

IMMUNOLOGIC INJURY IN MEASLES VIRUS INFECTION

II. Suppression of Immune Injury Through Antigenic Modulation*

BY BARRY S. JOSEPH† AND MICHAEL B. A. OLDSTONE

(From the Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California 92037)

Among the puzzles in contemporary medicine are disease states in which infectious virus persists in the presence of specific viral antibodies. For example, such a dichotomy may occur in human infections with herpes simplex virus, cytomegalovirus, rubella virus, and measles virus. Measles virus infection in humans usually runs an acute, self-limiting course as the patient mounts an immune response which clears this virus from his tissues. Convalescence is associated with low titers of antibodies to measles virus throughout life. In contrast, in the unusual instance of chronic measles virus infection (subacute sclerosing panencephalitis [SSPE])¹ the patient has high titers of antimeasles virus antibody and virus persists. Patients with SSPE most often have a progressive degenerative disease of the central nervous system (CNS), and 10-fold or greater titers of antimeasles virus antibody in their sera and cerebral spinal fluids than found in sera of patients recovered from acute measles virus infection. The virus can be isolated from the CNS (1, 2) and lymphoid tissues (3) in SSPE.

The persistence of virus during high antiviral antibody production in SSPE led to the hypothesis (4) that chronic measles virus infection was most likely associated with a specific defect in cellular immunity. However, evidence from several laboratories indicates that peripheral lymphoid cells from patients with SSPE retain responsiveness to measles virus antigens and can, under appropriate conditions, lyse target cells expressing measles virus antigens on their surface (5-7). Recently, the suggestion has been put forth that a blocking factor, either antibody or virus-antibody immune complexes, interferes with cell-mediated immune responsiveness to measles virus antigens in patients with SSPE (8). Alternatively antibodies against measles virus may strip or modulate viral antigens from the surface of infected cells, thus removing the recognition and binding sites to which the progenitors of immunological injury attach.

Antigenic modulation was first described by Boyse et al., who showed that surface isoantigens on mouse leukemic cells (specifically thymus-leukemia [TL] isoantigens) disappeared when TL-positive cells were exposed to antibody(s) against TL determinants in the absence of complement (C). Such phenotypic alteration occurred in vivo (9, 10), as well as in vitro (11), and was reversed by eliminating TL antibody(s). Other experiments indicated that antigenic modulation was an active process requiring metabolically intact cells and did not result from long-term masking of TL-antigenic sites by immunoglobulin (Ig) (11).

* This is publication no. 978 from the Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California 92037. Supported by U. S. Public Health Service grants AI-07007, AI-09484, and NS-12428.

† Recipient of National Multiple Sclerosis Society Fellowship Award.

¹ Abbreviations used in this paper: FITC, fluorescein isothiocyanate; MOI, multiplicity of infection; SSPE, subacute sclerosing panencephalitis; TL, thymus-leukemia.

Shortly thereafter it was demonstrated that anti-Ig's could redistribute surface Ig on murine lymphocytes into polar aggregates ("caps") (12, 13). After protracted incubation of lymphocytes under capping conditions, surface Ig totally disappeared through endocytosis and external stripping, but surface Ig reappeared shortly after reculturing denuded lymphocytes in medium lacking anti-Ig's. As in the TL system, modulation of surface Ig depended on active cell metabolism. Antibody-induced modulation has additionally been demonstrated for histocompatibility antigens (14-17), Burkitt lymphoma cell surface antigens (18), and Gross leukemia cell surface antigens (19).

Recently we reported that measles virus antigens at the surface of infected human cells in culture redistributed into polar aggregates after exposure to antibodies against measles virus (20, 21). Capping of measles virus antigens exhibited many of the same functional dependencies that have been defined for capping in other antigenic systems (20). The present study extends these earlier observations by demonstrating modulation of measles viral antigens on cultured human cells incubated with serum containing antibodies against measles virus in the absence of C. We relate this finding to the pathogenesis of SSPE and offer an hypothesis to explain why in certain diseases virus persists in the presence of antiviral antibodies.

Materials and Methods

Virus. Wild type Edmonston strain measles virus was obtained from the American Type Culture Collection, Rockville, Md. After repeated passage in HeLa cells, culture fluids from infected cells were clarified by centrifugation at 700 *g* for 10 min and were then stored in 3-cc aliquots at -70° C until use. This virus pool contained 3×10^6 plaque-forming U/ml on Vero cell monolayers.

Cell Lines. HeLa cells were obtained from Microbiological Associates, Inc., Los Angeles, Calif. These cells were found to be devoid of mycoplasma contamination. A line of HeLa cells persistently infected with measles virus was prepared according to the method described by Rustigian (22) and Minagawa (23). In brief, HeLa cells were infected with stock measles virus at a multiplicity of infection (MOI) of 0.2. Cultured monolayers were fed daily during the first 2 wk of infection and twice weekly thereafter. While the vast majority of cells died after acute virus infection, a few survived. After 1.5 mo, these cells multiplied sufficiently to permit serial repassaging. Such cells expressed virus antigens on their surface and released infectious virus into culture fluids and were called HeLa-P/M⁺.

Handling of Cells. Both infected and uninfected cell lines were grown routinely as monolayers in 75 cm² Falcon flasks (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) with Eagle's minimal essential medium supplemented with 10% heat-inactivated fetal calf serum, 1% glutamine, and antibiotics (growth medium). Flasks were incubated at 37° C in 5% CO₂ and cultures were divided weekly. Uninfected HeLa cells were fed weekly, whereas HeLa-P/M⁺ were fed twice per week.

In some experiments, HeLa cells were grown as suspension cultures in Spinner's medium in stoppered Erlenmeyer flasks on a rocker platform at 37° C. Conditions for infecting HeLa cells with stock measles virus, preparing cells for immunofluorescence, and cytotoxicity analysis have been described (20, 24).

Immune Reagents. Convalescent serum from an adult having antibodies to measles virus in a 1:64 cytotoxicity titer for HeLa cells infected with measles virus and a 1:64 hemagglutination inhibition titer was used in these studies. Fresh frozen aliquots of this immune serum, stored at -70° C, served as the source of antibody and C in cytotoxicity studies. For studying modulation of virus antigens, serum from 500 ml of fully clotted blood was passed through a 0.4 μ m filter into a sterile receptacle, heated at 56° C for 30 min (heat inactivated), and then stored at -20° C in 50-ml aliquots. In addition, the IgG fraction of this serum was purified, conjugated with fluorescein isothiocyanate (FITC), and absorbed with uninfected HeLa cells by methods detailed elsewhere (20). For radioisotopic studies, IgG from the same source was labeled with ¹²⁵I (25) and then centrifuged at 100,000 *g* for 30 min to remove aggregates. An IgG fraction from hyperimmune goat

sera against human IgG was prepared and labeled with FITC as described above.

Immunofluorescence. All immunofluorescence studies were performed on viable cell suspensions. The techniques and equipment used have been described (20). Capping reactions were assessed by mixing 1×10^5 washed and pelleted cells with 30- μ l aliquots of heat-inactivated fetal calf serum and FITC-conjugated human antibody against measles virus. Reaction mixtures were incubated at 37° C for 60 min, washed with medium, and a minimum of 100 cells were examined for capping in each test.

Cytotoxicity. A modification of the eosin micromethod was used and the cytotoxicity index calculated as previously described (24). Experimental reaction mixtures consisted of 300–400 target cells added to 8 μ l of freshly thawed immune serum. Nonspecific lysis was monitored by reacting target cells with heat-inactivated immune serum. In some instances, trypan blue dye exclusion was used to monitor cell viability.

Capping and Lysis of Cells Infected with Measles Virus. HeLa cells were infected with measles virus at an MOI of 0.2 and were then plated in replicate in T-75 culture flasks. On the 2nd, 3rd, 4th, and 5th days of culture, cells from individual flasks were suspended by treatment at room temperature with 0.05% trypsin-EDTA, then assessed for their ability to be capped, and lysed with humoral antimeasles virus reagents (20). In some experiments culture medium was not changed after initial cell plating while in other experiments, medium was changed daily.

In other studies, infected cells grown for 6 days without medium change were assessed simultaneously for the rates at which capping and lysing occurred at 24 and 37° C. Test cells were labeled with FITC-conjugated human antibody to measles virus, washed once, and then resuspended in freshly thawed immune serum (5,000 cells/100 μ l serum). Reaction mixtures incubated under differing temperature conditions were serially assessed for cap formation and cell death. Control preparations consisted of labeled cells incubated with heat-inactivated immune serum.

Cells Cultured under Extended "Capping" Conditions. Both acutely and persistently infected HeLa cells were used and in each instance growth medium was changed daily for 3 consecutive days before experimentations. Cells from both cultures were harvested, and evaluated for the presence of measles virus antigens on the cell surface and ability to be lysed by antimeasles virus antibody and C. This constituted time zero of the sequential analysis. Concurrently 5×10^6 cells from each culture were mixed with 20 ml of undiluted, heat-inactivated immune human sera for 30 min at room temperature with frequent agitation, pelleted by centrifugation at 500 *g* for 10 min at 4° C, resuspended in 50 cc modified Spinner's medium containing 20% heat-inactivated immune serum, and grown in suspension under standard culture conditions. At various time intervals 5-cc aliquots (approximately 5×10^5 cells) were removed from each culture, washed three times in cold (4° C) growth medium, and evaluated for measles virus antigens on the cell surface and lysability with viral antibody plus C. 24 h later cells were washed and resuspended in Spinner's medium lacking antibodies to measles virus. After an additional day in culture, cells were reassessed for surface measles virus antigenicity and susceptibility to lysis with immune serum.

Radioisotopic Labeling. To determine the fate of antibody molecules attaching to viral antigens at the surface of persistently infected cells, 1×10^7 freshly suspended, washed cells were incubated with 1,000 μ g of human IgG containing antibodies to measles virus and radiolabeled with 125 I (sp act, 0.5 μ Ci/ μ g; vol, 500 μ l) for 30 min at room temperature in 500 μ l growth medium, washed five times with 15-cc aliquots of growth medium, and then cultured in 50 cc Spinner's medium. 5-cc samples were collected serially at various intervals over 48 h. Each sample was mixed vigorously with 10 cc of growth medium and centrifuged at 500 *g* for 10 min. The resulting supernate was decanted and saved; the cell pellet was resuspended in growth medium to a final vol of 2 cc and the cells counted. Tubes containing supernatant fluids and resuspended cells were counted in a Baird atomic gamma counter (Baird Atomic, Inc., Bedford, Mass.).

In other experiments HeLa-P/M⁺ cells were labeled with [125 I]measles antibody in a manner akin to that described above. After labeling and several washes, cells were divided into two aliquots—one of which was cultured in Spinner's medium containing 20% heat-inactivated fetal calf serum; and the other in Spinner's medium containing 20% heat-inactivated immune serum. Serial 5-cc samples were collected at timed periods over 18 h and processed as described above. The percent of labeled antibody released into the culture fluid at each interval was calculated by dividing the number of unbound counts in each sample by the total number of counts in each sample. Background unbound radioactivity at time zero was subtracted from counts recorded for

each supernate before calculating the percent radioactive counts released.

Cells Cultured for Extended Periods in the Presence of Measles Antibodies with or without C. Monolayer cultures acutely infected with measles virus and showing at least 95% of all cells expressing surface virus antigens were washed several times with buffered saline and treated with undiluted, freshly thawed measles immune human sera. The surviving cells (less than 10%) were cultured in growth medium supplemented with 10% immune serum. Cultures were divided as often as necessary to maintain subconfluent conditions and maintained in this manner for 6 wk. Persistently infected cultures were either treated in a similar manner or cultured with heat-inactivated immune serum. At various intervals after exposure to immune serum, samples of cells from each test condition were removed and grown in suspension with medium lacking antibodies to measles virus. These cells were tested by immunofluorescence for re-expression of measles virus antigens on the surface of cells.

Growth rates were determined for uninfected cells, persistently infected cells unexposed to measles antibody, and persistently infected cells pretreated for several weeks with immune serum. Cells were grown in suspension in Spinner's medium supplemented with 10% heat-inactivated fetal calf serum. In addition, we monitored the change in growth rate and cell viability after acutely infected cells (pretreated for 6 wk with immune serum) were washed and then placed in Spinner's medium lacking measles antibody.

Results

Relationship between Capping and Immune Cytolysis. Initial studies indicated a direct relationship between redistribution (capping) of measles virus antigens on cell surfaces and lysis of virus infected cells by antibody and C. Fig. 1 shows that at temperatures of 24 and 37° C, within the same cell population over a 240 min period of observation, the numbers of infected cells lysed by antibody and C closely paralleled the numbers of cells that capped. A similar correlation between capping and antibody-mediated lysis was seen in replicate cultures sampled over a 5 day period. Fig. 2 shows that 3 days after acute viral infection, 65% of HeLa cells were capped and 60% lysed, and on the 5th day 90% were capped and 95% lysed. Capping occurred with equal efficiency in the presence or absence of C.

Despite the close correlation observed between capping and lysis, it was possible to disassociate these two events. When HeLa cells infected with measles virus were recultured daily in fresh growth medium, antibody-induced redistribution of measles virus antigens on the cell surface was markedly inhibited. In

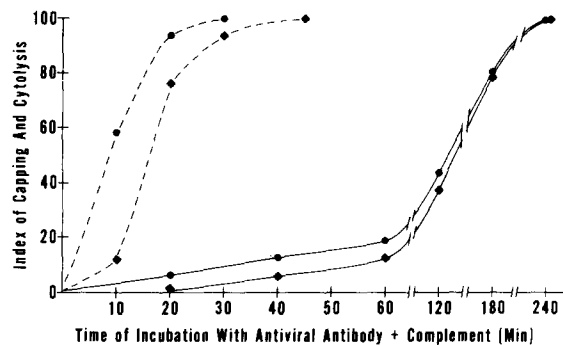


FIG. 1. Temporal association between redistribution of measles virus antigens on the surfaces of infected HeLa cells (●) and the ability of antimeasles virus antibody and C to lyse such cells (◆). Experiments done at 24° C (solid lines) and 37° C (dashed lines).

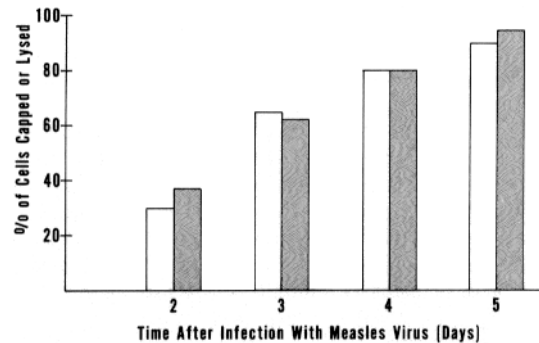


FIG. 2. Maximum degrees of redistribution of measles virus antigens on the surface of infected HeLa cells (*opened bars*) and lysis of such cells by antimeasles virus antibody and C (*stippled bars*) at 37° C. HeLa cells were infected with measles virus at a MOI of 0.2 and were assayed on successive days after virus adsorption.

contrast this treatment did not abrogate antibody-mediated C-dependent lysis of virus-infected cells. Evidently under these conditions lysis occurred independently of cap formation.

Relationship between Modulation of Measles Virus Antigens from the Cell's Surface and Antibody-Mediated C-Dependent Lysis. Preliminary studies indicated that measles virus antigens were removed from the surface of infected HeLa cells cultured in suspension with medium supplemented with heat-inactivated human serum containing antibodies to measles virus. In more detailed experiments, marked suppression of surface measles virus antigenic expression occurred in both acutely and persistently infected cells within 12 h after the addition of antimeasles virus antibodies (Figs. 3 and 4). In each instance the time-course associated with the loss of surface measles virus antigens paralleled the inability of antiviral antibody and C to lyse infected cells. Removal of measles virus antigens and suppression of antibody-mediated lysis were more complete in persistently infected (Fig. 4) than in acutely infected cells (Fig. 3).

Modulating of surface measles virus antigens proved to be completely reversible. Expression of measles virus antigens on the cells' surfaces and susceptibility of infected cells to injury by antiviral antibody and C recurred spontaneously in the preparation of acutely infected cells (Fig. 3). The reversal was similar when antibody-treated, persistently infected HeLa cells were washed and recultured for 24 h in medium lacking antimeasles virus antibodies (Fig. 4). No significant alterations in cell viability or concentration occurred during these experiments.

Only minimal degrees of cap formation were evident at each interval sampled despite modulation of surface viral antigens. This is in agreement with others who showed that modulation of TL surface antigens could occur independently of any major redistribution of these antigens along the cell surface (26, 27).

Fate of Antimeasles Virus Antibodies after Attachment to Cells Infected with Measles Virus. Radiolabeling experiments indicated that the majority of [¹²⁵I]antimeasles virus IgG attached to the surface of infected cells was shed into the culture fluid. A representative experiment is shown in Table I in which 67% of [¹²⁵I]antimeasles virus IgG attached to the surface of persistently infected

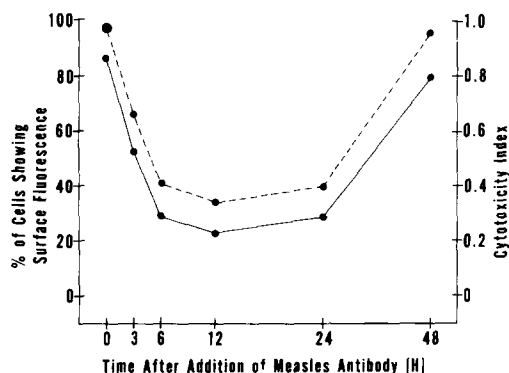


FIG. 3. Alteration of surface expression of measles viral antigens (*dashed line*) and antibody-mediated C-dependent lysis (*solid line*) after incubation of acutely infected HeLa cells with measles virus antibodies. HeLa cells were infected with measles virus at an MOI of 0.2 and on the 4th day of infection were cultured in suspension with antiviral antibody. Surface expression of measles virus antigens and susceptibility to antibody-mediated C-dependent lysis reverted spontaneously in this test system.

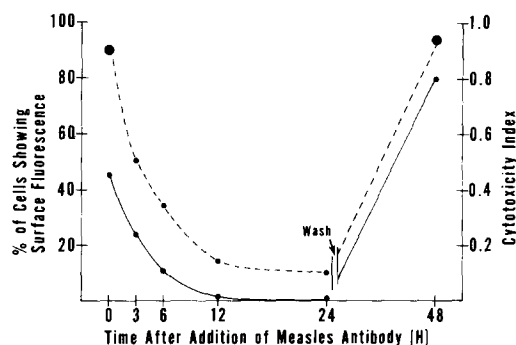


FIG. 4. Alteration of surface expression of measles viral antigens (*dashed line*) and antibody-mediated C-dependent lysis (*solid line*) after incubation of persistently infected HeLa cells with antibodies to measles virus. This persistently infected line was derived from cultured cells surviving acute measles infection and was maintained in culture 3 mo before beginning these experiments. Cells were incubated with medium containing antibodies to measles virus for 24 h, washed, and then recultured in medium lacking antibodies to measles virus.

HeLa cells was released from the cell surface into culture medium during the first 24 h of culture. Release of label was most pronounced during the first 12 h of culture when 55% of originally cell-bound radioactive counts were dissociated from the cell surface; by 48 h, 85% of radioactive counts had been released. Similar results were observed in acutely infected cells cultured in suspension, where 61 and 79% of originally cell-bound radioactive counts dissociated from the cell surface at 12 and 24 h, respectively.

Other experiments showed that the rate of release of radiolabeled measles virus antibody from the surface of persistently infected cells did not significantly vary as the concentration of antibody increased. As shown in Table II, at 6 h 26%, at 12 h 39%, and at 18 h 50% of total cell-bound [125 I]Ig counts were released

TABLE I
Fate of Antibody Attaching to HeLa Cells Persistently Infected with Measles Virus

Time after addition of [¹²⁵ I]antibody	Cell pellet	Supernate	[¹²⁵ I]antibody dissociated from cell surface*
<i>h</i>	<i>cpm per sample</i> ‡		<i>%</i>
0	130,624	0	0
3	92,676	36,164	28
6	77,429	50,838	40
12	57,282	68,650	55
24	41,494	84,930	67
48	17,584	100,942	85

* The percent [¹²⁵I]antibody dissociated from the cell surface was calculated at each interval by the formula: (cpm supernate)/[(cpm cell pellet) + (cpm supernate)] × 100.

‡ Infected cells (1 × 10⁷) were labeled with [¹²⁵I]measles antibody, then washed repeatedly, suspended in 50 cc Spinner's growth medium, and grown in suspension on a rocker platform at 37°C. At each interval shown, a 5 cc sample was removed from the cell culture. The sample was clarified by centrifugation and radioactivity was then determined independently for the cell pellet and the supernate. Background radioactivity in supernate at time zero, 1,102 cpm, was subtracted from the total radioactivity recorded for supernate at each interval.

TABLE II
*Rate of Release of [¹²⁵I]Antibody to Measles Virus from HeLa Cells Persistently Infected with Measles Virus**

Time after placing labeled cells in culture	Total cell bound counts released into:	
	Regular growth medium	Growth medium supplemented with antibodies to measles virus
<i>h</i>	<i>%</i>	<i>%</i>
0	0	0
3	19	21
6	26	30
12	39	42
18	50	50

* The experimental protocol was similar to that outlined in Table I with the exception that half of the labeled cells were cultured in standard medium while the remainder were cultured in medium supplemented with 20% heat-inactivated immune serum rather than fetal calf serum. The method for determining percent [¹²⁵I]antibody release is described in Table I.

into the medium when cells were cultured in the absence of additional antiviral antibody as compared to 30% at 6 h, 42% at 12 h, and 50% at 18 h, respectively, in the presence of supplemental antiviral antibody. Thus, release of antibody is independent of antibody concentration. At present it is not clear whether antibody enhances the release of viral antigens from the cell surface as no direct measurement of viral antigen or viral antigen-antibody complexes was made. Analogous results occur in the TL system (28).

Fate of HeLa Cells Infected with Measles Virus and Cultured for Prolonged

Times with Antimeasles Virus Antibody in the Presence or Absence of C. Culturing persistently infected cells with serum containing antimeasles virus antibodies and C produced a population of cells devoid of surface measles virus antigens. As exposure to antiviral antibody and C lengthened, the rate at which measles virus antigens returned to the cell surface, once antibody was removed, progressively diminished. Hence, after only 1 day incubation with antiviral antibody, viral antigen reappeared on 50% of the cells within 24 h; after 5 days incubation it took an additional 6 days before surface viral antigen was detected on 50% of the cells. With 6 wk of antibody and C treatment, we were unable to detect a return of measles virus antigens on the surfaces of HeLa cells over a subsequent 6 wk period in which these cells were washed and cultured in medium lacking antibodies to measles virus. In addition to not expressing surface viral antigens, the latter cell line also failed to express internal measles antigens or measles virus structures when cells were fixed on cover slips with ether-alcohol, washed and stained with FITC antimeasles antibody reagent, and studied by electron microscopy (Joseph, B. S., P. W. Lampert, and M. B. A. Oldstone, unpublished observations).

In contrast, with similar culture conditions, measles virus antigen-negative cell lines were not produced by treatment with antiviral antibody alone or in acutely infected cells treated with antibody and C. Acutely infected cells that survived treatment with antibodies to measles virus plus C re-expressed full surface antigenicity and were lysed by antimeasles viral antibodies and C within 4–7 days after antibody withdrawal regardless of the treatment's duration (Fig. 5).

As shown in Fig. 6, the growth rate of persistently infected cells cultured with antibodies to measles virus was similar to that of uninfected control HeLa cells. In contrast the growth rate of persistently infected HeLa cells in the absence of antimeasles virus antibody was retarded. Acutely infected cells which survived incubation with antiviral antibody and C exhibited growth kinetics similar to that of uninfected HeLa cells as long as the infected cells were cultured with antiviral antibody. Once antibody was removed from the culture medium,

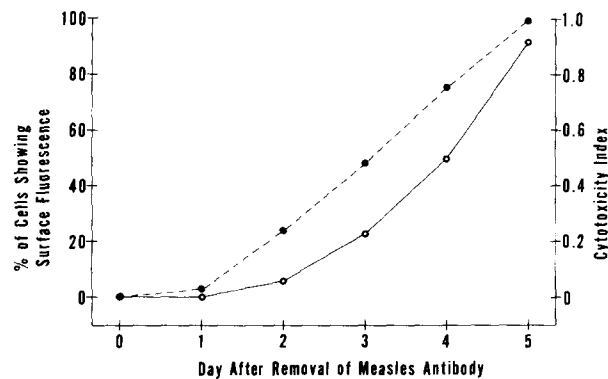


FIG. 5. Return of surface measles virus antigens (*dashed line*) and of lytic susceptibility to antibody and C (*solid line*) after culturing acutely infected HeLa cells for 2 wk with measles virus antibodies and C. Comparable results were obtained after 6 wk of culturing with antibody and C.

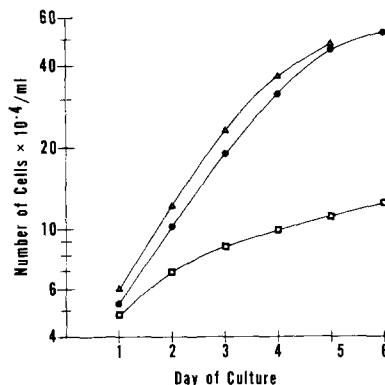


FIG. 6. Growth rates of normal HeLa cells (●), persistently infected HeLa cells (□), and persistently infected cells pretreated for 4 wk with measles virus antibodies (△). The growth rate of acutely infected HeLa cells was likewise normalized by antibody treatment.

growth lagged and ultimately the entire culture degenerated, showing focal areas of giant cell and syncytial formation, thus appearing cytopathologically similar to HeLa cells acutely infected with measles virus.

Acutely or persistently infected HeLa cells cultured in the presence of anti-measles virus antibodies showed normal cytomorphology. No giant cell or syncytial formation was evident in these cultures, and the cells grew to confluency.

Discussion

Antibody to measles virus removed measles virus antigens off the surface of infected cells. Surface viral antigenic expression of both acutely and persistently infected cells was greatly diminished within 12 h after exposure to measles virus antibody. As judged by immunofluorescence, removal of measles virus antigens from the surface of persistently infected cells was more complete than that obtained with acutely infected cells, possibly because the former contain two to three times less surface viral antigens,² or because of differences in factors such as membrane or cellular metabolic function. In addition, the majority of radioactive antimeasles virus antibody that had attached to infected HeLa cells was exogenously released over a 12–24 h period. This agrees with our earlier observation by electron microscopy that infected HeLa cells only rarely endocytosed measles virus-antibody immune complexes (21). Preliminary studies² showed that radiolabeled antibody released from infected cells had a higher sedimentation coefficient than IgG and contained antibodies specifically reactive with measles virus hemagglutinin, indicating that antibody is probably extruded complexed with surface viral antigen.

Once measles virus antigens were removed from the surface of infected cells, the cells were no longer susceptible to antibody-mediated C-dependent lysis. Thus, we devised an experimental situation in which persistently infected cells and high titers of antibody coexisted. Possibly this model is the counterpart of persistent measles virus, rubella virus, herpes virus, and cytomegalovirus

² Perrin, L. H., B. S. Joseph, and M. B. A. Oldstone. Manuscript in preparation.

infections in humans where antiviral antibody and a functional C system occur in the face of persistent virus infection. In all these instances, antiviral antibody may be instrumental in maintaining viral persistence; neither humoral nor cellular-mediated immunity would be expected to function against infected cells denuded of surface viral antigens. In our investigations, when antibody treatment of infected cells was discontinued, measles virus antigens usually became fully re-expressed on plasma membranes. With reintroduction of antiviral antibody and C, these cells were again susceptible to antibody-mediated C-dependent lysis. Hence theoretically, a disease of remissions and exacerbations could occur dependent on fluctuations in the concentrations of either antibody or C in regions of viral persistence.

Biologically, removal of measles virus antigens from the surface of acutely infected cells changed on expected lethal infection associated with such membrane events as giant cell and syncytial formation, into a persistent, nonlethal infection with neither event. Continuous exposure to measles antibody also normalized the growth and cytomorphology of persistently infected cells. Cells treated in this manner continued to express measles virus antigens in their cytoplasm but not on their surface and accordingly were not susceptible to antibody-mediated C-dependent lysis. We have termed this phenomenon "sustained antigenic modulation." Sustained antigenic modulation of acutely infected cells apparently did not detectably promote viral defectiveness, since cells treated in this manner produced infectious measles virus and exhibited complete cytopathic degeneration shortly after withdrawal of antibody to measles virus from the culture medium. Thus, antibody-induced removal of surface viral antigens apparently can directly normalize the function of cells that remain residually infected with virus. In this respect, our observations parallel the recent findings of Stevens and Cook (29) regarding the mechanisms of herpes simplex virus latency within murine neural tissues *in vivo*.

Extended treatment of persistently infected HeLa cells with antibody to measles virus and C generated a cell population which by use of infectivity assay, immunofluorescence, and electron microscopy appeared to be cured of virus infection. In contrast, similar treatment of acutely infected cells or treatment of persistently infected cells with antibody and no source of C failed to produce such cells. Virus-negative or nonyielding cell lines have been previously developed from HeLa cells persistently infected with measles virus but only through the use of cell cloning combined with protracted antibody treatment (23, 30). The fact that we were able to obtain such a line without cloning suggests that a comparable suppressive mechanism could operate *in vivo*.

Recent studies show that there is probably no defect in either humoral or cell-mediated immunity to measles virus in patients with SSPE (5-7, 24, 31, 32). It has been argued that persistence of measles virus-infected cells in patients with SSPE may be related to circulating factors which selectively block cell-mediated cytotoxicity (8). But such an hypothesis does not explain why the humoral immune system fails to rid the body of persistently infected cells. Our results indicate that antibody to measles virus is capable of modulating measles virus antigens from the surface of infected cells, thereby rendering both humoral and cell-mediated immune responses against such cells ineffective. This denuding is

associated with a release of virus-antibody complexes from the cell surface. These complexes might be expected to further nullify immunologic defenses against persistently infected cells (33) and could in fact constitute the "blocking factor" recorded by others (7, 8). In addition, such complexes may deposit in tissues causing local phlogogenic responses (33).

Antigenic modulation might prevail in regions of the body that are relatively deficient in C. In such areas, antiviral antibody could interact with virus-infected cells in a nonlytic manner. Since C in brain fluids is relatively inefficient in promoting lysis of susceptible cells (34), although antibodies can reach high titers in these fluids, possibly virus-infected brain cells would be particularly amenable to antigenic modulation. Furthermore, brain cells infected with measles virus might be particularly affected, since antibody-mediated lysis of measles virus-infected cells occurs by the alternative C pathway (24), a route of activation that is more easily rendered nonoperational by dilution than the classical C pathway. Denuding of measles virus-infected brain cells through antigenic modulation would render these cells resistant to immunologic assault, would prevent virus-associated cell fusion and death, and would favor the establishment of viral persistence. Such a mechanism may play a central role in the pathogenesis of SSPE and other persistent virus infections associated with high antibody responses in humans. Whether antigenic modulation could be brought under clinical control and whether such manipulation would favorably affect the course of persistent viral infection remain subjects for future studies.

Summary

Upon the addition of antibody to measles virus, measles virus antigens expressed on the surface of infected cells can be modulated from the cell's membrane in vitro. Removal of measles virus antigens from the surface of cells occurs relatively rapidly and is accompanied by a parallel reduction in the ability of antibody and complement to lyse these cells. Modulation of surface viral antigens can occur in the absence of cap formation and is fully reversible once measles virus antibodies are removed from culture medium.

Protracted exposure of acutely infected cells to measles virus antibodies results in a population of cells that exhibit normal cytomorphology and growth behavior. These cells continue to express measles virus antigens internally, but not at the cell surface, and are refractory to immune lysis. Once antiviral antibody is removed, measles virus antigens again appear on the cell surface, giant cell and syncytial formation occur, and cell death follows. These observations may explain the persistence of virus in spite of a vigorous host antiviral immune response in certain chronic infections of man.

The authors thank Ms. Hendrix and Ms. Minick for assistance in preparation of this manuscript.

Received for publication 14 May 1975.

Bibliography

1. Horta-Barbosa, L., D. A. Fuccillo, J. Sever, and W. Zeman. 1969. Subacute sclerosing panencephalitis: isolation of measles virus from a brain biopsy. *Nature (Lond.)*. 221:974.

2. Payne, F. E., J. V. Baublis, and H. H. Itabashi. 1969. Isolation of measles virus from cell cultures of brain from a patient with subacute sclerosing panencephalitis. *N. Engl. J. Med.* 281:585.
3. Horta-Barbosa, L., R. Hamilton, B. Wittig, D. A. Fuccillo, and J. L. Sever. 1971. Subacute sclerosing panencephalitis: isolation of suppressed measles virus from lymph node biopsies. *Science (Wash. D. C.)*. 173:840.
4. Burnet, F. M. 1968. Measles as an index of immunological function. *Lancet*. 2:610.
5. Valdimarsson, H., G. Agnarsdottir, and P. Lachmann. 1974. Cellular immunity in subacute sclerosing panencephalitis. *Proc. R. Soc. Med.* 67:1125.
6. Kreth, W. H., M. Käckell, and V. ter Meullen. 1975. Demonstration of *in vitro* lymphocyte-mediated cytotoxicity against measles virus in SSPE. *J. Immunol.* 114:1042.
7. Sever, J. L., and K. W. Sell, editors. 1975. Conference on cellular immunity and SSPE. *Arch. Neurol.* 32:48.
8. Sell, K. W., G. B. Thurman, A. Ahmed, and D. M. Strong. 1973. Plasma and spinal-fluid blocking factor in SSPE. *N. Engl. J. Med.* 288:215.
9. Boyse, E. A., L. J. Old, and S. Luell. 1963. Antigenic properties of experimental leukemias. II. Immunological studies *in vivo* :with C57BL/6 radiation-induced leukemias. *J. Natl. Cancer Inst.* 31:987.
10. Boyse, E. A., E. Stockert, and L. J. Old. 1967. Modification of the antigenic structure of the cell membrane by thymus-leukemia (TL) antibody. *Proc. Natl. Acad. Sci. U. S. A.* 58:954.
11. Old, L. J., E. Stockert, E. A. Boyse, and J. H. Kim. 1968. Antigenic modulation. Loss of TL antigen from cells exposed to TL antibody. Study of the phenomenon *in vitro*. *J. Exp. Med.* 127:523.
12. Taylor, R. B., P. H. Duffus, M. C. Raff, and S. de Petris. 1971. Redistribution and pinocytosis of lymphocyte surface immunoglobulin molecules induced by antiimmunoglobulin antibody. *Nat. New Biol.* 233:225.
13. Loor, F., L. Forni, and B. Pernis. 1972. The dynamic state of the lymphocyte membrane. Factors affecting the distribution and turnover of surface immunoglobulins. *Eur. J. Immunol.* 2:203.
14. Schlesinger, M., and M. Chaouat. 1972. Modulation of the H-2 antigenicity on the surface of murine peritoneal cells. *Tissue Antigens*. 2:427.
15. Edidin, M., and C. S. Henney. 1973. The effect of capping H-2 antigens on the susceptibility of target cells to humoral and T cell-mediated lysis. *Nat. New Biol.* 246:47.
16. Cullen, S. E., D. Bernoco, A. O. Carbonara, H. Jacot-Guillarmod, G. Trinchieri, and R. Ceppellini. 1973. Fate of HL-A antigens and antibodies at the lymphocyte surface. *Transplant. Proc.* 5:1835.
17. Kourilsky, F. M., D. Silvestre, C. Neauport-Sautres, Y. Loosfelt, and J. Dausset. 1972. Antibody-induced redistribution of HL-A antigens at the cell surface. *Eur. J. Immunol.* 2:249.
18. Aoki, T., G. Geering, E. Beth, and L. J. Old. 1971. *In Recent Advances in Human Tumor Virology and Immunology*. W. Nakahara, K. Nishioka, T. Hirayama, and Y. Ito, editors. University Park Press, Tokyo, Japan. 425.
19. Aoki, T., and P. A. Johnson. 1972. Suppression of Gross leukemia cell-surface antigens: a kind of antigenic modulation. *J. Natl. Cancer Inst.* 49:183.
20. Joseph, B. S., and M. B. A. Oldstone. 1974. Antibody-induced redistribution of measles virus antigens on the cell surface. *J. Immunol.* 113:1205.
21. Lampert, P. W., B. S. Joseph, and M. B. A. Oldstone. 1975. Antibody-induced capping of measles virus antigens on plasma membrane studied by electron microscopy. *J. Virol.* 15:1248.

22. Rustigian, R. 1966. Persistent infection of cells in culture by measles virus. I. Development and characteristics of HeLa sublines persistently infected with complete virus. *J. Bacteriol.* 92:1792.
23. Minagawa, T. 1971. Studies on the persistent infection with measles virus in HeLa cells. I. Clonal analysis of cells of carrier cultures. *Jap. J. Microbiol.* 15:325.
24. Joseph, B. S., N. R. Cooper, and M. B. A. Oldstone. Immunologic injury of cultured cells infected with measles virus. I. Role of IgG antibody and the alternative complement pathway. 1975. *J. Exp. Med.* 141:761.
25. McConahey, P. J., and F. J. Dixon. 1966. A method of trace iodination of proteins for immunologic studies. *Int. Arch. Allergy Appl. Immunol.* 29:185.
26. Lamm, M. E., E. A. Boyse, L. J. Old, B. Lisowska-Bernstein, and E. Stockert. 1968. Modulation of TL (thymus-leukemia) antigens by Fab-fragments of TL antibody. *J. Immunol.* 101:99.
27. Stackpole, C. W., J. B. Jacobson, and M. P. Lardis. 1974. Antigenic modulation in vitro. I. Fate of thymus-leukemia (TL) antigen-antibody complexes following modulation of TL antigenicity from the surfaces of mouse leukemia cells and thymocytes. *J. Exp. Med.* 140:939.
28. Yu, A., and E. P. Cohen. 1974. Studies on the effect of specific antisera on the metabolism of cellular antigens. II. The synthesis and degradation of TL antigens of mouse cells in the presence of TL antiserum. *J. Immunol.* 112:1296.
29. Stevens, J. G., and M. L. Cook. 1974. Maintenance of latent herpetic infection: an apparent role for anti-viral IgG. *J. Immunol.* 113:1685.
30. Rustigian, R. 1966. Persistent infection of cells in culture by measles virus. II. Effect of measles antibody on persistently infected HeLa sublines and recovery of a HeLa clonal line persistently infected with incomplete virus. *J. Bacteriol.* 92:1805.
31. Kibler, R., and V. ter Meulen. 1975. Antibody-mediated cytotoxicity after measles virus infection. *J. Immunol.* 114:93.
32. Oldstone, M. B. A., V. A. Bokisch, F. J. Dixon, L. H. Barbosa, D. A. Fuccillo, and J. L. Sever. 1975. Subacute sclerosing panencephalitis: destruction of human brain cells by antibody and complement in an autologous system. *Clin. Immunol. Immunopathol.* 4:52.
33. Oldstone, M. B. A. 1975. Virus neutralization and virus induced immune complex disease: virus-antibody union resulting in immunoprotection or immunologic injury—two different sides of the same coin. *Prog. Med. Virol.* 19:84.
34. Petz, L. D., G. C. Sharp, N. R. Cooper, and W. S. Irvin. 1971. Serum and cerebral spinal fluid complement and serum autoantibodies in systemic lupus erythematosus. *Medicine (Baltimore).* 50:259.