

Antibody-independent activation of the alternative complement pathway by measles virus-infected cells

(antibody-and-complement-mediated lysis/properdin/viral glycoproteins)

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ABSTRACT When HeLa cells acutely infected with measles virus were incubated in a mixture containing only the six proteins of the alternative pathway of complement activation (C3, factors B and D, β 1H, C3b inactivator, and native properdin) without antibody, there was activation of the alternative pathway as shown by progressive uptake of ^{125}I -labeled C3b onto the cell surface. This C3b uptake was blocked by EDTA and was not shown by uninfected cells. The rate of ^{125}I -labeled C3 uptake by infected cells was the same in the absence and presence of properdin; however, when antiviral IgG was bound to the cell surface, the rate of C3 uptake was increased in the presence of properdin. Significant ^{125}I -labeled C3 uptake was first detectable when cells were studied at 12 hr after infection, when all cells expressed viral polypeptides on their surface. There was also progressive uptake of ^{125}I -labeled C3 onto measles virus-infected cells incubated in human serum depleted of both IgG and C4. Hence, the human alternative pathway of complement activation can be initiated on the surface of measles virus-infected cells independent of IgG antibody. However, lysis of the infected cells only occurs when antiviral antibody is present.

Antibodies play a crucial role in limiting the spread of infection by viruses (reviewed in refs. 1 and 2). Cells infected with a wide variety of viruses are lysed by antiviral antibody and complement (3-5). This lysis is specific in that the antibody must be directed against viral antigens on the cell surface (3-6), and it is efficient in that virus-infected cells can be lysed early in infection, prior to the release of progeny virus (5).

The requirements for lysis of virus-infected cells by human immune serum have been studied in detail in this laboratory (6-8). Previous work has shown that lysis of several human cell lines infected with a number of RNA and DNA budding viruses is dependent on the presence, in serum, of both antiviral IgG and an intact alternative pathway of complement. No lysis occurs when the specific IgG is absent or when individual alternative pathway components are immunochemically depleted from serum. In contrast, lysis is undiminished in the absence of early components of the classical pathway of complement activation (6, 7). We have recently shown (8) that measles virus-infected HeLa cells can be lysed by IgG and the purified cytolytic alternative pathway, composed of the 11 isolated proteins of the alternative pathway of activation and membrane attack pathway of complement, with an efficiency comparable to that of serum.

Current evidence indicates that initiation of the alternative pathway occurs on the surface of activators such as rabbit erythrocytes (9, 10), zymosan (11), and certain strains of *Escherichia coli* (12) because C3b bound from the fluid phase is relatively protected from its serum inactivators, β 1H and C3b inactivator, allowing generation of the alternative pathway C3 convertase C3bBb. Initiation thus resides in the discriminating

ability of activator-bound C3b, and there is no demonstrated requirement for immunoglobulin. In contrast, both IgG and IgM, when complexed with antigen, have a well-characterized role in initiating the classical complement pathway by binding C1q.

The dependence of complement-mediated lysis of virus-infected cells on both the alternative pathway and IgG antibody is clearly established (6-8). However, the absence of any requirement for IgG for initiation of the alternative pathway by other known activators (9-12) poses the question of whether IgG is required for alternative pathway activation in the virus system or whether activation is effected by the surface of the virus-infected cell itself.

This paper presents evidence that cells infected with measles virus (an enveloped RNA virus) can initiate the alternative pathway of complement activation on their surface in the absence of IgG antibody. However, no lysis of the virus-infected cell ensues unless IgG antibody is present.

MATERIALS AND METHODS

Virus and Cells. HeLa cells were grown in monolayers in Eagle's minimal essential medium supplemented with 10% fetal bovine serum, glutamine, and antibiotics. The cells were infected in suspension with the Edmonston strain of measles virus at a multiplicity of infection of 1 and maintained in suspension culture in the above medium for 40 hr; 100% of cells were then expressing measles antigen as determined by surface staining with fluorescein-conjugated measles antibody (7). Surface radioiodination and sodium dodecyl sulfate/polyacrylamide gel electrophoresis of infected cells showed that both the surface polypeptides of the virus—the M_r 80,000 HA (hemagglutinin) and the M_r 41,000 F1 (fusion) protein—were present on the cell membrane (13). Before use in the assays described below, infected cells were washed in serum-free Eagle's medium and in Veronal-buffered saline containing 1.2 mM MgCl_2 , 0.15 mM CaCl_2 , and 0.1% ovalbumin.

Purified Complement Components. C3 (14), factor B (15), factor D (16), C3b inactivator (17), β 1H (17), and native properdin (18) were prepared as described. Purity of the isolated proteins was assessed, in part, by sodium dodecyl sulfate/polyacrylamide gel electrophoresis. Representative gel patterns have been published (19). Proteins were radioiodinated by using the lactoperoxidase method (20).

Preparation of the Purified Alternative Pathway. A mixture of the above six purified components at their physiological concentrations was prepared as described (19). The final concentrations of components were: C3, 1200 $\mu\text{g}/\text{ml}$; factor B, 200 $\mu\text{g}/\text{ml}$; factor D, 2 $\mu\text{g}/\text{ml}$; β 1H, 470 $\mu\text{g}/\text{ml}$; C3b inactivator, 34 $\mu\text{g}/\text{ml}$; and native properdin, 20 $\mu\text{g}/\text{ml}$. Possible C3b contamination of C3 preparations was eliminated by incubation of C3 with appropriate amounts of β 1H and C3b inactivator just prior to addition of the three other proteins. The function

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of the component mixture was tested by assaying the uptake of ^{125}I -labeled C3 (^{125}I -C3) from the mixture onto rabbit erythrocytes (19).

Preparation of C4- and IgG-Depleted Serum. Serum immunochemically depleted of C4 was prepared by adsorption with the IgG fraction of monospecific antiserum to C4 coupled to Sepharose 4B (Pharmacia) (21). IgG was depleted from serum by adsorption with a monospecific anti-human IgG similarly coupled. Serum depleted of C4 and IgG retained alternative pathway activity as assessed by its ability to lyse rabbit erythrocytes. Classical pathway activity (assessed by hemolytic titer against antibody-coated sheep erythrocytes) was fully reconstituted by addition of physiologic concentrations of C4. After adsorption, the IgG-depleted serum gave no precipitation line against anti-human IgG serum on analysis by double diffusion in agar.

Antibody to Measles Virus. The IgG fractions of serum from patients with subacute sclerosing panencephalitis were isolated by anion exchange chromatography and concentrated to 12–20 mg/ml.

Studies of ^{125}I -C3 Uptake. Measles virus-infected HeLa cells were suspended in the Veronal-buffered saline at a final concentration of 1×10^7 cells per 100 μl . If required for the experiment, cells were pretreated with antiviral IgG by incubation with purified subacute sclerosing panencephalitis IgG under conditions resulting in binding of approximately 5×10^7 molecules of IgG per cell (8). Cells were added to an equal volume of the purified alternative pathway mixture containing ^{125}I -C3 that had previously been centrifuged in a Beckman Microfuge to remove any aggregated ^{125}I -C3. The mixture was incubated with shaking at 37°C , and aliquots (2×10^6 cells) were removed at various times. These cells were layered on 300 μl of 20% sucrose in Veronal-buffered saline in a Microfuge tube and centrifuged for 1.5 min; then, the tip of the tube containing the cell pellet was cut off, and the radioactivity in the pellet was determined. The uptake of ^{125}I -C3 by cells in the presence of 0.01 M EDTA and uptake by uninfected cells were determined in the same way.

Lysis of Virus-Infected Cells. This was assessed by a small-scale ^{51}Cr release assay as described (8).

RESULTS

Uptake of ^{125}I -C3 by Measles Virus-Infected HeLa Cells.

In order to determine whether measles virus-infected HeLa cells could activate the alternative pathway in the absence of IgG, infected cells were incubated with the purified alternative pathway mixture containing ^{125}I -C3. Fig. 1 shows the kinetics of ^{125}I -C3 uptake onto infected cells from the purified alternative pathway, compared to the uptake of ^{125}I -C3 onto uninfected cells. There was a time-dependent increase in uptake of ^{125}I -C3 by the infected cells, with 30×10^5 C3b molecules ultimately bound per cell. In contrast, ^{125}I -C3 uptake by uninfected cells was 1×10^5 molecules per cell or less and did not change with time. Even when the number of uninfected cells used in the experiment was double the number of infected cells, there was no significant increase in ^{125}I -C3 uptake by the uninfected cells.

These observations were confirmed by using human serum immunochemically depleted of C4 and IgG with ^{125}I -C3 added. Uptake of ^{125}I -C3 onto measles virus-infected cells increased progressively up to 70 min, when 7.5×10^5 molecules of C3b were bound per cell (Fig. 2). At the final point of the time course, ^{125}I -C3 uptake by virus-infected cells coated with IgG was identical to that by infected cells alone; ^{125}I -C3 uptake by uninfected cells was 1×10^5 molecules per cell or less throughout.

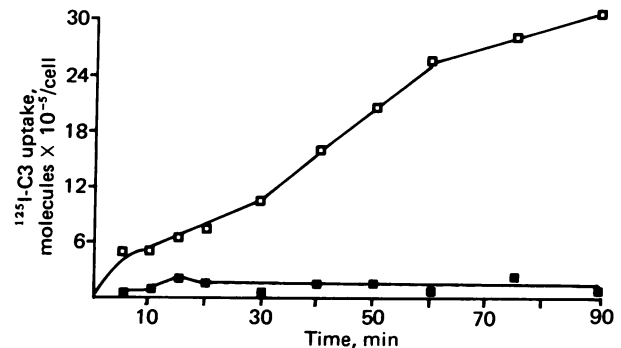


FIG. 1. Uptake of ^{125}I -C3 from the purified alternative pathway (C3, factors B and D, $\beta 1\text{H}$, C3b inactivator, and properdin) onto measles virus-infected HeLa cells (□) and uninfected HeLa cells (■). ^{125}I -C3 uptake in the presence of 0.01 M EDTA—determined simultaneously was $\approx 2 \times 10^5$ molecules per cell throughout for infected cells and $\approx 1 \times 10^5$ molecules per cell for uninfected cells—has been subtracted at each point.

Effect of Properdin on ^{125}I -C3 Uptake by Measles Virus-Infected Cells in the Presence and Absence of IgG. Because previous experiments (8) had shown that properdin was required for lysis of measles virus-infected cells, we studied the effect of properdin on C3 uptake by infected cells. When properdin was omitted from the purified alternative pathway, the uptake of ^{125}I -C3 by infected cells coated with IgG was retarded and the rapid early component of the uptake curve was lost (Fig. 3). The uptake of ^{125}I -C3 from the purified alternative pathway by infected cells without IgG closely paralleled that shown by infected cells with IgG in the absence of properdin.

The results of a separate experiment comparing the uptake of ^{125}I -C3 from the purified alternative pathway by measles virus-infected cells alone with and without properdin are shown in Fig. 4. The uptakes were similar and, in particular, there was no rapid early uptake of ^{125}I -C3 in the presence of properdin. These results indicate that C3 uptake by infected cells in the absence of IgG is independent of properdin and that antiviral IgG does not increase the rate of C3 uptake onto infected cells in the absence of properdin. The presence of both IgG and properdin increased the rate of ^{125}I -C3 uptake (Fig. 3); however, after 60–90 min, the amounts of C3b ultimately bound to infected cells with and without IgG were similar (15×10^5 molecules per cell).

Effect of Time Elapsed After Infection on ^{125}I -C3 Uptake. The uptake of ^{125}I -C3 from the purified alternative pathway onto HeLa cells infected with identical amounts of measles virus and maintained in culture for varying lengths of time was determined. Cells acquired the ability to bind ^{125}I -C3 between

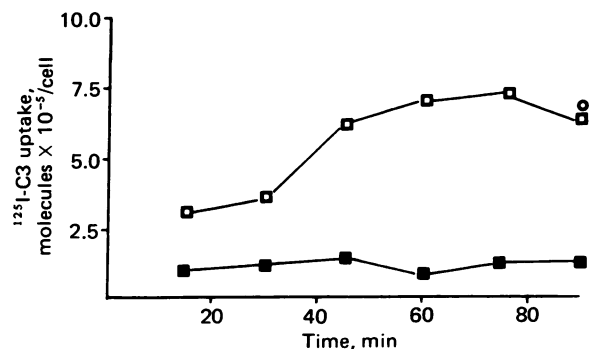


FIG. 2. Uptake of ^{125}I -C3 from human serum immunochemically depleted of C4 and IgG onto measles virus-infected HeLa cells (□), uninfected HeLa cells (■), and measles virus-infected HeLa cells coated with antiviral IgG (○). ^{125}I -C3 uptake in the presence of 0.01 M EDTA has been subtracted at each point.

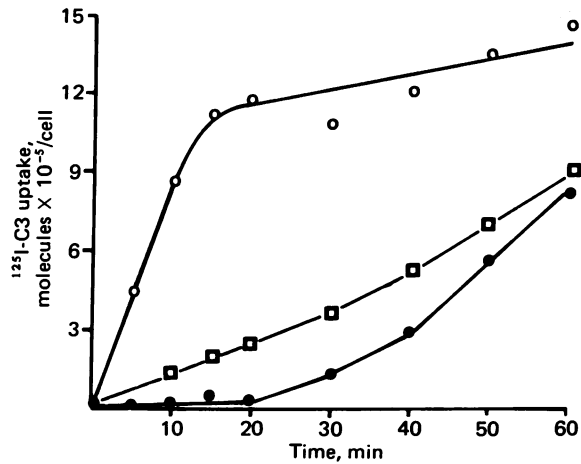


FIG. 3. Uptake of ^{125}I -C3 onto measles virus-infected HeLa cells coated with IgG, from the purified alternative pathway (○) and from the purified alternative pathway without properdin (●). □, Uptake of ^{125}I -C3 from the purified alternative pathway onto measles virus-infected uncoated HeLa cells.

12 and 18 hr after infection (Fig. 5). The uptakes of ^{125}I -C3 onto infected cells alone and onto cells coated with antiviral IgG were similar after the 90-min incubation used in this experiment. Viral antigens, detected by surface immunofluorescent staining and surface radioiodination, appeared on these cells between 6 and 12 hr after infection; by 18 hr, all cells were expressing viral antigens.

Lysis of Measles Virus-Infected Cells. In agreement with previous results, there was no lysis of measles virus-infected cells by serum in the absence of antibody. In additional experiments, infected cells were incubated in human serum with no antibody to measles for periods up to 18 hr. Even after this time there was no specific lysis of infected cells, despite the fact that lysis in the presence of antibody is usually completed within 1 hr.

DISCUSSION

These experiments demonstrate that measles virus-infected HeLa cells activate the human alternative complement pathway without any requirement for antibody. Second, despite this ability to activate the alternative pathway, virus-infected cells are not lysed unless antibody is present. Third, this antibody-independent activation of the alternative pathway by measles virus-infected cells does not require properdin; however, properdin enhances the rate of C3 uptake by infected cells in the presence of antiviral antibody.

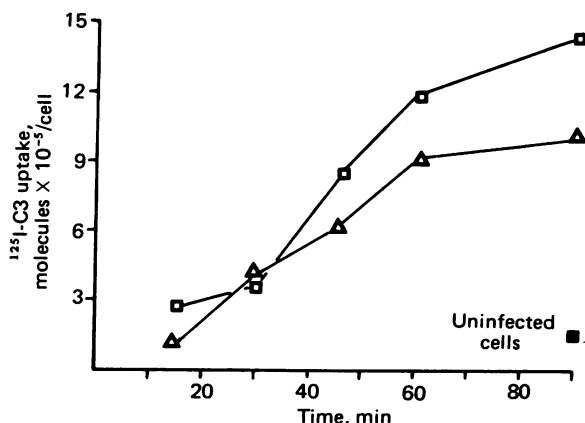


FIG. 4. Uptake of ^{125}I -C3 from the purified alternative pathway onto measles virus-infected HeLa cells in the presence (□) and absence (△) of properdin. ■, Uptake of ^{125}I -C3 from the purified alternative pathway onto uninfected HeLa cells.

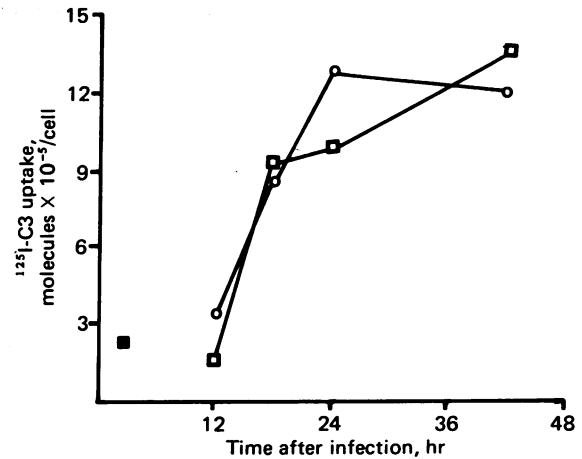


FIG. 5. Effect of time elapsed after infection on the uptake of ^{125}I -C3 from the purified alternative pathway onto measles virus-infected HeLa cells alone (□) and onto the same cells coated with antiviral IgG (○). ■, ^{125}I -C3 uptake by uninfected HeLa cells. Cells were incubated in the purified alternative pathway for 90 min.

There was progressive uptake of ^{125}I -C3 onto measles virus-infected cells when they were incubated either in the purified alternative pathway (a mixture composed only of the six highly purified proteins of the alternative pathway of complement activation) or in human serum depleted of both C4 and IgG. The use of the purified alternative pathway indicates that no other serum factor is required for pathway activation. Uninfected HeLa cells showed very low ^{125}I -C3 uptake. Significant ^{125}I -C3 uptake by infected cells was first detected more than 12 hr after infection, when all cells were expressing viral antigen, indicating that virus replication is a prerequisite for activation. ^{125}I -C3 uptake by infected cells approached 1.5×10^6 molecules per cell whereas the uptake in the presence of EDTA was approximately one-seventh of this, indicating that the bulk of C3 uptake resulted from activation of the alternative pathway and deposition of C3b rather than direct binding of ^{125}I -C3 to the cell membrane.

The mechanism by which infection with measles virus confers the ability to activate the alternative pathway on HeLa cells remains to be determined. It has been shown that C3b deposited on the surface of pathway activators exhibits decreased ability to bind $\beta 1\text{H}$. This decreased interaction between C3b and $\beta 1\text{H}$ results in decreased control of the activator bound C3b and thereby facilitates formation of C3 convertase: C3bBb (9-12). It has been suggested that specific surface molecules on activators of the alternative pathway might restrict the binding of $\beta 1\text{H}$ to surface-bound C3b and thereby act as " $\beta 1\text{H}$ antagonists" (10). The observation that insertion of lipopolysaccharide from *E. coli* O-4 into the membrane of sheep erythrocytes converts them from a nonactivator to an activator of the alternative pathway lends support to this concept (22). It is possible that the two measles virus glycoproteins, which are inserted into the cell membrane as integral membrane proteins, could function in this way by restricting the binding of $\beta 1\text{H}$ to C3b. Alternatively, some other virus-induced change in the cell membrane could initiate the alternative pathway.

^{125}I -C3 uptake onto infected cells occurred in the absence of properdin; this agrees with the known role of properdin in the alternative pathway. Native properdin binds to C3b, and multiple C3b molecules must be deposited on a surface in close spatial association for binding to occur. Once bound, properdin retards decay of the intrinsically labile C3bBb (23, 24). Initiation of the alternative pathway, with consequent membrane deposition of C3b, hence is a necessary step prior to the recruit-

ment of properdin and is independent of properdin. However, it was surprising that C3 uptake by infected cells was not accelerated in the presence of properdin. In contrast, when IgG was bound on the membrane of the infected cell, ^{125}I -C3 uptake was clearly accelerated in the presence of properdin. This fact, taken with the absolute requirement for both IgG and properdin for lysis (8), suggests that some interaction between the two could be of importance in the mediation of cell lysis. One possibility is that IgG facilitates the formation of C5 convertase on the cell membrane by permitting incorporation of additional C3b. This would allow both binding of properdin and assembly of the membrane attack complex. Any effect of IgG and properdin seems unlikely to be due to increasing the absolute uptake of C3 because the amounts of ^{125}I -C3b ultimately bound to infected cells with and without IgG and properdin were similar.

The requirement of IgG for lysis of measles virus-infected cells by human serum has been clearly documented (6, 7, 25). IgG bound to either viral glycoprotein is effective, $\text{F}(\text{ab}')_2$ fragments are as effective as whole IgG, and Fab' fragments are inefficient in inducing lysis (13). The requirement for divalency suggests that patching of viral antigen on the cell membrane may be required for induction of lysis. The fact that measles virus-infected HeLa cells can be lysed by IgG antibody and the purified cytolytic alternative pathway (8) shows that no serum factor other than IgG is required for lysis. The role of IgG in inducing lysis remains to be defined, but the present results clearly demonstrate that it is not required for initiation of the alternative pathway on the surface of measles virus-infected cells.

Several other reports are relevant to our results. A number of human lymphoblastoid cell lines can activate the alternative pathway in human serum (26, 27), and it has been reported that this ability correlates with transformation by Epstein-Barr virus. Cell lines positive for the nuclear antigen determined by this virus can activate the alternative pathway whereas negative cell lines cannot but they do acquire the capacity after transformation by Epstein-Barr virus. However, these cell lines do not actually express detectable Epstein-Barr virus antigens on their surface, and the capacity to activate the alternative pathway may reside in transformation antigens (28). A recent preliminary report (29) indicated that cells infected with Sendai virus (another paramyxovirus) can activate the alternative pathway in C4-deficient guinea pig serum. There is also evidence that Newcastle disease virus (as free virions) can be lysed by the alternative pathway in human serum (ref. 30; reviewed in ref. 31).

Our findings further support the concept of the alternative pathway as a system of host defense against microorganisms which can operate in the absence of specific immunity, initiation of the pathway being induced by the microorganism itself. They suggest the possibility that acutely infected cells expressing viral antigen on their membrane could activate the alternative pathway *in vivo*; the resultant surface deposition of C3b could mediate the phagocytosis or killing of these cells by cytotoxic cells bearing C3b receptors.

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