977), although the biochemical structure of such selected viruses has not been examined carefully.

2. *Fate of Virus Antigen–Antibody Complex on Infected Cells*

The fate of the virus antigen–antibody complex on the infected cell is important to any understanding of the mechanism of antigenic modulation. In general, the approach used to study ligand interactions with cell surface molecules has been to label one or the other component of the complex and follow its fate. When ^{125}I-labeled IgG antibody was bound to the surface of measles virus-infected cells at the start of the culture period, under the conditions described above for antigenic modulation, about 40% of the radioactivity was still cell associated at 12 hours and nearly all was protein bound (Joseph and Oldstone, 1975; Perrin and Oldstone, 1977). The fate of the antibody

was followed in detail by Perrin and Oldstone (1977). After 3 hours in culture 30% of the [^{125}I]IgG was present in the supernatant, and analysis on linear sucrose density gradients showed that a third of this labeled IgG sedimented in a peak heavier than 7 S IgG. This peak of heavier IgG also contained measles virus HA but not nucleocapsid, as detected by immunoprecipitation with specific antibodies. Material from this peak bound to rheumatoid factor and C1q, and to Raji cells, indicating the presence of immune complexes. Thus, these findings suggested that a significant amount of complexed antibody and viral glycoprotein was shed from the cell surface, and the absence of nucleocapsids indicated that the antibody was unlikely to be complexed with whole virus. In contrast, only a small amount of antibody appeared to be endocytosed. This was shown by studying cell lysates in SDS in which most of the [^{125}I]IgG was 7 S, but some non-TCA precipitable material was present, suggesting intracellular degradation in lysosomes (Perrin and Oldstone, 1977).

Immunoelectron microscopy studies of measles-infected cells capped with IgG antibody to measles and then labeled with peroxidase conjugated anti-IgG rarely showed any internalized peroxidase (Lampert *et al.*, 1975). Hence, endocytosis of the viral antigen–antibody complex appears to be unusual in the 80-minute duration of these latter experiments and accounts for only a minority of the complex during the longer interval of modulating conditions studied by Perrin and Oldstone (1977). Some endocytosis of peroxidase label was seen in electron microscopy studies of antibody-treated measles-infected syncytial cells (P. W. Lampert and M. B. A. Oldstone, unpublished observations; Hooghe-Peters *et al.*, 1979) and influenza-infected cells (Rutter and Mannweiler, 1976) after several hours of incubation. However, the use of the second peroxidase-coupled antibody has been shown to favor endocytosis of membrane proteins, at least in B lymphocytes (Schreiner and Unanue, 1976) and makes the system more artificial compared to the situation *in vivo*.

In recent experiments the fate of viral cell surface antigens was followed by surface radioiodination of measles virus-infected cells and maintenance of the cells in culture with or without antibody. The rate of disappearance of ^{125}I-labeled viral glycoproteins from the cell was then determined by running cell lysates on SDS–PAGE and excising and counting the viral glycoprotein bands. After an initial acceleration, the turnover rate of the labeled viral glycoprotein in cells cultured with antiviral antibody was no greater than in cells cultured in the absence of antibody (Fujinami and Oldstone, 1980; Fujinami *et al.*, 1980). This suggests that, after initial shedding, antibody does not

subsequently enhance the net rate of viral glycoprotein loss from the cell under the culture conditions used.

In contrast to the fate of viral antigen–antibody complexes on the plasma membrane, ligand interaction with surface Ig or Fc receptors on B lymphocytes results primarily in their endocytosis (reviewed Schreiner and Unanue, 1976). The fate of other cell surface molecules complexed with antibody is less defined, although it appears that MHC molecules on B cells are less likely to be endocytosed and may be shed (Unanue *et al.*, 1972; reviewed by Schreiner and Unanue, 1976).

3. *Antigenic Modulation in Other Viral Systems*

The only other viral systems in which antigenic modulation has been studied involve oncogenic murine retroviruses. Gross virus leukemia antigens can be modulated *in vivo* in immunized mice (Aoki and Johnson, 1972) and *in vitro* (Ioachim and Sabbath, 1979), as shown by resistance to antibody and complement-dependent lysis. There is also evidence for antigenic modulation in FLV-induced leukemia. This was demonstrated *in vitro* with FLV-infected cells by Genovesi *et al.* (1977) and is likely to play a role *in vivo* (Doig and Cheseboro, 1979). Presence of antiviral antibody, loss of FLV antigens from the leukemic cell surface, and recovery from viremia were all associated with a single genetic locus, named *RFV-3*, which is not linked to the MHC. It was suggested that *RFV-3* acts as an immunoregulatory gene influencing production of cytotoxic anti-FLV antibodies. Nevertheless, despite this evidence for antigenic modulation, progressive fatal leukemia occurred unless appropriate separate *H-2*-linked genes, influencing recovery from leukemia, were associated with the *RFV-3* locus (Doig and Cheseboro, 1979).

Mouse mammary tumor virus antigens can also be specifically modulated *in vitro* (Calafat *et al.*, 1976). Shedding of viral antigen–antibody complexes was also prominent in this study, as assessed by immunoelectron microscopy. These studies are further reviewed by Oldstone *et al.* (1980) and Stackpole and Jacobson (1978).

C. Effect of Antibody Binding to Infected Cells on Synthesis and Expression of Viral Proteins

Antibody in the medium of infected cultured cells results in a decrease of detectable virus in the medium. The cause is in part complexing of antibody with free virus released from the cell, but also cross-linking of viral glycoproteins on the cell surface by antibody, thereby inhibiting release of virus, probably by interfering with the

budding process. The release of influenza virus was inhibited by antibody to the HA and, less effectively, by antibody to neuraminidase (Dowdle *et al.*, 1974); Fab fragments of antiviral IgG had earlier been reported not to inhibit release of virus (Becht *et al.*, 1971).

In addition to these effects of antibody on the release of virus, there is evidence to suggest that antibody may also affect the synthesis and/or expression of viral proteins inside the cell. Fujinami and Oldstone (1979) have recently studied the effect of specific antiviral antibody [under the conditions used by Joseph and Oldstone (1975)] on the expression of individual measles virus polypeptides in acutely infected HeLa cells. After culture in the presence of antiviral antibody in the medium for 6–12 hours, the cells were washed and their incorporation of various labeled precursors into viral polypeptides was studied and compared to controls. It was found that expression of the F protein was diminished as detected by decreased incorporation of [^{35}S]methionine. Since turnover of F protein at the cell surface was not appreciably accelerated (Fujinami *et al.*, 1980), this observation most likely represents diminished synthesis of F protein. Interestingly, expression of a cytoplasmic viral polypeptide, the measles virus phosphoprotein (P) was also diminished (as assessed by [^{35}S]methionine and ^{32}P incorporation) (see Fig. 4). P protein is associated with the transcriptive complex and is probably necessary for replication of the viral genome (Mountcastle and Choppin, 1977). The effects were specific for antiviral antibody, as they could be produced exclusively by purified antiviral IgG, but not by incubation with antibody to the HeLa cell surface or nonimmune IgG. Hence, regulation of an internal viral polypeptide by antibody initially present outside the cell can occur. Whether a transmembrane signal is produced by antibody binding to the plasma membrane or whether the effect is mediated by pinocytosed antibody acting inside the cell is not known.

Yagi *et al.* (1978) observed changes in the polypeptide profile of mouse mammary tumor virus when tumor cells were cultured in the presence of antibody *in vitro,* although these changes were not analyzed in detail with regard to individual proteins.

There is evidence that antibodies can inhibit viral transcription and translation in cell-free systems. Thus, antibody to the VSV viral transcriptase immediately inhibited RNA transcription by VSV nucleocapsids (Imblum and Wagner, 1975), and antibody to Newcastle disease virus nucleocapsid had the same effect (Miller and Stone, 1979). These results, presumably produced by direct binding of antibody to viral enzymes, may be useful tools in molecular virology. However, the observation that antibody can affect viral polypeptide expression

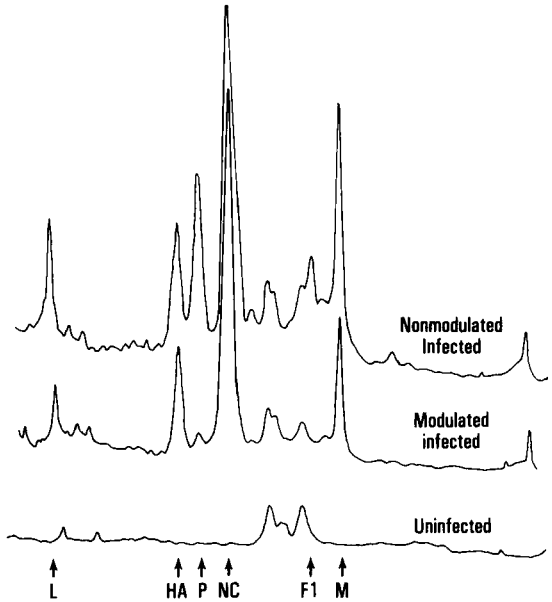


FIG. 4. Inhibition of expression of measles virus phosphoprotein (P) and fusion (F) protein in infected cells by antibody to measles virus. Polypeptide profile of modulated and nonmodulated infected HeLa cells, represented by a densitometer scan of reduced SDS-PAGE of cells pulse labeled with [^{35}S]methionine for 2 hours. Prior to labeling, cells were incubated with or without antibody for 6 hours. P protein and F (seen here as its major reduction fragment F1) are reduced in cells cultured with antibody. Peak found in uninfected cells migrating just before F1 probably represents cellular actin (P is found inside the cell; F is expressed on the cell's surface). (From Fujinami and Oldstone, 1979, 1980.)

in intact cultured cells suggests that the synthesis, transport, or assembly of viral polypeptides could be significantly affected by antibody *in vivo*.

D. Interaction of IgG and the Herpes Simplex Virus-Induced Fc Recptor

Both HSV and cytomegalovirus can induce formation of an Fc receptor for IgG on the surface of infected cells. Costa *et al.* (1977) cultured Vero cells infected with HSV-2 in the presence of nonimmune rabbit IgG or its Fc fragment at concentrations of 5–10 mg/ml and found a marked reduction in virus production. Since the IgG had no neutralizing effect on free virus or cytotoxic effect on infected cells, it was suggested that binding to the Fc receptor inhibited the growth of

HSV. Costa *et al.* did not record the effect of $F(ab')_2$ fragments or of the same IgG preparation on other virus-infected cells. Adler *et al.* (1978) reported that preincubation of HSV-1 infected rat neuroma cells with aggregated nonimmune IgG resulted in loss of susceptibility to lysis by antibody and complement. The effect of native nonaggregated IgG was not reported; although this report mentions that no capping was observed when FITC-aggregated IgG was used, there have been no systematic studies of the fate of HSV Fc receptors binding IgG. In contrast, Rager-Zisman *et al.* (1976) reported that aggregated nonimmune IgG could mediate cytotoxicity of HSV-infected cells by normal mouse peritoneal macrophages. The authors postulated that the cause was cross-linking of Fc receptors on the HSV-infected targets and the effector cytotoxic cells by the aggregated IgG. These reports thus suggest that the presence of the HSV Fc receptor may inhibit viral replication on the one hand, and might interfere with or enhance immune cytolysis on the other. Clearly, more detailed studies in this system are warranted, and the presence of the HSV Fc receptor must be taken into account in any assessment of immune cytolysis of herpes virus-infected cells.

E. Summary and Conclusions

Antibody binding to the surface of virus-infected cells can affect virus production and release in the absence of an effector system. A complex and dynamic interaction exists between antibody and the viral polypeptides expressed on the plasma membrane. Clearly, antibody can induce redistribution and capping of viral proteins on the cell surface. Most of the measles viral antigen and antibody complexed on the plasma membrane is subsequently shed rather than endocytosed; however, it is difficult to know whether shedding of complexes is more likely to occur with viral membrane proteins on the cell surface than with other membrane proteins. Although IgG–anti-IgG complexes on the B lymphocyte membrane are predominantly endocytosed, this may be peculiar to signaling in the B cell. While other host membrane proteins complexed with ligands may not be as rapidly endocytosed, endocytosis, with subsequent degradation in lysosomes, is believed to be the normal route of metabolism for host cell membrane proteins as indicated by the studies of surface radioiodinated mouse L cells by Hubbard and Cohn (1976). Hormone receptors complexed with their specific ligands probably are endocytosed and degraded in the same way (Catt *et al.*, 1979). Viral glycoproteins, which are destined for export from the membrane, may be more sus-

ceptible to shedding; when they are actually in segments of the membrane where virus budding has commenced, this might be even more likely. Electron microscopic studies on measles virus-infected cells undergoing capping show that budding virus is visible in the microvilli forming the cap (Lampert *et al.*, 1975).

Antibody-induced viral antigenic modulation as described for measles virus-infected cells has some similarities to the original TL system of Boyse *et al.* (1963, 1967). Hence, capping is not essential and some antibody and viral antigen is still present on the cell at the time of maximal resistance to antibody- and complement-mediated lysis. However, Yu and Cohen (1974) could not detect any change in TL synthesis in TL^+ cells undergoing modulation, but showed by surface radioiodination that there was accelerated removal of TL from the cell surface, at least during the early time course of modulation. In contrast, diminished synthesis may contribute to the loss of surface viral antigen in the presence of antibody in measles virus-infected cells. There is no suggestion of any requirement for C3 to produce modulation of measles virus-infected cells, whereas there is one in the TL system (Stackpole *et al.*, 1978). It may be concluded that the loss of surface viral antigen induced by antibody and leading to antigenic modulation could result from a combination of (*a*) shedding of antigen–antibody complexes; (*b*) blocking of antigenic sites by IgG remaining on the membrane; and (*c*) diminution of synthesis of surface viral polypeptides. Because cells can generally be rendered resistant to lysis by antibody and complement via relatively modest reduction of surface antigens, it is important to note that, in addition to being resistant to antibody-dependent complement-mediated lysis, modulated measles virus-infected cells are also resistant to antibody-dependent cell-mediated cytotoxicity (Oldstone and Tishon, 1978).

Antibody binding to intact cells can affect the intracellular expression of viral polypeptides as shown in measles virus-infected HeLa cells (Fujinami and Oldstone, 1979). This area has just begun to be studied at a biochemical level, and it will be interesting to see the parallels and contrasts that arise with other membrane receptor–ligand interactions, such as those between hormones and their receptors. The major interest in these observations lies in the possibility that such effects of antibody may represent mechanisms whereby virus persistence could be established *in vivo*. The consequence would be a cell rendered resistant to immune injury because of lost surface viral markers, but still retaining the potential to shed virus once antibody is removed or to infect adjacent cells by cell-to-cell spread. This possibility is further discussed in Section VI.

IV. Antibody- and Complement-Mediated Lysis of Virus-Infected Cells

Virus-infected cells can be lysed by specific antibody and complement. The mechanism of this reaction is discussed in detail in this section. Emphasis is given to work with homologous human systems, and with measles virus-infected cells in particular. Enveloped viruses, as free virions, can also serve as targets for antibody- and complement-mediated lysis; this topic has been reviewed by Oldstone (1975) and by Cooper and Welsh (1979).

A. Complement

The human complement system is composed of some 20 plasma proteins, including its intrinsic components and the proteins that regulate activation of the pathways. The mechanisms by which the system is activated, produces membrane lesions and mediates some of its biological effects are now relatively well understood. The mechanism of activation of the classical pathway (Müller-Eberhard, 1975) and the mechanism by which the membrane attack pathway (C5-9) produces membrane lesions (Podack and Müller-Eberhard, 1980) have been reviewed recently. The alternative pathway of complement activation is the subject of a review in this volume by Schreiber and Müller-Eberhard. Only points that are relevant to the present discussion are mentioned here.

Activation of the classical pathway is usually dependent on the interaction of the Fc portion of IgG or IgM complexed to antigen with the C1q subunit of C1. C1 is activated and enzymically catalyzes assembly of the classical pathway C3 convertase $\overline{C4b2a}$ by sequential proteolytic cleavage of C4 and C2. After cleavage of C3, association of C3b with $\overline{C4b2a}$ gives the classical pathway C5 convertase $\overline{C4b2a3b}$.

The alternative pathway of complement activation consists of six proteins: C3, factors B and D, β1H, C3b inactivator, and properdin. Current evidence indicates that initiation of the alternative pathway on the surface of known activators, such as rabbit erythrocytes (Fearon and Austen, 1977a; Pangburn and Müller-Eberhard, 1978), zymosan (Fearon and Austen, 1977b), and certain strains of *Escherichia coli* (Schreiber *et al.*, 1979), occurs because C3b from the fluid phase is bound there and relatively protected from its serum inactivators. There is probably a low level of background C3b generated from the interaction of native C3, factor B, and factor D. In the fluid phase or on the surface of nonactivators, β1H and C3b inactivator rapidly inactivate this C3b, whereas the access of β1H to C3b bound to the surface of an activator is restricted: factor B can then bind to C3b and is

cleaved by factor D generating the alternative pathway C3 convertase $\overline{C3bBb}$, the active site being on the Bb fragment of factor B. Amplification of C3 turnover with further surface deposition of C3b and acquisition of C5 cleaving activity by $\overline{C3bBb}$ then occur. Native properdin binds to C3b and retards the otherwise rapid decay of enzymic activity of $\overline{C3bBb}$. Properdin is thus a regulatory protein and is not required for initiation of the pathway [see Schreiber and Müller-Eberhard (this volume) for further details].

Consequent upon activation of either pathway, C5 is cleaved and the membrane attack complex is assembled from C5-9. This complex is inserted into the lipid bilayer of cell membranes as a dimer of C5b-9 (Biesecker *et al.*, 1979), giving the typical electron microscopic complement membrane lesion. Osmotic lysis of the cell then results, either because a hydrophilic protein channel is created by insertion of the membrane attack complex, or because its insertion results in rearrangement of the phospholipids and creation of a lipid channel in the bilayer (Podack and Müller-Eberhard, 1980).

B. Lysis of Virus-Infected Cells by Antibody and Heterologous Complement

Many reports of antibody- and complement-dependent lysis of virus-infected cells involved heterologous sources of antibody and complement. A number of studies from 1963 onward showed that cells infected with togaviruses, ortho- and paramyxoviruses, rhabdoviruses, arenaviruses, retroviruses, coronaviruses, and enteroviruses (all enveloped RNA viruses) and with the DNA HSV and poxviruses, could all be lysed by antibody and heterologous complement. These studies are summarized in Table II, and by Rawls and Tompkins (1975). These reports demonstrate convincingly that cells infected with a variety of RNA and DNA viruses are susceptible to lysis by antibody and complement. However, heterologous sera, particularly rabbit serum, which is frequently used as a complement source, may contain natural antibodies reacting with heterologous cell surface or viral antigens. For example, Rawls and Tompkins (1975) found that rabbit serum, as a complement source, lysed certain HSV-infected cells in the presence of antibody, whereas guinea pig or human serum did not. A prozone phenomenon is often observed with rabbit or guinea pig serum as a complement source (Ehrnst, 1977; Hicks *et al.*, 1976). These are not particular problems if the principal purpose is to assay potentially cytotoxic antiviral antibodies, as it was in a number of the studies in Table II. For instance, in cytotoxicity testing for H-2 or HLA typ-

TABLE II
SUDIES OF ANTIBODY- AND COMPLEMENT-MEDIATED LYSIS OF VIRUS-INFECTED CELLS USING HETEROLOGOUS COMPLEMENT SOURCES[a]

Virus	Antibody source	Complement source	References
DNA			
Herpes simplex	Rabbit	Guinea pig	Roane and Roizman (1964)
Herpes simplex	Human	Guinea pig	Smith *et al.* (1972)
Herpes simplex	Rabbit	Rabbit	Brier *et al.* (1971)
Shope papilloma	Rabbit	Guinea pig	Wahren (1963)
Vaccinia	Rabbit	Rabbit	Brier *et al.* (1971)
RNA			
Friend leukemia	Mouse	Guinea pig	Wahren (1963)
Influenza	Rabbit	Rabbit	Brier *et al.* (1971)
Lymphocytic choriomenigitis virus	Mouse	Guinea pig	Oldstone and Dixon (1971)
Measles (persistently infected cells)	Human (normal and SSPE[b] sera)	Guinea pig	Kibler and ter Meulen (1975)
Measles (persistently infected cells)	Human	Rabbit/guinea pig	Ehrnst (1977)
Measles (persistently infected cells)	Rabbit	Rabbit	Minagawa and Yamada (1971)
Mumps	Human/guinea pig	Guinea pig	Oldstone and Dixon (1971)
Rabies	Human and mouse	Guinea pig	Wiktor *et al.* (1968)
Sendai/Newcastle disease virus	Rabbit/mouse horse	Guinea pig/rabbit	Eaton and Scala (1970), Brier *et al.* (1971)
Simian virus 5	Rabbit	Guinea pig	Holmes *et al.* (1969)

[a] Summary of earlier studies of antibody- and complement-dependent lysis of virus-infected cells, in which heterologous sources of antibody and complement were used. Most of these studies were designed primarily to detect cell surface viral antigens or antibody to them, rather than to analyze the mechanism of cell lysis.

[b] SSPE, subacute sclerosing panencephalitis.

ing, the presence of natural antibodies in the complement source is often used to "rig" the system to produce maximum lysis (Ferrone *et al.*, 1974). However, the presence of such natural antibodies makes it difficult to analyze the mechanism of antibody- and complement-mediated lysis of virus-infected cells. Thus, use of heterologous systems provides results that are of doubtful relevance to the situation *in vivo*.

C. Lysis of Virus-Infected Human Cells by Human Serum

The lysis of virus-infected human cells by human serum has been studied in detail in a homologous system by Oldstone, Cooper, and colleagues (Joseph *et al.*, 1975; Perrin *et al.*, 1976; Sissons *et al.*, 1979a). In addition to having obvious direct relevance to human infection, the use of a homologous system avoids the problems inherent in the use of heterologous sera as a complement source referred to above.

Lysis by human serum of cells infected with HSV types 1 and 2, influenza A, parainfluenza 1, 2, 3, and 4, mumps, and measles viruses was dependent on the presence in serum of IgG antibody specific for the relevant virus and of complement (reviewed by Oldstone and Lampert, 1979). In addition to infected human epithelioid cell lines (HeLa, HEP 2), virus-infected lymphoblastoid, neural and glial cell lines of human origin were also lysed. No lysis occurred in serum from agammaglobulinemic children, despite an intact complement system. Serum lacking antibody to a particular virus would not lyse cells infected with that virus, but would do so after addition of IgG containing specific antibody to that virus (Perrin *et al.*, 1976). Acquisition by serum of the ability to lyse virus-infected cells has been demonstrated after immunization with mumps virus and measles virus in individuals without preexisting antibody. Sera obtained before immunization and 3 days after it would not lyse infected cells, but became able to produce specific lysis of mumps virus or measles virus-infected cells 6 days afterward, maximum lytic ability being attained 10 days after immunization (Perrin *et al.*, 1976; and unpublished observations).

A consistent finding in these studies was the requirement for an intact alternative pathway of complement activation in human serum for lysis to occur (Joseph *et al.*, 1975; Perrin *et al.*, 1976; Sissons *et al.*, 1979a). Thus the ability of serum to lyse virus-infected cells was extremely sensitive to dilution, 90% of the cytolytic activity being lost at a 1:6 dilution, and was abrogated by heating serum at 50°C (which inactivates factor B). In contrast, lysis still occurred in serum with 0.01 *M* EGTA and 1 m*M* Mg^{2+}, which selectively chelates Ca^{2+} and thus inactivates the classical pathway (Joseph *et al.*, 1975). This dependence on an intact alternative pathway was confirmed by using human sera that were immunochemically depleted of specific complement components by affinity column chromatography with monospecific antisera coupled to Sepharose 4B (Perrin *et al.*, 1976). Lysis of cells infected with mumps, herpes simplex, influenza, or measles viruses

TABLE III
ROLE OF ALTERNATIVE COMPLEMENT PATHWAY IN ANTIBODY- AND COMPLEMENT-DEPENDENT LYSIS OF VIRUS-INFECTED HELA CELLS BY SERUM[a]

Depletion experiments	Reconstitution experiments	
Component depleted	Percent lysis in depleted serum	Percent lysis on addition of depleted component
None (normal serum)	95	—
Factor B	5	95
Factor D	7	95
Properdin	20	95
C4	95	—
C2	95	—

[a] Human sera containing IgG antibody to measles virus were immunochemically depleted of various complement components by affinity chromatography, except for C2, which was obtained from a patient with genetic deficiency of C2. Depleted sera were reconstituted with a physiologic concentration of the purified depleted component. Lysis was assessed by the eosin microcytotoxicity assay (1). Data represent the results of three experiments. Spontaneous lysis of infected cells in MEM was 5 ± 10%; lysis of uninfected HeLa cells in reagents was 5 ± 5% (mean ± 2 SD). In similar additional experiments cells infected with mumps, influenza, parainfluenza, and HSV types 1 and 2 were lysed by antibody and C4-depleted, but not factor B-depleted, serum. Reconstitution with factor B restored lysis (see Perrin *et al.*, 1976; Sissons *et al.*, 1979a).

was completely abrogated in serum thus depleted of factor B or of factor D and diminished by 80% in properdin-depleted serum. Reconstitution of the depleted component with physiologic concentrations of the purified protein restored the lytic ability in each case (see Table III) (Perrin *et al.*, 1976; Sissons *et al.*, 1979a).

In contrast, serum immunochemically depleted of C4 or genetically deficient in C2, both components necessary for formation of the classical pathway C3 convertase $C\overline{42}$, was as efficient as whole serum in lysing measles virus-infected cells (Perrin *et al.*, 1976). Furthermore, dose related lysis curves showed that C4 depleted serum with no measles virus antibody was quantitatively equal to the original whole serum in its ability to lyse antibody coated measles virus-infected cells (Fig. 5). The kinetics of lysis in whole and C4-depleted serum were also identical (Fig. 6) (Sissons *et al.*, 1979b).

These observations thus showed that the lysis of virus-infected cells by human serum depends on antibody and the alternative comple-

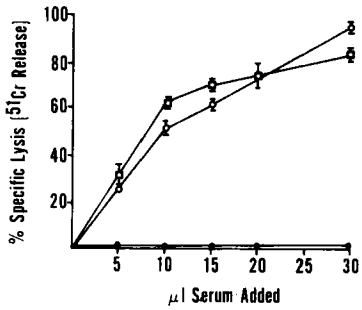


FIG. 5. Equivalent dose-related immune lysis of measles virus-infected HeLa cells coated with antibody by whole and C4-depleted serum. Lysis of HeLa cells acutely infected with measles virus and coated with antiviral IgG by whole serum lacking antibody to measles (○); by the same serum immunochemically depleted of C4 (●); or by the serum immunochemically depleted of factor B (□). Uninfected cells or infected cells without antibody were not lysed. (From Sissons *et al.*, 1979b.)

ment pathway. The lack of any requirement for C4 or C2 showed that activation of the classical pathway is not necessary for lysis, and that the alternative pathway must therefore be initiated by a mechanism other than recruitment in consequence of classical pathway activation.

There are very few other reported studies of lysis of virus-infected cells by human serum. Hicks *et al.* (1976) reported lysis of measles virus-infected monkey (Vero) cells by antibody and human serum. Specific lysis was observed with both C2-deficient and Mg^{2+} EGTA-treated serum and in serum heated at 50°C or adsorbed with zymosan, although none of these was as effective as whole serum. These authors therefore concluded that lysis could be mediated by alternative and classical pathways, but that neither pathway alone was as effective as

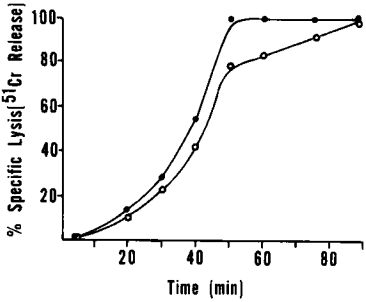


FIG. 6. Kinetics of immune lysis of measles virus-infected HeLa cells. Lysis of HeLa cells acutely infected with measles virus and coated with antiviral IgG by whole (○) and C4 depleted (●) serum; 20 μl of serum was used for each time point. (From Sissons *et al.*, 1979b.)

both combined. Again, human serum produced no lysis in the absence of antibody in these studies. The finding of some classical pathway mediated lysis, in contrast to the findings discussed above, is probably related to the use of a different cell line and a heterologous system.

D. Analysis of the Requirement for Antibody in Complement-Mediated Lysis

The absolute requirement for antibody in complement-mediated lysis of virus-infected cells by serum is documented above. The amount of cell bound antibody required to induce lysis was determined by using measles virus-infected HeLa cells as targets and employing a binding assay with radiolabeled IgG. No lysis occurred until a mean of greater than 5×10^6 to 1×10^7 molecules of IgG had bound per infected cell (Joseph *et al.*, 1975; Perrin *et al.*, 1976; Sissons *et al.*, 1979a) (Fig. 5). This is a large amount of IgG, although the measles virus infected HeLa cell has a large surface area.

The $F(ab')_2$ fragment of IgG can also induce lysis of mumps virus- and measles virus-infected cells (Perrin *et al.*, 1976), and the dose related lysis curves for whole IgG and $F(ab')_2$ in whole and C4 depleted serum are near-identical (Sissons *et al.*, 1979b). However, Fab' fragments are ineffective in inducing lysis. At least 10 times more Fab' than $F(ab')_2$ or IgG molecules bound per infected cell were required to produce 50% lysis, which was the maximum attainable with Fab' in these experiments. At this high density it is difficult to exclude the possibility that Fab' fragments reassociated despite being initially alkylated (see Fig. 7) (Sissons *et al.*, 1979b). These experiments thus

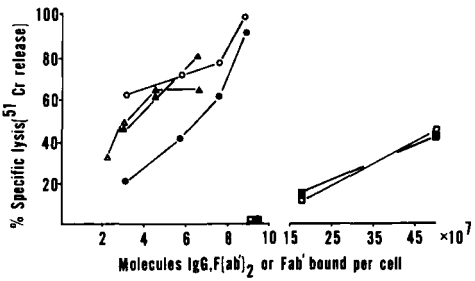


FIG. 7. Comparison of the dose related immune lysis of measles virus-infected HeLa cells produced by IgG and its $F(ab')_2$ or Fab' fragments. Lysis induced by IgG in whole (○) and C4 depleted (●) serum; by $F(ab')_2$ in whole (△) and C4-depleted (▲) serum; and by Fab' in whole (□) and C4-depleted (■) serum. Production of 50% lysis required binding of at least 10 times more Fab' than $F(ab')_2$ or IgG molecules.

show a requirement for divalent antibody, but no requirement for the Fc portion, for IgG to induce lysis of virus-infected cells by the alternative pathway. The fact that $F(ab')_2$ (which cannot bind C1q) is as effective as whole IgG is further proof of the lack of requirement for classical pathway activation for lysis.

Fab' and Fab in addition to $F(ab')_2$ fragments were reported as able to induce lysis of measles virus-infected cells in one other study, but rabbit or guinea pig sera were used as complement sources (Ehrnst, 1978). These findings are difficult to interpret because a heterologous complement source was used, and no determination was made of the amount of Fab bound to the cell. In contrast, Fab' fragments blocked the lysis of HSV-infected cells by IgG antibody and rabbit complement in a study by Brier *et al.* (1971).

This requirement for divalency in the homologous human system suggests that antibody-induced redistribution of viral antigens on the cell membrane may be required for production of lysis. Prevention of capping with cytochalasin D or sodium azide does not abrogate complement-mediated lysis (Perrin *et al.*, 1976). However, these inhibitors of actin polymerization do not prevent local patching of viral antigen on the cell membrane by antibody.

Antibody directed against different viral polypeptides expressed on the cell surface can mediate lysis of measles virus-infected cells. Measles virus has two surface glycoproteins, HA and F. Using a persistently infected cell line and a heterologous complement source, Ehrnst (1977) reported that antibodies to HA induced lysis by the alternative pathway, whereas antibody to F induced lysis by the classical pathway. Again, the amount of antibody bound per cell was not determined in these experiments; by immunoprecipitation and SDS-PAGE analysis of infected cells, the antisera used by Ehrnst were not monospecific (J. G. P. Sissons and M. B. A. Oldstone, unpublished observations), making these results at best difficult to interpret. In contrast, using cell surface labeling and immunoprecipitation to assess the site of antibody binding on acutely infected cells, Sissons *et al.* (1979b) found that human IgG antibodies that bound specifically only to HA or only to F protein were equally effective in inducing lysis by the alternative pathway (in C4-depleted serum) and that neither antibody would induce lysis by the classical pathway alone (in factor B-depleted serum). An equivalent amount of antibody bound to either viral polypeptide induced an equal degree of lysis.

The subclass of IgG seems unlikely to be of direct importance in the induction of lysis, especially since the Fc region is not required. Although some experiments indicate that IgG1 is the major subclass in-

volved in lysis of measles virus-infected cells (Ehrnst, 1978; L. H. Perrin and M. B. A. Oldstone, unpublished observations), this probably reflects the fact that 70% of all serum IgG is of the IgG1 subclass.

There is virtually no information on whether other human immunoglobulin classes can induce complement-dependent lysis of virus-infected cells. This would be of interest insofar as most viruses induce an early IgM response on primary infection or immunization and may also produce a serum IgA response (Ogra *et al.*, 1975). Joseph *et al.* (1975) reported that, at amounts equivalent to or greater than cytolytic IgG, IgA, and IgM isolated from convalescent sera of patients with measles or after vaccination (unpublished observations) did not induce lysis of measles virus-infected cells. However, as might be expected, measles virus antibody titers in the IgM and IgA preparations were very low compared to titers in the IgG fraction. Rabbit IgM antibody appears to be relatively ineffective in inducing lysis of virus-infected cells when compared to IgG (Brier *et al.*, 1971; Ehrnst, 1975).

The precise molecular mechanism by which antibody produces lysis of virus-infected cells in conjunction with complement remains to be determined. However, the findings described in the next section show that IgG is not required for initiation of the alternative pathway by measles virus-infected cells, although it is required for cell lysis.

E. Analysis of the Requirement for the Alternative Pathway

Recent work has further confirmed the role of the alternative pathway in lysing virus-infected cells. These experiments have again used measles virus-infected HeLa cells as a representative model.

The alternative pathway of complement activation has recently been assembled *in vitro* from its isolated constituent proteins in highly purified form. A mixture composed of C3, factors B and D, native properdin, and the two control proteins, β1H and C3b INA, all at physiologic concentrations, can mediate deposition of C3b onto activators of the alternative pathway, such as rabbit erythrocytes, with an efficiency equal to serum (Schreiber *et al.*, 1978). Addition to this mixture of the five purified proteins of the membrane attack pathway, C5-9, creates a system with cytolytic capacity. This purified cytolytic alternative pathway can lyse rabbit erythrocytes and (with lysosyme) *E. coli* as efficiently as C4-depleted serum (Schreiber and Müller-Eberhard, 1978; Schreiber *et al.*, 1979). In recent experiments it was shown that the purified cytolytic alternative pathway could lyse antibody-coated measles virus-infected cells with an efficiency comparable to that of C4-depleted or whole human serum. As in serum, no lysis

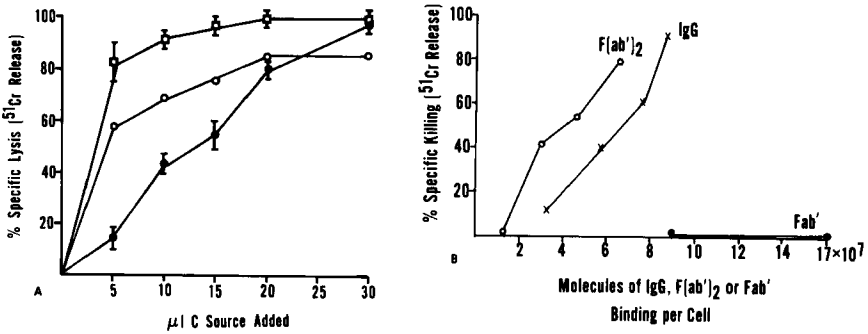


FIG. 8. (A) Dose-related immune lysis of measles virus-infected HeLa cells coated with IgG antibody to measles virus by the following sources of complement: whole human serum lacking antibody to measles (□); human serum immunochemically depleted of C4 (●); and the purified cytolytic alternative pathway (○). There was no lysis of uninfected HeLa cells or infected cells without antibody. (B) Requirements for IgG, $F(ab')_2$, and Fab' for immune lysis of measles virus-infected cells via the purified cytolytic alternative pathway. Measles virus-infected HeLa cells (2×10^5) were preincubated with increasing amounts of IgG (×) or its $F(ab')_2$ (○) and Fab' (●) fragments. Then 5×10^3 cells were incubated with 20 μl of the purified complement components. Data for lysis are plotted against the number of molecules of IgG, $F(ab')_2$, or Fab' binding per measles virus-infected cell, as determined in a parallel binding assay using ^{125}I-labeled antibody or fragments. (A and B reproduced from Sissons *et al.*, 1979a.)

occurred in the absence of antibody, and $F(ab')_2$ and IgG gave near identical dose related lysis curves, but Fab' produced no lysis. Approximately 5×10^7 molecules of IgG or $F(ab')_2$ bound per infected cell were required to induce 50% lysis (Sissons *et al.*, 1979a) (see Fig. 8).

Omission of properdin from the purified cytolytic alternative pathway totally abrogated lysis of measles virus-infected cells. This is noteworthy insofar as properdin was not essential for the lysis of rabbit erythrocytes or *E. coli* by the purified cytolytic alternative pathway (Schreiber and Müller-Eberhard, 1978; Schreiber *et al.*, 1979). This difference may well reflect the fact that nucleated cells require more complement-mediated membrane damage to produce lysis. The use of this system, composed of 11 highly purified complement proteins, provided conclusive evidence that the known proteins of the alternative and membrane attack pathways with IgG antibody are sufficient for lysis of the measles virus-infected cell, without other serum factors. These findings and the efficacy of $F(ab')_2$ also excluded any possibility that alternative pathway activation by antibody-coated virus-infected cells could be occurring by the "C1 bypass activation pathway" described by May and Frank (1973), in which sheep erythrocytes bearing rabbit antibody apparently initiate the alterna-

tive pathway by a mechanism involving C1. Hence, a dominant role is established for the alternative pathway in the complement-dependent lysis of virus-infected cells by human serum.

The requirement for both specific antibody and an intact alternative pathway for lysis of virus-infected cells at first suggested that antibody might be required for activation of the alternative pathway in this system. This would be distinctly unusual, as activation of the alternative pathway by known particulate activators (rabbit erythrocytes, zymosan, *E. coli* 04) is independent of immunoglobulin. The question therefore arose whether, despite being required for lysis, IgG was actually necessary for activation of the pathway on the virus-infected cell. Recent experiments show that measles virus-infected cells activate the alternative pathway independently of antibody. Sissons *et al.* (1980) found that measles virus-infected HeLa cells incubated in a mixture of highly purified C3, factors B and D, native properdin, β1H, and C3b INA (the purified alternative pathway of complement activation, without C5-9) showed progressive linear uptake of [^{125}I]C3b onto the cell surface (Fig. 9). This C3b uptake, which averaged 1 to 1.5×10^6 C3b molecules per cell after 90 minutes, was specific in that it was not shown by uninfected HeLa cells and was blocked by 0.01 *M* EDTA. Measles virus-infected HeLa cells also showed progressive uptake of [^{125}I]C3b from human serum immunochemically depleted of C4 and IgG. In addition to being independent of IgG, [^{125}I]C3b uptake from the purified alternative pathway onto measles-infected cells in the absence of antibody was also independent of properdin, the rate of uptake being the same in the absence and in the presence of properdin, and similar to the rate of [^{125}I]C3 uptake by infected cells in the presence of IgG but the absence of properdin. These experiments are summarized in Fig. 9. Thus, the rate of [^{125}I]C3b uptake by infected cells was increased only in the presence of IgG and properdin together. Because both IgG and properdin are also required for lysis, this suggests that some interaction between the two, possibly affecting the rate of C3b uptake, is important in the mediation of lysis.

In these experiments, significant [^{125}I]C3 uptake by measles virus-infected cells was first demonstrable 12–18 hours after infection, by this time all cells were expressing viral polypeptides on their surface, indicating that viral replication must occur before alternative pathway activation becomes detectable. In all the above experiments the absolute amount of C3b ultimately bound by infected cells alone was similar or identical to the amount bound by infected cells coated with IgG, only the rate of uptake being influenced by IgG.

Further work is needed to determine whether this ability to activate

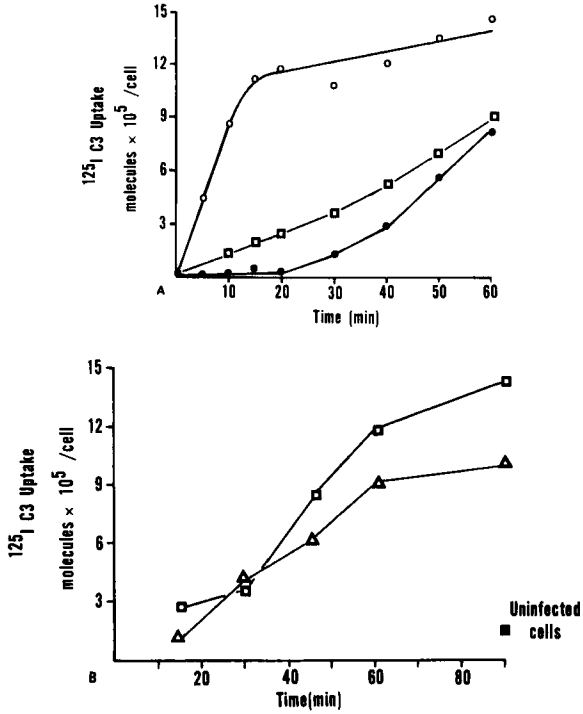


FIG. 9. (A) Uptake of ^{125}I-labeled C3 on to measles virus-infected HeLa cells coated with IgG from the purified alternative pathway (○) and from the purified alternative pathway without properdin (●). □: Uptake of [^{125}I]C3 onto measles virus-infected HeLa cells, without IgG, from the purified alternative pathway. [^{125}I]C3 uptake in the presence of 0.01 *M* EDTA has been subtracted at each time point. (B) Uptake of [^{125}I]C3 on measles virus-infected HeLa cells via the purified alternative pathway in the presence (□) or the absence (△) of properdin. (■)[^{125}I]C3 uptake on uninfected HeLa cells via the purified alternative pathway. (A and B from Sissons *et al.*, 1980.)

the alternative pathway independent of antibody is a general property of cells infected with viruses. However, it should be noted that a number of human lymphoblastoid cell lines can activate the alternative pathway in human serum (Budzko *et al.*, 1976; Theofilopoulos and Perrin, 1976; McConnell and Lachmann, 1976; McConnell *et al.*, 1978). McConnell *et al.* (1978) have reported that this ability correlates with transformation by Epstein–Barr (EB) virus. They found that cell lines positive for EB virus-determined nuclear antigen (EBNA) activated the pathway, as shown by C3 conversion in Mg-EGTA serum and deposition of C3 on cells detected by immunofluorescence. EBNA negative lines did not deposit C3 on their membranes but acquired the ability to do so after EB virus transformation and ac-

quisition of EBNA. EB virus-transformed cells are mostly nonproducing and do not express detectable structural viral antigens on their surfaces. There presumably must be changes in the membrane as shown by the presence of the uncharacterized lymphocyte-determined membrane antigen (Svedmyr and Jondal, 1975). Despite the ability of Raji cells, one of these EBNA-positive lymphoblastoid lines, to activate the alternative pathway, lysis of the cells occurred only after prolonged incubation in serum for 7–8 hours and was independent of antibody (Theofilopoulos and Perrin, 1977). In contrast, measles virus-infected HeLa cells did not lyse (as measured by specific ^{51}Cr release) even after 18 hours of incubation in serum without antibody to the virus (J. G. P. Sissons, unpublished observations).

There is supportive but less direct evidence suggesting that measles virus-infected cells (Hicks *et al.*, 1976) and Sendai virus-infected cells (Okada *et al.*, 1979) can activate the alternative pathway. This is implied by their lysis in C4-deficient guinea pig serum independent of antibody. It was also suggested that retrovirus-infected cells can activate the alternative pathway, as evidenced by using Mg-EGTA serum and a rosetting assay to detect cell-bound C3 (Okada and Baba, 1974). However, it seems equally likely that retrovirus-infected cells might activate the classical pathway, since it is already known that free retrovirus activates the classical pathway independent of antibody by binding C1q via its surface glycoproteins (Welsh *et al.*, 1975; Cooper *et al.*, 1976; Bartholomew *et al.*, 1978).

F. Aspects of Target Cell Structure Affecting Antibody- and Complement-Mediated Lysis

The principal aspects of target cells affecting lysis are the density and presentation of viral antigen expression on the cell surface. Any factor decreasing surface antigen expression tends to decrease susceptibility to lysis. Thus, events that cause the virus-infected cell to produce mutants defective for surface glycoproteins or defective interfering virus, which suppresses replication of the standard virus, result in diminished viral antigen expression on the cell surface. Welsh and Oldstone (1977) found that the susceptibility of cultured neuroblastoma cells infected with LCMV to lysis by antiviral antibody and heterologous complement declined with increasing time in culture. This was related to diminished LCMV surface antigen expression, which resulted from generation of defective interfering LCMV. The resistance to lysis was specific for LCMV in that both acutely and persistently infected cells were still susceptible to lysis by anti-H-2 and complement.

Antigenic modulation of measles virus-infected cells by antibody,

as described in Section III, also renders cells resistant to human antibody- and complement-mediated lysis. Joseph and Oldstone (1975) found that after 12 hours of culture in the presence of antibody, cells both acutely and persistently infected with measles virus lost their susceptibility to antibody- and complement-dependent lysis.

At certain doses addition of interferon to cultured cells expressing LCMV antigens is followed by a reduction in the expression of viral antigens on the cell surface and susceptibility to immune lysis (M. B. A. Oldstone and T. Merigan, unpublished observations).

A further mechanism whereby infected target cells could escape lysis by antibody and complement is the expression of new membrane proteins that block access of antibody to surface antigens. Celis *et al.* (1979) showed that murine myeloma LPC-1 cells grown as an ascites tumor express increased amounts of a surface glycoprotein (gp100) that seemed able to block binding of cytotoxic antibodies to H-2 antigens. Removal of this protein with proteases restored susceptibility to immune lysis by anti-H-2 and complement or cytotoxic lymphocytes. Whether similar events can occur with viral antigens is unknown.

The cell cycle can also influence susceptibility to lysis. Cikes and Friberg (1971) found that Moloney virus-transformed lymphocytes were susceptible to lysis by antibody and complement only in the G1 phase, not during S, G2, or M. Yet viral antigen was present and accessible to antibody, and complement was activated throughout the cell cycle (Lerner *et al.*, 1971). Complement membrane lesions were also present on the cells at different phases of the cell cycle, although immune lysis was again restricted to G1 (Cooper *et al.*, 1974). Consequently, the plasma membrane may vary in its ability to withstand complement-mediated damage at various phases of the cell cycle, presumably because of factors affecting membrane repair.

Susceptibility of nucleated cells in general to complement-mediated lysis is probably related in part to their ability to effect membrane repair. For instance, complex changes in the synthesis and release of several lipid classes have been reported in tumor cell lines upon their exposure to antibody and complement (Schlager *et al.*, 1978). However, there is no direct information on how virus infection can affect the cell's ability to repair its membrane.

G. Summary and Conclusions

In summary, cells infected with a number of different RNA and DNA budding viruses are lysed by human serum, and this lysis is dependent on IgG antibody and the alternative pathway of complement. More detailed studies using measles virus have shown that $F(ab')_2$ is

as effective as whole IgG in inducing lysis but that Fab′ is ineffective. Furthermore, IgG is not required for activation of the alternative pathway, which occurs on the surface of measles virus-infected cells independently of antibody. Properdin is not required for activation of the alternative pathway, but is required with IgG for lysis of infected cells. The major remaining questions concerning the mechanism of this reaction are as follows: Do cells infected with other viruses activate the alternative pathway? How do virus-infected cells activate the alternative pathway? What is the mechanism by which IgG induces lysis?

The mechanism by which measles virus-infected cells activate the alternative pathway may be similar to that described for other activators, activator-bound C3b being protected from β1H. It has been proposed that specific surface molecules on activating particles might act as "β1H antagonists," restricting access of β1H to C3b (Pangburn and Müller-Eberhard, 1978; Pangburn *et al.*, 1979). It is conceivable that the viral glycoproteins, which are inserted in the membrane as integral membrane proteins, could function in this way. Alternatively, some other virus-induced change in the membrane may be responsible. For example, virus infection could result in expression or release of membrane proteases that might cleave C3. Immunoelectron microscopy studies in which measles virus-infected cells were incubated in human serum containing antibody to measles, and then stained with ferritin-conjugated antisera to C3, factor B, or properdin, showed that the antisera bound to the viral antigen–antibody complex on the cell membrane. Adjacent sites on the membrane, where viral spikes were not apparent, showed no labeled antibody binding. This suggests that membrane deposition of C3 occurs at the sites of insertion of viral glycoproteins (Oldstone and Lampert, 1979).

The fact that properdin is not required for activation of the pathway by measles virus-infected cells accords with its known function, which is to delay intrinsic decay of $\overline{C3bBb}$. Properdin can be recruited only when multiple C3b molecules are deposited on a surface in close spatial association, and activation of the pathway must therefore precede its recruitment (Fearon and Austen, 1975; Medicus *et al.*, 1976). The requirement for properdin in complement-dependent lysis of measles virus-infected cells, in contrast to its nonessential role in lysing other activators, may reflect the fact that a nucleated cell with the capacity for membrane repair requires more extensive complement-mediated membrane damage before lysis results. Properdin would be expected to enhance C3 and C5 turnover and membrane deposition.

The fact that virus-infected cells are not lysed by antibody and the

classical pathway alone in human serum requires explanation. The classical pathway is *activated* by IgG antibody bound to virus-infected cells, as shown by uptake of C4 and C3 (assessed by immunofluorescence) from factor B-depleted serum (Perrin *et al.*, 1976) and binding of [^{125}I]C3 following assembly of $C\overline{42}$ on the surface of IgG bearing measles-infected cells (M. K. Pangburn and J. G. P. Sissons, unpublished observations); however, it is not known whether membrane lesions result. It is possible that, despite activation of the classical pathway, insufficient membrane damage occurs to achieve lysis in the absence of the amplification provided by the alternative pathway C3b-dependent feedback mechanism.

The mechanism whereby IgG induces lysis by the alternative pathway remains to be determined. The requirement for divalent antibody suggests that patching of viral polypeptides on the membrane may be important. The fact that the rate of C3 uptake onto measles-infected cells is enhanced only when IgG and properdin are both present suggests that IgG may facilitate the uptake of properdin. Further experiments are needed to test these possibilities.

There are a few other instances in which antibody and the alternative pathway are both required to lyse cells. Human lymphocytes coated with human alloantisera can be lysed by the alternative pathway in human serum, although not all the alloantisera used in this study had this ability and some were effective in inducing lysis only by the classical pathway (Ferrone *et al.*, 1973). *Trypanosoma cruzii* are also lysed by IgG antibody and the alternative pathway (Krettli and Nussenzweig, 1977). Additionally, there is some evidence to suggest that a serum factor, probably IgG, enhances the alternative pathway-dependent lysis of rabbit erythrocytes by human serum (Polhill *et al.*, 1978; Nelson and Ruddy, 1979), although IgG is not essential for this reaction. It is apparent that considerably more surface-bound IgG is required to induce complement-dependent lysis of virus-infected cells than is required for antigenic modulation or antibody-dependent cell-mediated cytotoxicity. The possible implications of this are discussed in Section V.

Further work showing that cells infected with other viruses can activate the alternative pathway independent of antibody would strengthen the evidence for viewing the alternative pathway as a means of nonspecific host defense against microorganisms. It is also important to determine whether free viruses can activate the pathway. The observations of Wedgewood *et al.* (1956) and of Welsh (1977) suggest that Newcastle disease virus may be inactivated by the alternative pathway independent of antibody in human serum (reviewed by Cooper and Welsh, 1979).

V. Antibody-Dependent Cell-Mediated Cytotoxicity of Virus-Infected Cells

A. Introduction

The main emphasis in this review is directed toward the effect of antibody alone or antibody and complement on virus-infected cells. Now we discuss antibody-dependent cell-mediated cytotoxicity (ADCC) briefly, both to illustrate the range of actions that antibody may have on virus-infected cells and to place all these actions of antibody in a comparative context. This section deals only with ADCC as applied to virus-infected cells, with emphasis on human systems and the role of antibody, rather than on detailed discussion of the effector cells involved.

1. General Aspects of ADCC

ADCC has been studied in a number of different systems (reviewed by McLennan, 1972; Perlmann *et al.*, 1972; Lovchik and Hong, 1977; Perlmann and Cerottini, 1979). Although the lysis of antibody-coated erythrocytes can be mediated by phagocytic cells—polymorphonuclear leukocytes (PML) and macrophages—the lysis of nucleated cells sensitized with antibody by nonimmune, human peripheral blood lymphocytes (PBL) is mediated predominantly by lymphocytes that have Fc receptors. These cells do not have conventional B or T lymphocyte markers (surface Ig and sheep erythrocyte (E) rosette negative) and thus fall within the "null cell" population. However, there is evidence that some PBL able to mediate ADCC can be found in the subpopulation of T cells with Fc receptors for IgG (see Perlmann and Cerottini, 1979) as has also been reported for virus-infected targets (Santoli and Koprowski, 1979). This T cell subset also contains cells able to suppress antibody production by B cells (Moretta *et al.*, 1977). Although cells that can mediate ADCC are often referred to as K cells, this is a functional definition and is not based on the presence of unique surface markers. ADCC is usually rapid; membrane damage can be detected 10 minutes after the reaction's inception, and cell lysis is usually completed by 4–8 hours. There is evidence to suggest that the effector cell is inactivated by interaction with its targets (Ziegler and Henney, 1975), which may result from modulation of the Fc receptors on the effector cell surface (Perlmann and Cerottini, 1979).

In assaying for ADCC, exogenous antibody is conventionally added to the cytotoxicity assay in the presence of PBL. However, PBL can exhibit cytotoxicity for a range of target cells in the absence of added antibody, and this activity is mediated, in part, by a class of cells func-

tionally designated as natural killer (NK) cells. There has been some uncertainty over whether or not this spontaneous or natural cytotoxicity could be dependent on small amounts of antibody present in the assay system. For this reason, recent observations concerning human NK cells are summarized below.

2. *Lysis of Virus-Infected Cells by Natural Killer Cells*

The spontaneous cytotoxicity exhibited by unsensitized human PBL for various cultured cell lines and virus-infected cells *in vitro* has received increasing attention. This lysis, mediated by NK cells, has been recently reviewed in human (Herberman *et al.*, 1979; Saksela *et al.*, 1979; Santoli and Koprowski, 1979; Perlmann and Cerottini, 1979) and murine (Herberman *et al.*, 1979; Kiessling *et al.*, 1979; Welsh, 1978) systems.

The bulk of evidence indicates that human NK cells cannot be easily separated from cells mediating ADCC, both being chiefly null cells with Fc receptors. Depletion of NK cells by target binding and cross competition experiments suggests that the same cell population mediates both activities. However, the mechanism of target recognition differs, and a functional distinction between cells mediating ADCC and NK activity can be made on this basis. The available evidence (summarized in the reviews above) suggests that NK cells do not recognize and bind to target cells via their Fc receptors or depend on antibody for cytotoxicity. Furthermore, NK cells do not exhibit conventional immunologic specificity but can lyse a wide range of infected or uninfected target cells. In contrast, cells mediating ADCC recognize and bind to target cells sensitized with antibody via their Fc receptors and require antibody for cytotoxicity, which thus confers immunologic specificity on the reaction. An obvious and important question is whether small amounts of IgG antibody secreted during the course of a cytotoxicity assay, or bound to cytotoxic cells via their Fc receptors, could account for NK cell cytotoxicity. The case against a role for IgG in NK cell cytotoxicity is supported by experiments showing a failure to block NK cell activity by F(ab')2 or Fab anti-IgG, anti-Fab or protein A; all these reagents can block the IgG-Fc receptor interaction and effectively inhibit ADCC by exogenous antibody (Kay *et al.*, 1978; Trinchieri *et al.*, 1978). However, Pape *et al.*, (1979) could inhibit a significant part, but not all, of NK cell activity against K-562 cells by these methods. The question of whether ADCC can account for some NK cell cytotoxicity is thus not resolved.

Interferon enhances the activity of human NK cells *in vitro* (Trinchieri and Santoli, 1978; Herberman *et al.*, 1979) and *in vivo* (Hudd-

lestone *et al.*, 1979). However, it is not clear whether interferon can also enhance the ability of PBL to mediate ADCC (Trinchieri and Santoli, 1978; Herberman *et al.*, 1979). Virus-infected cells (and tumor- and virus-transformed cell lines) can induce interferon release from PBL. Interferon can then enhance the activity of NK cells within the PBL population and contribute to lysis of target cells in the course of a cytotoxicity assay (Santoli *et al.*, 1978). Thus, distinguishing NK cell activity from ADCC presents difficulties. Unless carefully defined reagents are used to block IgG-Fc receptor interactions with uninfected and infected cell targets, it is difficult to interpret their relative importance during lysis of virus-infected cells by PBL.

When NK activity is induced *in vitro* by interferon released during a cytotoxicity assay, cytotoxicity is maximal at 16–18 hours, whereas ADCC in the presence of exogeneously added antibody is usually maximal by 4–8 hours. However, killing by NK cells already induced by preexposure to interferon *in vivo* or *in vitro* is maximal at 6–8 hours, whereas ADCC dependent on antibody secreted during an *in vitro* assay might be expected to have slower kinetics. Hence, clearly distinguishing between NK cytotoxicity and ADCC on the basis of kinetics is also difficult. It is possible that if the same cell is involved in both NK cell killing and ADCC, as current evidence suggests, then attachment to target cells sensitized by antibody via its Fc receptor may induce a more efficient cytotoxic mechanism than binding via the putative NK cell receptor.

B. ADCC of Virus-Infected Cells

The mechanism of ADCC appears to be the same for different cells infected with a variety of viruses and is unlikely to differ from that involved in ADCC of other nucleated cells. Although there are numerous descriptions of virus-infected cells killed by human PBL, only those reports in which ADCC activity was deliberately assayed by addition of exogenous antibody are discussed below.

The first examples concerned ADCC of HSV-infected cells by human PBL (Shore *et al.*, 1974, 1976; Rager-Zisman and Bloom, 1974) as well as mouse peritoneal exudate cells (Rager-Zisman and Bloom, 1974). Both reports emphasized that ADCC could be produced by antibody concentrations several hundredfold less than those needed to produce antibody and complement dependent lysis of the same virus-infected cells. Extending these findings, Shore *et al.* (1978) and Kohl *et al.* (1977) noted that the effector cell in the human PBL population could be either an adherent cell or a nonadherent Fc receptor-positive lymphocyte. The adherent cell required higher antibody concentra-

tion and displayed slower kinetics of killing than the nonadherent cell. This is the only report of an adherent mononuclear cell mediating ADCC of virus-infected cells in a human system; others have found that eliminating adherent cells did not diminish or might enhance ADCC. Other studies with HSV (Möller-Larssen *et al.*, 1977) suggested that trace amounts of antibody present in the medium used for washing PBL could mediate ADCC by PBL in the absence of added IgG. The added complication of the HSV-induced Fc receptor (discussed in Section III) should be recalled. Lysis of HSV-infected cells may occur in part by interaction involving this receptor. Rager-Zisman *et al.* (1976) suggested that even traces of aggregated IgG in fetal bovine serum could cross-link target and mouse peritoneal effector cell Fc receptors to produce apparent ADCC of HSV infected cells, although the possibility of NK cell killing was not considered.

In studies with mumps virus-infected cells (Andersson *et al.*, 1975; Härfast *et al.*, 1977), high concentrations of rabbit antibody to mumps virus inhibited, but lower concentrations enhanced, PBL-mediated cytotoxicity. Antibody independent but virus specific cytotoxicity mediated by Fc receptor bearing PBL was also noted in these studies (Härfast *et al.*, 1978).

Several laboratories have independently assayed PBL-mediated killing of measles virus-infected targets. Antibody to measles virus enhanced the killing of measles virus-infected cells by PBL harvested from normal immune subjects (Perrin *et al.*, 1977a; Kreth and ter Meulen, 1977; Ewan and Lachmann, 1977). Antibody from normal immune subjects or patients with subacute sclerosing panencephalitis (SSPE) also enhanced the cytotoxicity of PBL obtained from patients with SSPE (Kreth and ter Meulen, 1977; Perrin *et al.*, 1977a; A. Tishon, J. H. Huddlestone, and M. B. A. Oldstone, unpublished observations). Since patients' sera enhanced cytotoxicity, these observations are at variance with the earlier report of Ahmed *et al.* (1974) of a "blocking factor" in the sera of SSPE patients that inhibited lymphocyte-mediated activity (migration inhibition, proliferation) against measles virus-infected cells, although Ahmed *et al.* did not assay cytotoxicity. Perrin *et al.* (1977a) calculated that an average of 4 to 5 $\times$ 10^5 antibody molecules bound per measles virus-infected target (HeLa) cell was required to induce ADCC. This contrasts with the much greater (at least 10-fold) amount of cell-bound antibody required to induce antibody- and complement-dependent lysis in the same system (see Section IV).

Respiratory syncytial virus-infected cells were lysed by ADCC (Scott *et al.*, 1977) using colostrum as well as serum as sources of IgG

antibody. ADCC has also been used to detect IgG antibodies to EB virus (Jondal, 1976), but only lytically infected cells that express virus-specific surface antigens, not transformed cells, can be used as targets.

C. Summary and Conclusions

1. Role of Antibody in ADCC of Virus-Infected Cells

It is clear that addition of specific antibody can enhance the cytotoxicity of PBL for virus-infected cells. Minimal amounts of antibody are effective for ADCC activity. Because the kinetics of ADCC are usually rapid, increased ionic efflux from target cells being observable at 10 minutes in some systems (Ziegler and Henney, 1975), it seems unlikely that antibody would produce significant modulation during the course of a cytotoxicity assay. Nevertheless, it is possible that the inhibitory effects of high doses of antibody on ADCC might be related to capping or redistribution of viral antigens by antibody; inhibition might also be produced by modulation and loss of Fc receptors on the effector cells by aggregated IgG (Perlmann and Cerottini, 1979). IgG antibody has been responsible for ADCC of virus-infected cells in all the human studies cited. At present there is no published work on the relative efficacy of antibodies directed against different viral antigenic determinants on the cell surface in mediating ADCC. However, unpublished work from this laboratory (L. H. Perrin and M. B. A. Oldstone) suggests that there may be differences in the ability of antibody to measles virus HA and antibody to measles virus F protein to produce ADCC.

If spontaneous cytotoxicity by NK cells does involve recognition of target antigens by a receptor distinct from the Fc receptor on cells within the null cell population, it is conceivable that antiviral antibody on the target cell surface might sterically hinder binding of such cells to their target antigen, whatever that antigen is, on the infected cell. Antibody might thus inhibit NK cell binding as it simultaneously facilitated K cell binding.

2. Role of Antibody in Spontaneous PBL-Mediated Cytotoxicity of Virus-Infected Cells

There are a number of reports that evaluate the spontaneous cytotoxicity of human PBL for virus-infected targets without added antibody. Some selected reports are discussed that indicate a possible role for antibody. In some cases there is evidence that cytotoxicity is dependent on antibody adsorbed to effector cells as suggested by studies of Greenberg *et al.* (1977) with influenza and Möller-Larssen *et al.*

(1977) with HSV. An alternative explanation would be that antibody is produced by plasma cells in the course of the cytotoxicity assay. After vaccination of human subjects with measles virus or vaccinia virus, Perrin *et al.* (1977b, 1978) found that cytotoxic PBL were generated. Enhanced lysis of virus-infected targets by PBL began at day 5, peaked by day 7, and was rapidly dissipated by day 15 after immunization. These induced cytotoxic PBL showed immune specificity. PBL generated after vaccination with measles virus killed measles virus-infected target cells preferentially, but not vaccinia virus-infected targets. Similarly, cytotoxic PBL generated after vaccinia vaccination showed enhanced killing of vaccinia-infected targets, but not of measles virus-infected targets. The target cells used were autologous fibroblasts and homologous and xenogeneic cultured cell lines. PBL cytotoxicity induced by measles virus or vaccinia virus vaccines was not mediated by T cells (E-rosetting cells), but by Fc receptor positive, E rosette negative cells. The specific cytotoxicity for virus-infected target cells was inhibited 95% by $F(ab')_2$ antihuman IgG. Further, experiments using different subsets of PBL from immune and nonimmune subjects showed that Fc receptor bearing lymphocytes from either immune or nonimmune persons lysed virus-infected targets in the presence of B cells from immune donors. Hence, two populations within PBL participated in the killing of virus-infected targets. In addition to the Fc receptor positive effector lymphocytes, it is likely that B lymphocytes producing antibody were present. The evidence for antibody playing a role in these circumstances is compelling, but synthesized antibody has not been identified directly. However, PBL from subjects 7 days after immunization or natural infection with influenza virus have recently been shown to secrete specific antibody to the viral hemagglutinin *in vitro*, coinciding with a peak in cytotoxicity by these PBL against influenza infected target cells (Greenberg *et al.*, 1979). However, in other instances the enhanced cytotoxicity against virus-infected targets shown by human PBL in the absence of added antibody is likely to result from interferon-induced NK cell activity (Santoli and Koprowski, 1979).

3. *Effector Cell in ADCC of Virus-Infected Cells*

The abundance of evidence indicates that the effector cells mediating ADCC of virus infected targets are the same as those mediating ADCC of other nucleated cell targets by human PBL, namely, nonadherent cells with Fc receptors that do not rosette with sheep E. The report of an adherent cell mediating ADCC against an HSV-infected target cell (Kohl *et al.*, 1977) could relate to special circumstances in the HSV system. However, in most other studies the cytotoxicity of

adherent cells had not been studied directly, their ineffectiveness being indirectly deduced from the fact that their depletion from PBL does not diminish cytotoxicity by the remaining PBL. Human adherent mononuclear cells can reportedly mediate ADCC against uninfected sensitized nucleated targets (K562 and CLA-4 cells) (Horwitz *et al.*, 1979).

Rouse *et al.* (1976) noted that neutrophils were the predominant effector cell in a bovine system, killing sensitized targets infected with a bovine herpes virus. They also reported that complement enhanced ADCC mediated by neutrophils in this system (Rouse *et al.*, 1977). However, no clear distinction was made between an effect of complement enhancing ADCC, or causing independent membrane damage that summated with that produced by ADCC. There are no reports of neutrophils mediating ADCC of virus-infected targets in a human system. Enhanced ADCC activity against measles virus-infected targets by PBL (without neutrophils) in the presence of complement has been the subject of extensive experiments in this laboratory, but has not been demonstrated (J. Huddlestone, unpublished observations). Although some K cells probably have low affinity C3 receptors, no convincing role for C3 has been shown in ADCC of other nucleated cell targets (Perlmann and Cerottini, 1979).

4. Conclusion

IgG antibody is highly efficient in its ability to sensitize virus-infected cells for lysis by human PBL. The amount of cell-bound specific antiviral antibody required for ADCC is at least 10- to 100-fold less than that required for antibody-dependent complement-mediated cytotoxicity and the kinetics of ADCC are faster than those of modulation. At present, in virus-infected human target models there is no evidence that complement potentiates ADCC activity. Although precise assessment of the role of ADCC in cytotoxicity assays is complicated by the presence of NK cell activity in the same lymphocyte population, ADCC appears to be a more efficient cytotoxic mechanism *in vitro* than NK cell cytotoxicity. The relative role of ADCC *in vivo* in man during acute viral infections, as well as that of NK cells induced by interferon and that of cytotoxic T cells, remains to be determined.

VI. Conclusions

There is ample evidence from *in vitro* studies that, in the absence of any cytotoxic effector system, antibody can inhibit virus production and can prevent infected cells from subsequent immunologic attack.

In the presence of complement- or antibody-dependent cytotoxic lymphocytes, antibody can mediate the destruction of virus-infected cells. As with other types of cytotoxicity demonstrated *in vitro*, it is difficult to be certain about the relative importance of these actions *in vivo*. Complement alone, NK cells, cytotoxic T cells, macrophages and interferon are all potentially able to contribute to the control of virus infection *in vivo*, in addition to any effects dependent on antibody.

A. Role of Viral Antigenic Modulation *in Vivo*

There is evidence that antibody-induced viral antigenic modulation occurs *in vivo*. This is well documented for the tumor virus models alluded to in Section III, namely for Gross leukemia virus (Aoki and Johnson, 1972) and FLV (Doig and Cheseboro, 1979), as it is for the TL system (Boyse *et al.*, 1963, 1967). The evidence is the loss of surface viral antigens *in vivo* despite the presence of cytoplasmic viral antigen and the reexpression of surface antigen when leukemic spleen cells from affected animals are transferred to an antibody-free environment. The reason why antigenic modulation, rather than immune lysis, of infected cells should occur is not clear. However, both complement-mediated lysis and ADCC are relatively inefficient in the mouse compared to human systems; for instance, the DBA/2 mice with dormant FLV erythroleukemia [studied by Wheelock *et al.* (1972) and Genovesi *et al.* (1977)] are C5 deficient. In addition, antibody might block T cell cytotoxicity (Welsh and Oldstone, 1977; Effros *et al.*, 1979), or the infection could adversely affect the function of cytotoxic effector cells.

It is not known whether antigenic modulation of virus-infected cells occurs *in vivo* in man. However, one situation with possible relevance to the *in vitro* antigenic modulation of measles-infected cells is the disease subacute sclerosing panencephalitis (SSPE). SSPE is a rare disease characterized by chronic progressive brain damage afflicting children or young adults. Measles virus has been isolated predominantly from the brain and lymphoid tissue by cocultivation techniques, and electron microscopy shows accumulations of measles viral nucleocapsids in brain cells (ter Meulen *et al.*, 1972). Thus, SSPE is a persistent measles virus infection in man, but the reasons for the latent period of several years between typical measles virus infection and the insidious onset of SSPE as well as for the localization in the brain are unknown. Patients with SSPE have high titers of antibody to measles in their sera and cerebrospinal fluids (CSF), but no evidence of impaired antibody and complement killing (Perrin *et al.*, 1976; Joseph *et al.*, 1975) or ADCC (Kreth and ter Meulen, 1977; Per-

rin *et al.*, 1977a) of measles virus-infected target cells. CSF has no detectable hemolytic complement, either in normal subjects or in patients with SSPE. We have postulated that in the presence of antibody, but absence of complement or paucity of cytotoxic lymphocytes in local areas of the central nervous system, antibody could modulate viral antigens expressed on infected cell surfaces. This could prevent cell fusion and death and initiate a state of viral persistence. These conditions would provide selective pressure favoring the emergence of mutant viruses, such as temperature-sensitive or defective interfering virus (Oldstone, 1981). There is experimental evidence suggesting that antibody can be associated with modification of measles infection and facilitation of a persistent infection. Albrecht *et al.* (1977) found that an SSPE isolate produced a chronic encephalitis in animals with preexisting antibody to measles, compared to an acute fatal disease in animals without antibody. Similarly, Wear and Rapp (1971) found that maternal antibody was required to produce latent brain infection upon inoculation of newborn suckling hamsters with an adapted strain of human measles virus.

HSV is commonly maintained in a latent state within nerve ganglia in the central nervous system. It has been suggested that antibody to HSV could maintain HSV latency in infected ganglia, as evidenced by passively transferred antibody preventing reactivation in latently infected ganglia implanted into nonimmune mice (Stevens and Cook, 1974). This has been interpreted as a possible example of antibody-induced antigenic modulation (Stevens and Cook, 1974; Joseph and Oldstone, 1975). Others have suggested that an additional mechanism may maintain latency, because in their experiments reactivation of HSV occurred despite high titers of HSV antibody both *in vitro* and in immunosuppressed mice (Openshaw *et al.*, 1979).

B. Role of Antibody-Dependent Killing of Virus-Infected Cells *in Vivo*

In murine models of virus infection, there are a number of reports of protection from acute virus infection by passive transfer of antibody (reviewed in part by Allison, 1974), although the protection is not invariable. However, the protective effect of antibody could result from neutralization of free virus rather than any cytotoxic effect on infected cells. Hicks *et al.* (1978) showed that decomplementation with cobra venom factor increased the mortality and severity of pneumonia from influenza in mice (disease was also more severe in C5-deficient animals) despite serum neutralizing antibody responses equivalent to controls. The mechanism, whether by direct lysis of infected cells or

chemotaxis of inflammatory cells, or an effect on free infectious virus, is unknown.

There is evidence that tissue injury at sites of virus persistence in chronic LCMV infection in mice or Aleutian disease of mink can be mediated by passively transferred antibody (Oldstone and Dixon, 1970; Porter *et al.*, 1972), and that decomplementation with cobra venom factor protects from death adult mice acutely infected with LCMV (Oldstone and Dixon, 1971). IgG and C3 have been demonstrated by immunofluorescence on neuronal cells infected with measles, in perivascular areas of the brains of patients with SSPE (Vandvik, 1973; Jenis *et al.*, 1973). This type of evidence, suggesting that antibody and complement can induce tissue damage by reacting with virus-infected cells, provides the strongest evidence for antibody-mediated destruction of virus infected cells *in vivo*.

The evidence provided by human immunodeficiency syndromes suggests that patients with T cell deficiencies are more likely to develop severe infections from common viral agents (e.g., measles virus, herpes virus, vaccinia virus infections). Many of the immunodeficiencies characterized by T cell defects are also accompanied by deficiency in antibody production, and many viral antigens are T dependent. Hence, antibody deficiency cannot be totally excluded from contributing to the severity of virus infections in these circumstances. Patients with hypogammaglobulinemia generally recover normally from virus infections, although whether their small amount of IgG effectively mediates ADCC is not known. Patients with deficiencies of C3 itself, or of C5-8, do not seem to be unduly predisposed to virus infections, although C3-deficient patients are prone to recurrent pyogenic bacterial infections (Lachmann and Rosen, 1979). Nevertheless, passively administered antibody can clearly exert a protective or therapeutic effect in a number of human virus infections. This applies also to immunodeficient patients who contract vaccinia virus or varicella-zoster virus infections (Pahwa *et al.*, 1979).

C. Conclusion

In this review we have documented, particularly by reference to work in human systems, that antibody exerts a number of significant effects on virus-infected cells. IgG antibody can mediate the destruction of virus-infected cells in conjunction with complement or cytotoxic lymphocytes. In addition, at a conceptual level there is evidence to suggest that antibody may enhance and confer specificity on basic nonspecific humoral and cell-mediated defense mechanisms. Thus, virus-infected cells can activate the alternative complement pathway

independent of IgG, but antibody is required for subsequent lysis of the cell. Similarly, NK cells can lyse virus-infected target cells independent of antibody and without immunologic specificity, but antibody enhances, and confers specificity on, cytotoxicity by what is probably the same class of effector cells. The importance of these reactions *in vivo*, particularly in comparison with cytotoxic T cells, remains to be uncovered, as does the possibility that antibody can block cytotoxic T cell function *in vivo* as it can *in vitro* (Welsh and Oldstone, 1977; Effros *et al.*, 1979; Zinkernagel and Doherty, 1979). Because there is limited information on the relative importance of these cytotoxic mechanisms in acute virus infections in humans, future collection and analysis of data in this area are necessary.

Finally, the ability of antibody to affect the synthesis and intracellular and surface expression of viral proteins has only recently been investigated on a molecular basis. There is already evidence that antibody can act at this level, and such mechanisms may play an important role in the establishment and maintenance of viral persistence.

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