IMMUNOLOGIC INJURY OF CULTURED CELLS INFECTED WITH MEASLES VIRUS

I. Role of IgG Antibody and the Alternative Complement Pathway*

By BARRY S. JOSEPH,‡ NEIL R. COOPER,§ AND MICHAEL B. A. OLDSTONE

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

Measles virus infection in man is commonly an acute, self-limiting disease not associated with viral persistence (1). After infection, which occurs through the respiratory tract, measles virus replicates in respiratory epithelial cells and disseminates throughout the body infecting lymphoid organs and other tissues. During the initial 9–14 days after infection, there is little or no overt evidence of clinical disease. The advent of clinical symptoms and tissue injury is thought to be, at least in part, a direct consequence of the virus and/or virus-infected cells interacting with sensitized lymphocytes and antimeasles virus antibody which are generated during the incubation period. Such interactions are also recognized as prerequisites for viral clearance and clinical recovery. As convalescence proceeds, circulating antibody to measles virus increases, and the virus becomes undetectable. After recovery, the patient has circulating antibody and clinical resistance to measles virus reinfection throughout life.

Other less common forms of measles virus infection are also recognized. Post-infectious encephalomyelitis usually follows the outbreak of rash after measles virus infection (2). Failure to recover virus from diseased tissues associated with an inflammatory response at the site of tissue injury has led to the suggestion that this form of measles-associated disease is basically allergic in nature (3). A chronic degenerative disease of the central nervous system, subacute sclerosing panencephalitis (SSPE)[1] is recognized as a late sequel of measles virus infection (4). In this disorder the host makes a continuous, heightened immune response against measles virus and virus persists in various tissues. SSPE is currently viewed as a prototype of human "slow" virus disease.

In this report we characterize interactions between cells infected with measles virus and the humoral immune system. We have determined the time-course required for infected cells to express cytoplasmic and surface measles virus antigens and studied the lysis of such cells by antibody to measles virus and complement (C). In this in vitro model of acute measles virus infection IgG was the only class of immunoglobulin (Ig) found to initiate injury of cells bearing

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[1] *Abbreviations used in this paper:* FITC, fluorescein isothiocyanate; MOI, multiplicities of infection; NeF, nephritic factor; SSPE, subacute sclerosing panencephalitis.

measles virus antigens on their surface in conjunction with C and cell lysis was found to be mediated by the alternative C pathway. In contrast, purified measles virus in conjunction with antibody was found to activate the classical C pathway. Various parameters of these immune reactions were quantitated and their implications discussed.

Materials and Methods

Virus. Wild Edmonston strain measles virus was obtained from the American Type Culture Collection, Rockville, Md. After several passages at low multiplicities of infection (MOI) in HeLa cells, a pool of infectious virus was collected, separated from cellular debris by centrifugation at 700 *g*, divided into small aliquots, and frozen at −70°C until use. The infectious titer of this pool was 5 × 10^6 tissue culture infective $dose_{50}$ U ($TCID_{50}$)/ml. Two attenuated strains of measles virus (M-VAC vaccine, American Cyanamid Co., Lederle Laboratories Div., Pearl River, N.Y.; Lirugen Schwarz strain vaccine, Dow Chemical Co., Midland, Mich.) were obtained commercially and passed at low MOI in HeLa cells. SSPE virus (strain Halle) was kindly provided by Monique Dubois-Dalq, National Institute of Neurological Diseases and Stroke, Bethesda, Md. This isolate, previously passed twice in Vero cells, was adapted to grow in HeLa cells in our laboratory. Purified measles virus was kindly supplied by John Holland, University of California at San Diego, La Jolla, Calif. In brief, virus was obtained from infected HeLa cells and freed from contamination by initial low speed centrifugation and by subsequent banding in 5-40% sucrose and 20-45% potassium tartrate gradients. The virus obtained was dialyzed against barbital saline buffer with gelatin, calcium chloride, and magnesium chloride (5).

Cell Lines. HeLa, KB, and H.Ep. no. 2 epithelial cell lines were obtained from Microbiological Associates, Los Angeles, Calif. Human neuroblastoma cells (line IMR-32) and glioma cells (line 339 MG) were furnished, respectively, by Harvey Herschman, University of California at Los Angeles, Calif. and Jan Ponten, University of Uppsala, Uppsala, Sweden. Human lymphocyte lines RPMI 8866 and Raji were provided by Richard Lerner, Scripps Clinic and Research Foundation, La Jolla, Calif.

Culturing, Infecting, and Handling of Cells. HeLa, KB, H.Ep. no. 2, IMR-32, and 339 MG cells were each grown as monolayers in 75 cm^2 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% CO_2 and cultures were divided weekly. Lymphocyte lines were placed in suspension cultures in Spinner's medium supplemented with nutrients and antibiotics as above. These cultures grew in stoppered Erlenmeyer flasks on a rocker platform at 37°C and were divided weekly.

Monolayer cells to be infected or to be analyzed for surface immunofluorescence or cytotoxicity were washed repeatedly in situ with phosphate-buffered saline to remove loose cells. The adherent cells were covered with 0.05% trypsin EDTA solution (Grand Island Biological Co., Grand Island, N. Y.) and gently agitated at room temperature for 1–2 min until the cells were completely suspended. After repeated washing in growth medium, such cells showed greater than 95% viability by trypan blue dye exclusion. To achieve maximal viability, cells were washed with cold (4°C) growth medium in a refrigerated (4°C) centrifuge. Cells grown in suspension culture were washed once in growth medium before viral infection and three times before experimental testing.

For infection with virus, washed cells were mixed with measles virus at MOIs of 0.2–12.0 depending on the experimental protocol. Viral adsorption was carried out in plastic tubes at 37°C for 2 to 4 h with frequent mixing. Infected cells were then replated or resuspended under standard culture conditions. In some instances, infected cells were grown on glass cover slips held in Petri dishes. Monolayer cover slip cultures were prepared for immunofluorescence analysis by previously published methods (6).

Immune Sera. Antisera used in this study were derived from human subjects with varying histories of exposure to measles virus infection: (*a*) 12-mo old infant, prevaccination; (*b*) same child, 3 wk postmeasles vaccination; (*c*) 3 children, acute epidemic measles; (*d*) 3 male adults, distant convalescent; (*e*) 3 female adults, distant convalescent; and (*f*) placental cord blood from newborns from each of the preceding women. In addition sera and cerebrospinal fluid from several patients with SSPE were obtained from John Whitaker, University of Tennessee, Memphis, Tenn.; Robert Roelofs, Vanderbilt University, Nashville, Tenn.; and John Sever, National Institute of Neurological

Diseases and Stroke, Bethesda, Md. In most cases, the fresh sera and spinal fluid samples were stored at −70°C.

Ig fractions from convalescent sera were prepared by previously reported techniques (7) and were tested for purity at a concentration of 10 mg/ml by Ouchterlony analysis. Whereas IgG and IgM isolates were immunochemically pure by these criteria, IgA isolates were contaminated with trace amounts of IgG. Heavy chain-specific antisera to human IgG and IgA were obtained from Miles Laboratories, Inc., Miles Research Div., Kankakee, Ill. Purified human myeloma IgA was provided by Geraldine Meinke, Scripps Clinic and Research Foundation, La Jolla, Calif. All reagents were tested for specificity by Ouchterlony analysis or immunoelectrophoresis. Igs were quantitated by the Mancini radial immunodiffusion assay using Hyland test plates (Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif.).

Antisera to HeLa cells were prepared in rabbits. Viable suspensions of 1×10^8 HeLa cells were intraperitoneally injected into rabbits each week for 8 consecutive wk after an initial subcutaneous priming with 1×10^8 HeLa cells in complete Freund's adjuvant. 1 wk after the final injection, sera were collected, heat inactivated at 56°C for 60 min, and stored in small aliquots at −70°C until use.

C and C Reagents. Freshly collected or fresh frozen human sera served as the sources of measles antibody and of C for most of these studies. For some experiments, sera from humans congenitally deficient in the second component of C but containing measles antibody were employed. Our other C sources for studies with Ig fractions, cerebrospinal fluids (CSF), or human immune sera previously heated at 56°C for 30 min included serum from rhesus monkeys which lacked antibody to measles virus and guinea pig serum which lacked antibody to measles virus and was deficient in the fourth component of C.

In order to assess the participation of the alternative C pathway, human serum was heated at 50°C for 20 min, a procedure known to inactivate C3 proactivator, an essential component of the alternative C pathway. This treatment yielded serum that was unable to sustain consumption of C3 on addition of inulin although full restoration of this activity was achieved on addition of isolated C3 proactivator. A partial loss of classical pathway function resulted, since there was a 40–60% impairment in ability to lyse sheep erythrocytes sensitized with antibody. C activity was interrupted by incubation of 100 μl of serum with 6 μl of 0.2 M EGTA, pH 7, and alternative pathway function reconstituted by adding 6-μl aliquots of magnesium chloride at varying molarities as specified in the Results. Both C pathways were inactivated by chelation of serum with 0.2 M EDTA, pH 7, or by heating of the sera at 56°C for 30 min.

Highly purified C3 proactivator was isolated from human serum according to the method of Götze and Müller-Eberhard (8) and added in varying concentrations to serum depleted of alternative pathway function by heating. Consumption of selected C components was determined after isolated measles virus (approximate concentration of 500 μg/ml) was incubated with an equal volume of fresh human serum for 60 min at 37°C or after 1×10^5 HeLa cells infected with measles virus were incubated with 400 μl of human serum for 60 min at 37°C. Methods employed to measure CH50, C1, C2, C3, C4, C5, C8, and C9 quantitatively by hemolytic titration have been published (5, 9, 10). The integrity of the alternative C pathway after treatment of fresh convalescent sera with either cell-bound or purified measles virus was assessed with three tests. First, inulin at a final concentration of 10 μg/ml was added to measles treated and control sera. Second, the sera were incubated with nephritic factor (C3NeF) (11) in a final concentration of 700 μg/ml. Third, the sera were incubated with cobra venom factor (12) in a final concentration of 400 μg/ml. After incubation at 37°C for 30 min, samples were assessed for residual C3 hemolytic activity.

Cytotoxicity Studies. A modification of the eosin micromethod (13) was used in most cases. Briefly, 6-μl aliquots of fresh antiserum (antibody and C source) followed by 1-μl aliquots of cell suspension (5×10^5/ml in growth medium) were discharged under mineral oil onto a Møller-Coates plate (HTI Corp., Associated Biomedic Systems, Buffalo, N. Y.). In all experiments a control consisting of cells and heated serum (56°C, 30 min) was included. The plates were then incubated for 90 min at 37°C after which 1 μl of 5% eosin and then 1 μl of undiluted formaldehyde were added to each reaction mixture. Viability was assessed by using a binocular inverted-phase microscope with an adjustable condenser (6-mm focal point) at a magnification of 200. When different sources of antibody and C were employed, the cells were incubated first with 2 μl of antibody solution (previously heated at 56°C for 30 min) for 30 min at 37°C after which 6 μ of C was added.

The results were expressed as a cytotoxicity index that was corrected for nonspecific background death of cells which never exceeded 5%. The cytotoxicity index is defined as the percentage of cell

death observed in the experimental samples minus the percent of cells killed with heat-inactivated human serum divided by 100 minus the percentage of cells killed with heat-inactivated serum.

Expression of Measles Virus Determined by Immunofluorescence. Fluoresceinated monospecific antisera to human IgG, IgA, IgM, IgD, and IgE were furnished by Thomas Edgington, Scripps Clinic and Research Foundation, La Jolla, Calif. Human IgG separated from sera of patients having measles antibody was conjugated with fluorescein isothiocyanate (FITC) as described elsewhere (14). The monospecificity of each antisera was verified by Ouchterlony analysis and by immunoelectrophoretic testing.

For staining measles virus antigens expressed on the cell surface, 1-4 $\times$ 10^5 infected HeLa cells were resuspended and washed as described earlier and incubated with 50 μl of FITC-IgG antibody to measles virus for 30 min at room temperature. Cells were then washed twice in medium and examined for surface fluorescence with a Zeiss RA fluorescence microscope (Carl Zeiss, Inc., New York). The expression of measles virus antigens inside the cell was determined as above except that the cells were fixed in ether: 95% ethanol before staining as described (6).

Expression of Measles Virus on the Cell Surface Determined by Binding of Radiolabeled Antibody. HeLa cells were infected with measles virus at a MOI of 12.0 and plated in separate Falcon flasks. At various intervals after initiation of infection the cells were dissociated, washed, and 1 million cells incubated with identical amounts of deaggregated (15) ^{125}I-radiolabeled antibody (16) to measles virus for 60 min at room temperature. The cells were then washed five times in growth medium and the cell pellet counted in a Baird Atomic gamma counter (Baird Atomic, Inc., Bedford, Mass.). Specific labeling was obtained by subtracting the number of counts adhering to 1 million uninfected HeLa cells treated in an identical manner. The amount of antibody molecules bound on the surface of the infected cells at each interval was determined from the specific activity of the radiolabeled antibody fraction and the number of cells used in the assay.

Immunoabsorption. Separate globulin fractions of goat sera containing antibody to human IgG and to human IgA were prepared by ammonium sulfate precipitation. After dialysis, lyophilization, and reconstitution to 500 μg/ml, 40 μl were mixed with a 15-μl aliquot of neat convalescent measles serum and a 5-μl aliquot of purified human [^{125}I]IgG or [^{125}I]IgA. A single SSPE serum was treated in an analogous manner. Each mixture was incubated in a Beckman microfuge tube (Beckman Instruments Inc., Fullerton, Calif.) at 4°C for 7 days after which the precipitates were removed by centrifugation. Under these conditions, over 99% precipitation of the radiolabeled IgG or IgA tracers was obtained. Supernates from each reaction mixture were titered for residual cytolytic activity using a source of C devoid of measles virus antibodies.

Results

Antibody and C-Mediated Lysis of Cells Infected with Measles Virus. HeLa cells were susceptible to infection with Edmonston strain measles virus as evidence by the appearance of viral antigens in the cytoplasm and on the cell surfaces within 24 h after the addition of virus to the cultures. With a low MOI, generalized cytopathic effects, i.e. giant cell formation and membrane fusion, were evident by the 5th and 6th days after infection. When fresh undiluted sera from patients who were convalescent from measles infection, chronically infected with measles (SSPE), or recently immunized against measles virus were added to infected cells 48 h or more after initiating infection, cell lysis occurred. Prior heating of these sera for 30 min at 56°C abrogated the lytic effect, although heated sera could initiate lysis on the addition of a fresh source of C devoid of antibody(s) to measles virus. The specificity of the lytic process was shown both by the failure of these fresh sera to lyse uninfected HeLa cells and by their inability to damage infected cells after absorption with measles virus. Similar results were obtained with sera having antibodies to measles virus and taken from infants shortly after birth. When dilutions of sera were employed with Rhesus monkey serum as the C source, the 50% cytotoxic end point varied from

1/4 to 1/64 for convalescent patients and from 1/256 to >1/1,000 for sera from patients with SSPE.

Other epithelial cells lines (KB, and H.Ep. no. 2) as well as neuroblastoma (IMR-32), glioma (339 MG), and lymphoid cells line (RPMI 8866, Raji) were also readily infected by Edmonston strain measles virus. Similarly, these cells were lysed by antimeasles virus antibody(s) in conjunction with C. HeLa cells infected with three other strains of measles virus (M-VAC, Schwarz, and SSPE-Halle) manifested the same phenomena.

Expression of Measles Virus Antigens in Relation to Antibody- and C-Mediated Lysis of Cells Infected with Measles Virus. HeLa cells were infected with a low MOI of Edmonston strain measles virus (0.2) and examined at various times for the presence of measles virus antigens. As seen in Fig. 1, viral antigens were first noted in the cytoplasm and on the plasma membrane approximately 18 h after infection began, and the percentage of cells expressing measles virus

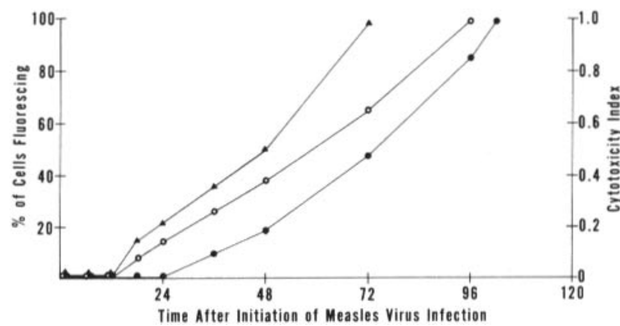

FIG. 1. Time-course of expression of measles virus antigens in the cytoplasm and on the surface of HeLa cells and relationship to susceptibility to antibody and C-mediated cytolysis. Cells were infected at an MOI of 0.2 and sampled at intervals. Cytoplasmic (▲) and surface (○) expression of measles virus antigens are shown as the percentage of cells demonstrating fluorescence while cytotoxicity (●) is shown as a cytotoxicity index.

antigens increased throughout the period of observation. In the same experiment, antibody- and C-mediated injury of these cells were first observed 36 h after the initiation of infection. Thereafter, the number of infected cells lysed increased directly in parallel with the number of cells showing measles virus antigens. By 96 h after infection began, all cells expressed virus antigens on their surface and 82% were lysed by antibody and C, and all cells were susceptible to lysis 100 h after infection.

Ig Class Initiating C-Mediated Lysis of Cells Infected with Measles Virus. In order to determine the class of antimeasles virus Ig(s) present in immune sera, infected HeLa cells were incubated with heated (56°C, 30 min) serum from individuals convalescent from measles infection, from patients chronically infected with measles virus (SSPE), and from children having an acute measles virus infection. After washing, the cells were incubated with FITC-labeled monospecific antisera to IgG, IgA, IgM, IgD, and IgE. These studies showed the presence of IgG, IgA, and IgE measles antibodies in all sera tested. Sera from

patients with acute measles contained, in addition, IgM antibody. No serum was found to contain antibody of the IgD class.

To determine whether IgG or IgA was responsible for antibody-initiated, C-mediated lysis in convalescent and SSPE sera, IgG and IgA were immunoprecipitated with monospecific antisera. As shown in Fig. 2, the removal of over 99% of IgG led to an eight-fold or greater reduction in the ability of sera to initiate lysis with a fresh source of monkey C devoid of measles virus antibodies. Studies performed with sera similarly depleted of IgA showed no decrease in the cytotoxic titer (Fig. 2).

In other experiments, IgG, IgA, and IgM were partially purified from convalescent sera, concentrated, and examined for their ability to induce lysis of infected HeLa cells in the presence of a source of human C lacking measles virus

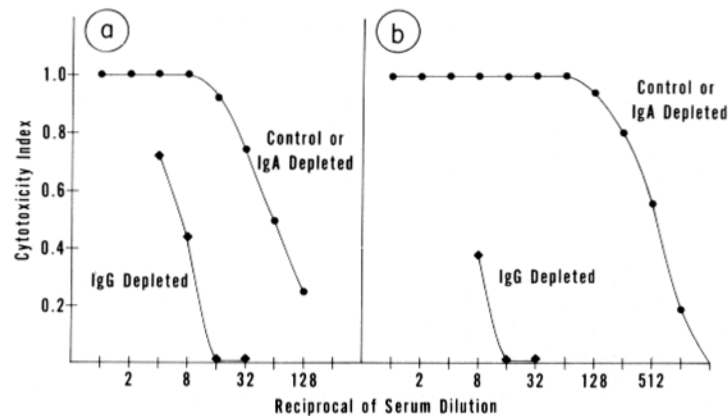

FIG. 2. Effect of depletion of IgG and IgA from sera containing antibodies to measles virus and the cytolytic potential of such sera in conjunction with a fresh source of C. Lysis associated with convalescent human serum (panel *a*) and SSPE serum (panel *b*) was significantly reduced by depletion of IgG but not depletion of IgA.

antibody. Only the IgG fraction was able to initiate lysis, although its titer in this regard was lower than that present in the starting serum. The IgG fraction contained over 90% of the hemagglutination inhibition activity present in the original convalescent serum. IgA and IgM fractions in the presence of C were unable to initiate lysis when examined in concentrations two- to threefold greater than those present in starting serum.

Quantitation of the Amount of IgG Antibody Required to Lyse Cells Infected with Measles Virus. HeLa cells in culture were sampled at varying times after infection (MOI, 12.0) and examined by immunofluorescence for surface expression of measles virus antigens. At the same time intervals, cells were incubated with [^{125}I]IgG isolated from a convalescent human serum. Concurrently, a paired aliquot of cells was assayed for lysis after the addition of antimeasles virus antibody and C. As shown in Table I, 85% of cells were found to have measles virus antigens on their surfaces by 20 h after infection. At this time, although these cells expressed sufficient measles virus antigens to bind 1.1×10^6 molecules of IgG per cell, antibody and C were not sufficient to lyse cells in the paired

TABLE I

Number of Antibody Molecules Required to Lyse Virus-Infected Cells

H after‡ infection	Percent of cells*		Molecules of specific§ antibody bound/cell
	Expressing viral antigens	Lysed by antibody + C	
4	0	<5	1.5×10^5‖
20	85	<5	1.1×10^6
24	95	20	2.0×10^6
36	100	70	ND
48	100	100	5.1×10^6

* Expression of virus antigen(s) on the surface of living cells was determined by direct immunofluorescence using FITC-labeled human antibody to measles virus, while immune lysis was observed with the microcytotoxicity assay. Approximately 200 cells were counted in each sample.

‡ HeLa cells were infected at MOI of 12.

§ Sp act of IgG used was 3.74×10^5 cpm/μg protein.

‖ Number calculated from the 15,488 ^{125}I-labeled cpm binding to 1×10^6 cells.

sample. C-mediated lysis was first observed 24 h after infection. At this time 2.0×10^6 molecules of IgG were bound per cell (Table I). C was able to induce lysis of all the cells by 48 h after infection when the cells bound at least 5.1×10^6 molecules of IgG per cell.

C Pathway Involved in Antibody-Initiated Lysis. The cytolytic activity of C for IgG antibody-coated HeLa cells infected with measles virus and expressing viral antigens on their surfaces (sensitized, infected HeLa cells) was extremely sensitive to dilution, with approximately half of the cytolytic activity being lost by using a 1:2 dilution of the C source and over 90% by using a 1:4 dilution. In contrast, cytolytic activity of the same C source for uninfected HeLa cells coated with rabbit anti-HeLa antibody(s) was considerably less sensitive to dilution since half of the original cytolytic titer remained with the C source diluted to 1:50.

Evidence that C-mediated lysis of sensitized, infected HeLa cells required magnesium but not calcium was obtained from studies in which increasing amounts of Mg were added to serum chelated with 1.1×10^{-2} M EDTA or EGTA. Whereas EDTA effectively chelates Ca and Mg, EGTA has a 10,000-fold lower affinity for Mg and thus is a relatively selective chelator of Ca. No lysis was observed with EDTA-chelated serum until a final concentration of 6.4×10^{-3} M Mg had been added, and complete lysis occurred only on addition of 1.28×10^{-2} M Mg (Fig. 3). In contrast, the addition of small amounts of Mg to EGTA-chelated serum rapidly restored lytic ability to the serum. Lysis was observed on addition of 7.5×10^{-4} M Mg and complete lysis occurred with 1.5×10^{-3} M Mg. No Mg ion dependency was noted in the lysis of HeLa cells during incubation with antibody to HeLa cell surface and C.

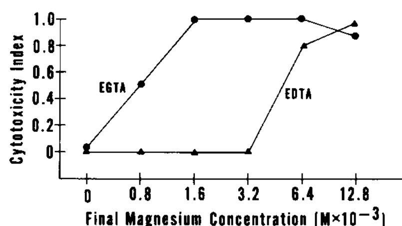

FIG. 3. Dependence of antibody-mediated C-dependent lysis of HeLa cells infected with measles virus on magnesium. Sera were chelated with 1.1×10^{-2} M EGTA (●) or EDTA (▲) and restored with varying concentrations of magnesium chloride. The abscissa shows the final concentration of added magnesium chloride.

These studies strongly suggested that the alternative C pathway was responsible for lysis of sensitized, infected HeLa cells. In order to confirm this impression, serum was heated at 50°C for 20 min, a treatment that leads to relatively selective inactivation of C3 proactivator, a central factor in the alternative C pathway. Serum heated in this manner was completely devoid of the ability to lyse sensitized, infected HeLa cells. Inactivation of the alternative C pathway was documented in independent studies in which the heated serum was unable to sustain conversion of C3 on addition of inulin; the classical pathway, however, was relatively intact since the heated serum retained approximately 40% of its original ability to lyse sensitized sheep erythrocytes. As shown in Fig. 4, the addition of amounts of highly purified C3 proactivator comparable to those present in normal serum (200 μg/ml) fully reconstituted the cytolytic ability of the heated serum.

To assess any role of the classical C pathway, human serum genetically deficient in the second component of C but containing antibody to measles virus was examined for its ability to lyse sensitized, infected HeLa cells. C2-deficient serum was fully active in this regard. As observed with normal serum, heating of C2-deficient serum at 50°C for 20 min abrogated its cytolytic ability, and this effect was reversed on addition of physiological amounts of purified C3 proactivator. Guinea pig serum deficient in C4 could also lyse infected HeLa cells presensitized with IgG antibody to measles virus.

Consumption of components in serum previously incubated with infected HeLa cells also revealed a pattern consistent with alternative pathway activation. Insignificant consumption of C1 (8%), C4 (0%), and C2 (14%) occurred, whereas consumption of C3 (37%) and C5 (42%) was significant.

C Pathway Involved in Lysis of Several Cell Lines Infected with Various Measles Viruses. Lysis of HeLa cells infected with M-Vac, Schwarz, or SSPE-Halle strains of measles virus and sensitized with IgG antibodies to measles virus was also found to proceed via the alternative C pathway since lysis was abrogated by heating of the C source at 50°C for 20 min and restored on addition of physiological amounts of purified C3 proactivator. Similar experiments showed that infected epithelial (HeLa, KB, and H.Ep. no. 2), neuroblastoma (IMR-32), glia (339 MG), and lymphoid (RPMI 8866, Raji) cell lines sensitized with antibody from convalescent human sera were lysed via the alternative C pathway.

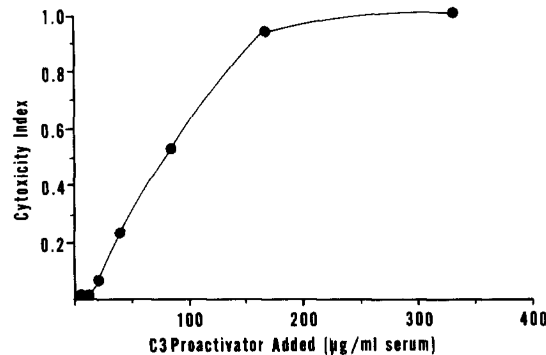

FIG. 4. Restoration by isolated C3 proactivator of antimeasles virus immunocytolytic activity to serum which had been previously heated at 50°C for 20 min. Full cytolytic activity was restored with physiological amounts of C3 proactivator (200 μg/ml).

Interaction of Antibody and C with Purified Measles Virions. Purified measles virus was found to activate the classical C pathway. Thus when purified virus was mixed with fresh human convalescent serum, significant consumption of CH50 (>90%), C1 (47%), C4 (70%), C2 (>96%), C3 (33%), C5 (31%), C8 (11%), and C9 (27%) occurred. Further, consumption of C1 (38%) and C4 (45%) was observed on incubation of measles virus with a C2-deficient human serum containing antibodies to measles virus; borderline consumption of C3 (19%) and C5 (10%) with no depletion of C8 or C9 was also observed. In order to determine whether components of the alternative C pathway had been similarly depleted, the ability of normal and C2-deficient sera previously incubated with isolated measles virus to sustain C3 consumption on incubation with three different activators of the alternative pathway was determined. Each of the activators selected, inulin, cobra venom factor, and C3NeF which initiate the alternative C pathway at different sites in the sequence was able to trigger comparable C3 depletion regardless of whether or not the sera had been previously incubated with measles virus (Table II). Reduced levels of consumption by one or more of these activators would have been expected if components of the alternative C pathway had been depleted by incubation with measles virus.

Discussion

A number of human cell lines including epithelial (HeLa, KB, and H.Ep. no. 2), neuroblastoma (IMR-32), glia (339 MG), and lymphoid (RPMI 8866, Raji) cells infected with wildtype Edmonston measles virus were lysed upon the addition of fresh convalescent serum. Antibody-initiated, C-mediated injury in this entirely homologous model of acute measles virus infection also occurred in cells infected with SSPE-Halle, Schwartz, or M-Vac measles virus. Immune sera from patients with either acute measles virus infection, SSPE, recent measles vaccinations, or histories of measles virus infection and convalescence lysed HeLa cells infected with Edmonston measles virus. Hence it is clear that humorally mediated injury of cells infected with measles virus is not restricted to one particular cell line, strain of virus, or clincal state of measles virus infection. Earlier studies mainly with heterologous systems showed that infected cells expressing virus antigens on their surfaces may be destroyed through the action of antiviral antibody and C. For example, humorally mediated injury has been reported with rabies (17), dengue

TABLE II

Integrity of the Alternative Pathway in Human Serum after Incubation with Purified Measles Virus

	First incubation	Second incubation	
		Activator	Depletion of C3*
			%
Normal serum:	Measles	Inulin	54
	Buffer	Inulin	36
	Measles	CoF	96
	Buffer	CoF	96
	Measles	C3NeF	92
	Buffer	C3NeF	97
C2-deficient serum:	Measles	Inulin	36
	Buffer	Inulin	40
	Measles	CoF	92
	Buffer	CoF	98
	Measles	C3NeF	71
	Buffer	C3NeF	81

* Normal or C2-deficient serum were incubated first with measles virus which led to 33 or 19% consumption of C3, respectively. The percent consumption of the residual amounts of C3 on addition of inulin, cobra venom factor (CoF), or C3NeF was then assessed.

(18), Newcastle disease (19), SV-5 (20), murine leukemia (21, 22), herpes simplex (19), vaccinia (19), influenza (19), lymphocytic choriomeningitis (23), mumps (23), and measles (24, 25) viruses.

In the present studies the mechanisms involved when HeLa cells acutely infected with measles virus were lysed by human antibody and C were analyzed. Immunofluorescent-staining experiments showed that convalescent and SSPE serum contained antibody directed against measles virus of the IgG, IgA, and IgE classes and serum from patients afflicted with an acute measles infection contained in addition IgM antibody. IgG antibody to measles virus initiated C-dependent lysis of infected cells as immunospecific depletion of IgG led to virtually total abrogation of cytolytic activity whereas similar depletion of IgA was without effect.

High concentrations of cell-bound IgG were associated with lysis. Lysis was first observed 24 h after infection when cells express sufficient virus antigens to bind at least 2×10^6 molecules of IgG per cell. Immune lysis was not obtained during measles virus adsorption even at high hemadsorption to target cell ratios or with using Rhesus monkey erythrocytes

as target cells (B. Joseph and M. B. A. Oldstone, unpublished data). All the cells were lysed 48 h after infection when test cells bound 5.1×10^6 or more molecules of IgG per cell. The requirement for high concentrations of cell-bound IgG may reflect an inefficiency of IgG in activation of C for cytolysis of infected HeLa cells, or the existence within the IgG fraction of only a minor proportion of IgG, perhaps subclass, which is capable of initiating lysis. Alternatively, it may be that measles virus antigens were initially expressed on portions of the cell surface which are resistant to C-dependent injury and only with prolonged infection is the virus expressed on regions of the membrane susceptible to lysis. It is also possible that virus directed alterations in the plasma membrane or in the ability to repair C-dependent damage modifies the susceptibility of the cell to immune attack. Recent studies with lymphocytes have demonstrated cell cycle-dependent alterations in antiviral immunolytic susceptibility which indicate that the cell membrane plays an active role in determining the outcome of a potentially cytolytic process (26–28). It is unlikely that this inefficiency in initiating lysis is due to saturation of the cell surface with antibody which is inefficient in triggering lysis because of the high concentration, since no prozone in regard to lysis was observed in dilutional studies of convalescent or SSPE sera which contained higher concentrations of IgG antibody than that present in the isolated fraction employed for the radioactive-binding studies.

Antibody-initiated injury of cells infected with measles virus was found to be mediated by the alternative C pathway. The evidence in support of this contention derives from studies which showed that brief heating at 50°C of the C source which leads to a relatively selective inactivation of an essential factor of the alternative C pathway, C3 proactivator, completely prevented lysis. Full cytolytic activity was restored by supplementation with physiological amounts of highly purified human C3 proactivator. Serum genetically deficient in C2 and possessing measles virus antibody showed full cytolytic potential, demonstrating no essential role for the classical C pathway in this lytic reaction. Lysis produced by C2-deficient serum also was abrogated on heating on 50°C for 20 min and restored by addition of purified C3 proactivator.

These studies represent the first demonstration of an alternative C pathway-mediated cytolytic event initiated by human antibodies of the IgG class. Other studies have shown that this pathway is directly activated by certain naturally occurring polysaccharides and by aggregates of human IgA[2] and IgE (29) myeloma proteins but similar aggregates of IgG myelomas have not been found to be effective.[2] In animals, however, guinea pig γ_1-Ig in complex with antigen has been shown to activate the alternative C pathway as has cell-bound rabbit IgG antibody in high concentration (30, 31).

All human sera tested with the exception of a 12-mo old infant which lacked measles-specific IgG antibody were shown to possess totally endogenous immunocytolytic potential against cells infected with measles virus. Lysis was also observed with serum taken from infants shortly after birth, documenting the integrity of the alternative C pathway at this stage in development. Sera and CSF from patients with SSPE was effective in lysing measles virus-infected cells indicating the presence of cytolytically effective antibody and the integrity of the alternative C pathway in patients with this disease. In other experiments not presented here, brief pretreatment of infected cells with

[2] Müller-Eberhard, H. J., O. Götze, and H. L. Spiegelberg. 1975. Activation of the alternative pathway by immunoglobulin aggregates and bacterial substances. Manuscript in preparation.

heated (56°C, 30 min) SSPE serum or CSF did not prevent subsequent lysis on addition of freshly thawed convalescent serum. Thus, no evidence for a defect in cytolytic antibody or the C system, or evidence for a blocking factor, was found in the serum or spinal fluid of patients with SSPE to account for the persistant viral infection which occurs in this disease.

Of interest is the fact that uninfected HeLa cells incubated with rabbit antibody prepared against HeLa cell surface antigens were found to be lysed by the classical C pathway. These studies amplify earlier work which showed that C and antibody to measles virus in a heterologous system could lyse HeLa cells infected with measles virus (25). In this study, lysis was abrogated at low dilutions of C in contrast with effective lysis with antibodies to HeLa cell surface at similar or greater dilutions of C. Equivalent results were obtained in our own studies. Differential activation of C by sensitized cells has also been noted in the HL-A system (32).

In contrast to the requirement for the alternative C pathway in lysing measles virus-infected cells, purified measles virus was found to trigger the classical C pathway on incubation with the same source of immune human serum. The C consumption studies failed to reveal significant evidence for activation of the classical C pathway by measles-infected cells and conversely, isolated measles virus appeared to be unable to significantly activate the alternative C pathway (Table II). The reason for this apparent utilization of different C pathways depending on whether measles virus is in the isolated form or expressed on cell membranes is not clear at present but it is tempting to speculate that the membranes of the infected cells provide an essential additional stimulus which serves to activate the alternative C pathway. This might occur if human sera possessed antibody to virus-induced but nonviral-associated antigens on the cell surface or antibody directed against unmasked surface antigens generated during virus maturation at the cell surface. We are currently evaluating whether this phenomenon occurs with other human viruses which bud from the cell surface.

Summary

In these studies, a number of human cell lines including epithelial, neural, glial, and lymphoid cells infected with several strains of measles virus were found to be lysed upon incubation with fresh sera from humans containing antibody to measles virus. In all instances, the cytolytic event was mediated by the alternative complement (C) pathway without a significant contribution from the classical pathway. In contrast, isolated measles virus in conjunction with antibody was found to selectively activate the classical C pathway.

Measles antibodies of the IgG class, but not the IgA class, possessed cytolytic potential against cells infected with measles virus. Human IgG antibodies could directly activate the alternative C pathway.

No defect was found in cytolytic measles antibody in sera or cerebrospinal fluid from patients with subacute sclerosing panencephalitis, nor was the alternative C pathway impaired in sera from these patients. Sera from newborn humans also exhibited a functional alternative C pathway.

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