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Regulation by membrane sialic acid of β1H-dependent decay-dissociation of amplification C3 convertase of the alternative complement pathway

(sialidase/$NaIO_4$/sheep erythrocyte/C3b inactivator)

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ABSTRACT Sheep erythrocytes in their native state did not activate the alternative complement pathway, as measured by lysis in dilutions of normal human serum containing [ethylenebis(oxyethylenenitrilo)] tetraacetic acid but acquired this capacity after membrane sialic acid residues had been removed (by sialidase) or modified (by $NaIO_4$). Activation of the alternative pathway by sheep erythrocytes required removal or modification of at least 40% of the membrane sialic acid to reach threshold, and it increased proportionately when larger amounts of sialic acid had been affected. Studies with isolated proteins of the alternative pathway demonstrated that the altered erythrocyte membranes resembled natural activators in protecting bound C3b from inactivation by C3b inactivator and β1H and protecting bound amplification C3 convertase (C3b,Bb) from decay-dissociation by β1H. A 1% decrease in intact sialic acid was associated with a 1% decrease in β1H activity in decay-dissociation of membrane bound C3b,Bb. Because removal of the C8 and C9 carbon atoms from the polyhydroxylated side chain of sialic acid by oxidation with $NaIO_4$ was functionally equivalent to removal of the entire sialic acid moiety, secondary effects of the latter reaction, such as diminution of the negative charge of the membrane or exposure of penultimate galactose residues, were not considered to be responsible for the altered activity of β1H. These studies suggest that facilitation, by membrane sialic acid residues, of the interaction between bound C3b and β1H is essential to prevent the particle from effectively activating the alternative pathway.

Activation of the classical complement pathway is dependent on primary recognition of foreign substances by IgG or IgM (1, 2), whereas activation of the alternative complement pathway is not necessarily dependent on antibody for formation of its C3 convertase. In the human, this latter pathway can serve a primary recognition function for various particulate microbial polysaccharides—such as zymosan (3), a derivative of yeast cell walls, and Gram-negative bacterial lipopolysaccharide (4)—and certain mammalian cells such as rabbit erythrocytes (5) and some human lymphoblastoid cell lines (6). Cleavage of C3, a β-globulin of M_r 185,000, by the alternative pathway occurs in two distinct phases: (*i*) continuous low-grade fluid phase generation of C3b, the 180,000 M_r cleavage fragment of C3 by interaction of native C3, B, $\overline{D}$, and properdin (7, 8) that is independent of an activating particle and (*ii*) subsequent C3b-dependent amplified cleavage of C3 by a particle-associated C3 convertase (9, 10). Significant C3b deposition occurs only in the latter phase in which C3b (11) reversibly binds B, a 90,000 M_r β-globulin that is then cleaved by $\overline{D}$ (11, 12), a 23,500 M_r serine protease (13) normally present in plasma, to uncover the C3-cleaving site in the residual 60,000 M_r Bb fragment that remains complexed with C3b to form the amplification C3 convertase (C3b,Bb) (14–16). This labile enzyme is stabilized by properdin, a 223,000 M_r gamma globulin (17, 18) that binds to C3b and retards spontaneous decay-dissociation of Bb (19–21).

Formation and function of the C3b,Bb does not occur as an automatic consequence of the presence of C3b but is restricted to C3b bound to surfaces of activators of the alternative pathway by the action of two regulatory proteins, β1H and C3b inactivator (C3bINA), β-globulins of M_r 150,000 and 93,000, respectively. The activity of C3b,Bb in the fluid phase or bound to nonactivating particles such as sheep erythrocytes, even in the presence of properdin, is rapidly abolished by binding of β1H to C3b (22) and dissociation of Bb (23, 24). The convertase formed on activating particles such as zymosan (9), rabbit erythrocytes (10), and *Escherichia coli* (25) is relatively resistant to decay-dissociation by β1H. Similarly, C3b is rapidly inactivated by C3bINA in a proteolytic reaction that is accelerated by β1H (24, 26); however, if C3b is bound to an activator, it is relatively protected (9, 10, 25). Thus, the regulatory proteins direct deposition of C3b to certain surfaces by selectively permitting assembly and function of particle-bound C3b,Bb.

The mechanism by which the regulatory proteins distinguished between various particle surfaces was not known, but several observations suggested a role for membrane sialic acid. Rabbit erythrocyte, an activator, has less membrane sialic acid content (27) than the nonactivating particle, sheep erythrocyte; and complement-mediated lysis of human erythrocytes from normal individuals (28) and from patients with paroxysmal nocturnal hemoglobinuria (29), of sheep erythrocytes (30, 31), and of murine (32) and guinea pig (33) tumor cells was enhanced by pretreatment of the cells with sialidase. The present study demonstrates that removal of sialic acid residues from sheep erythrocytes or degradation of the exocyclic polyhydroxylated side chain of sialic acid converts the cell into an activator of the alternative pathway by impairing β1H-mediated decay-dissociation of membrane-bound C3b,Bb.

MATERIALS AND METHODS

B (12), C3 (34), properdin (9), $\overline{D}$ (19), β1H (23), and C3bINA (35) were free of detectable contaminating proteins as assessed by polyacrylamide gel electrophoresis of their reduced forms

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Abbreviations: C3b,Bb, the amplification C3 convertase; C3bINA, C3b inactivator; GVB, Veronal-buffered saline/0.1% gelatin; GVB^{2+}, GVB/0.5 mM magnesium chloride/0.15 mM calcium chloride; $DGVB^{2+}$, half-isotonic GVB^{2+}/2.5% dextrose; GVB-EDTA, GVB/0.04 M ethylenediaminetetraacetate; GVB-MgEGTA, GVB/2 mM magnesium chloride/8 mM [ethylenebis(oxyethylenenitrilo)] tetraacetic acid.

in the presence of sodium dodecyl sulfate (36) and were quantitated as described. C3 was trace-labeled with ^{125}I (New England Nuclear, Boston, MA) by insoluble lactoperoxidase (Worthington Biochemical Corp., Freehold, NJ) (37) to a specific activity of 100,000 cpm/μg without loss of specific functional activity. For purification of *Clostridium perfringens* sialidase (acylneuraminyl hydrolase, EC 3.2.1.18), 323 mg containing 70 units (1 unit releases 1 μmol of *N*-acetylneuraminic acid from *N*-acetylneuraminlactose per min at 37°) of crude enzyme (type V, lot 96C-8125, Sigma Chemical Co., St. Louis, MO) was dissolved in 20 ml of ice-cold H_2O, dialyzed overnight at 4° against 0.05 M Tris·HCl/0.05 M NaCl, pH 7.5, and applied to a 2.5 × 40 cm column of QAE Sephadex A-50 (Pharmacia Fine Chemicals, Piscataway, NJ) equilibrated in the same buffer. Sialidase was eluted by a linear NaCl gradient, and the active fractions were pooled, dialyzed overnight at 4° against 0.05 M Na acetate (pH 6.0), and applied to a 2.5 × 15 cm column of CM-Sephadex C-50 (Pharmacia) equilibrated in the same buffer. Sialidase was eluted from this column by a linear NaCl gradient and had a specific functional activity of 16 units/mg. There was no detectable protease activity as assessed by release of non-trichloroacetic acid-precipitable radioactivity after treatment of ^{125}I-labeled casein for 60 min at 37°.

Sheep erythrocytes were collected in Alsever's buffer, washed twice in this buffer and three times in 0.15 M NaCl, and stored in 0.15 M NaCl at 2° for use within 1 week. For determination of total sialic acid associated with erythrocyte membranes, stroma from 3×10^{10} erythrocytes was hydrolyzed in 0.1 M HCl at 80° for 60 min, and free sialic acid was assayed by the thiobarbituric acid method (38). For treatment with sialidase, five samples of 2.8×10^{10} erythrocytes were washed with 0.05 M Na acetate/0.13 M NaCl, pH 6.5, pelleted by centrifugation for 10 min at 1300 × *g*, and resuspended in 1-ml portions of this buffer containing 0.37, 0.094, 0.023, and 0.006 unit of sialidase and 0.2 μmol of synthetic *N*-acetylneuraminic acid (Calbiochem, La Jolla, CA), respectively. After incubation for 30 min at 37°, the erythrocytes were sedimented by centrifugation at 1300 × *g* for 10 min at 2°, and the supernatants were removed for subsequent assay of sialic acid (38) (a standard reference curve was established with synthetic *N*-acetylneuraminic acid). The erythrocytes were washed four times with Veronal-buffered saline containing 0.1% gelatin (GVB) (39) and stored in this buffer at 2°. The total sialic acid released from each of the four samples of erythrocytes treated with sialidase was calculated on the basis of the recovery of the *N*-acetylneuraminic acid that was incubated with the fifth sample of sheep e.

For treatment with $NaIO_4$, five samples of 2×10^{10} erythrocytes were washed with 0.05 M Na acetate/0.13 M NaCl, pH 6.5, pelleted by centrifugation at 1300 × *g* for 10 min, and resuspended in 1-ml portions of the same buffer at 0° containing 3, 1.5, 0.75, and 0.375 μmol of $NaIO_4$ and 0.12 μmol of formaldehyde, respectively. After incubation at 0° for 10 min in the dark, the erythrocytes were sedimented by centrifugation at 1300 × *g* for 10 min at 2°, and the supernatants were removed for subsequent assay of formaldehyde. Each sample of erythrocytes was immediately resuspended in 10 ml of the acetate buffer to which was added 0.2 ml of 0.01 M NaOH containing 10 μmol of $NaBH_4$, incubated for 45 min at 22°, and washed three times with GVB. Formaldehyde was assayed in the supernatants after removal of trichloroacetic acid-precipitable protein as described (40) except that 1 ml of the chromotropic acid solution (325 mg of chromotropic acid in 100 ml of 16.4 M H_2SO_4) was added to 0.8 ml of the formaldehyde-containing samples, a modification that increased detection of formaldehyde by 4.4-fold. A standard reference curve was established with commercial formaldehyde (Merck and Co., Inc., Rahway, NJ); samples containing the various amounts of $NaIO_4$ served as blanks. The total formaldehyde released was calculated on the basis of the recovery of formaldehyde incubated with the fifth sample of erythrocytes.

Table 1. Removal of sialic acid from sheep erythrocytes and capacity of β1H to decay-dissociate Bb from membrane-bound C3b,Bb

Sialidase, (unit)	Sialic acid, μmol released/ 10^{10} erythrocytes	β1H (ng/ml) for 63% Bb dissociation*	Relative β1H activity
None		109	1.00
0.006	0.054	184	0.59
0.023	0.079	322	0.34
0.094	0.100	516	0.21
0.374	0.113	606	0.18

* From properdin-stabilized C3b,Bb sites.

Sialidase-treated, $NaIO_4$-treated, and untreated erythrocytes were converted to their respective EC3b intermediates by incubating 1×10^9 of each cell type with 700 μg of C3, 10 μg of ^{125}I-labeled C3, 1 mg of B, and 2 μg of $\overline{D}$ in 1 ml of GVB containing 0.5 μmol of Mg^{2+} and 0.15 μmol of Ca^{2+} (GVB^{2+}) (39) for 45 min at 30°. The cells were washed twice with GVB, incubated 120 min at 37° in GVB containing 0.04 M EDTA (GVB-EDTA) (39), and then washed twice with half-isotonic GVB^{2+} containing 5% dextrose ($DGVB^{2+}$) (39). The number of C3b molecules per erythrocyte was calculated from the ^{125}I bound in the presence of B and $\overline{D}$ after subtraction of the ^{125}I-labeled C3 nonspecifically bound in the absence of B and $\overline{D}$; the value was approximately 2000 C3b molecules per erythrocyte for all cell types. Cell-bound C3b hemolytic activity was assayed by incubating 1×10^7 EC3b of each cell type with 0.2 μg of properdin, 10 ng of $\overline{D}$, and various amounts of B (1–32 ng) in 0.2 ml of $DGVB^{2+}$ for 30 min at 30°. After addition of rat serum diluted 1:20 in GVB-EDTA and further incubation of the cells for 60 min at 37°, the percentage hemolysis was determined and the average number of hemolytic sites per cell was calculated. Although all cell types had equivalent numbers of cell-bound C3b, variable amounts of B were required for formation of one hemolytic site per cell: 3 ng of B for EC3b derived from untreated erythrocytes; 6, 12, 25, and 25 ng B for EC3b derived from erythrocytes treated with increasing amounts of sialidase, respectively; and 3, 2, 1.5, and 1.5 ng B for EC3b derived from erythrocytes treated with increasing concentrations of $NaIO_4$, respectively.

RESULTS

Removal of Sialic Acid from Sheep Erythrocytes by Treatment with Sialidase. Mean (±SD) total sheep erythrocyte membrane sialic acid in four experiments was 0.127 ± 0.007 μmol/10^{10} erythrocytes. Treatment of erythrocytes with increasing concentrations of purified sialidase released increasing amounts of sialic acid (Table 1). The effect of enzymatic removal of sialic acid on the capacity of erythrocytes to activate the alternative pathway was assessed by their lysis in human serum in the presence of [ethylenebis(oxyethylenenitrilo)] tetraacetic acid to prevent activation of the classical pathway (41). Samples (1×10^7 cells) of untreated and sialidase-treated erythrocytes in 0.2 ml of GVB containing 2 mM Mg^{2+} and 8 mM [ethylenebis(oxyethylenenitrilo)] tetraacetic acid (GVB-MgEGTA) were added to 0.3 ml of serial dilutions of normal

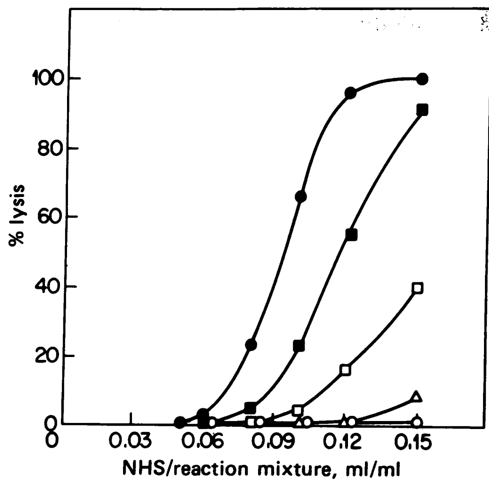

FIG. 1. Lysis of untreated sheep erythrocytes (○) and erythrocytes pretreated with 0.006 (△), 0.023 (□), 0.094 (■), and 0.374 unit (●) of sialidase, after incubation with various concentrations of normal human serum (NHS) diluted in GVB-MgEGTA.

human serum in GVB-MgEGTA. Treated and untreated erythrocytes added to dilutions of serum in GVB-EDTA, which prevents activation of both classical and alternative pathways, served as reagent blanks. All reaction mixtures were incubated for 45 min at 37°, and the extent of hemolysis was measured. No lysis of erythrocytes was observed after incubation with serum diluted in GVB-EDTA. In contrast, sialidase-treated erythrocytes were lysed by serum dilutions in GVB-MgEDTA, and the concentration of serum required for lysis was inversely related to the amount of sialic acid removed (Fig. 1).

To determine whether activation of the alternative pathway by desialated erythrocyte membranes reflected impaired action of a regulatory protein, the activity of β1H in decay-dissociation of membrane-bound C3b,Bb was assessed. EC3b (1×10^8) formed from untreated cells and from cells treated with increments of sialidase was incubated for 30 min at 30° in 1.0 ml DGVB^{2+} with 2 μg of properdin, 0.1 μg of $\overline{D}$, and sufficient B to yield two effective properdin-stabilized C3b,Bb sites per cell. The cellular intermediates were washed once with ice-cold DGVB^{2+} and resuspended in the same buffer, and 0.1-ml samples containing 1×10^7 of each cell type were added to 0.1 ml of ice-cold GVB-EDTA alone or containing 6, 12, 24, 48, 96, or 192 ng of β1H, respectively. The reaction mixtures were incubated for 25 min at 30°, and then 0.3 ml of rat serum diluted 1:20 in GVB-EDTA was added. Incubation was continued for 60 min at 37°, and lysis was measured. The concentration of β1H required to dissociate Bb from 63% of the cell-bound properdin-stabilized C3b,Bb sites was calculated for untreated and sialidase pretreated sheep erythrocyte intermediates (Table 1). There was direct relationship between the amount of sialic acid removed and the concentration of β1H required for decay-dissociation such that 5 times more β1H was necessary with the erythrocytes bearing the fewest sialic acid residues.

The relative capacities of C3bINA and β1H to inactivate C3b bound to untreated erythrocytes and to erythrocytes bearing the least residual sialic acid were compared by incubating 1×10^7 EC3b formed from untreated and sialidase-treated cells with 0.05 μg of C3bINA and 0.5 μg of β1H in 0.2 ml of GVB or in 0.2 ml of GVB alone for 30 min at 37°. The intermediates were washed three times with GVB and assayed for residual C3b activity. Whereas C3bINA and β1H inactivated 80% of the C3b on erythrocytes bearing normal amounts of sialic acid, these proteins inactivated only 19% of the C3b on those from

Table 2. Modification of sialic acid on sheep erythrocytes and the capacity of β1H to decay-dissociate Bb from membrane-bound C3b,Bb

$NaIO_4$, μmol/10^{10} erythrocytes	HCHO, μmol released/ 10^{10} erythrocytes	β1H (ng/ml) for 63% Bb dissociation*	Relative β1H activity
None		103	1.00
0.188	0.040	147	0.70
0.375	0.055	178	0.58
0.75	0.074	250	0.41
1.50	0.110	496	0.21

* From properdin-stabilized C3b,Bb sites.

which 0.113 μmol of sialic acid per 10^{10} erythrocytes had been removed.

Modification of Sialic Acid on Erythrocytes by $NaIO_4$. Mild oxidation of sialoglycoproteins, isolated or as membrane-associated proteins, with $NaIO_4$ selectively cleaves the polyhydroxylated exocyclic side chain of sialic acid (42–44), releasing 1 mol of formic acid and formaldehyde per mol of sialic acid. Treatment of erythrocytes with increasing concentrations of $NaIO_4$ generated increasing amounts of formaldehyde and yielded erythrocytes whose membranes differed in the number of modified sialic acid residues (Table 2). The capacity of these altered erythrocytes to activate the alternative pathway was assessed in normal serum containing GVB-MgEGTA as described for sialidase-treated erythrocytes. No lysis was observed with unmodified erythrocytes. Erythrocytes whose membrane sialic acid had been modified with $NaIO_4$ were lysed, and the concentration of serum required for lysis was inversely related to the number of membrane sialic acid residues that had been modified (Fig. 2). To exclude the possibility that modification of the membranes by formaldehyde generated during the $NaIO_4$ reaction was responsible for alternative pathway activation, 1×10^{10} erythrocytes were incubated with 0.1 and 0.5 μmol of formaldehyde, respectively, in 1 ml of acetate buffer for 10 min at 0°, washed, and assayed for their capacity to activate the alternative pathway as above. No lysis was observed.

The capacity of β1H to dissociate Bb from properdin-stabilized C3b,Bb sites on the $NaIO_4$-treated erythrocytes was assessed with the same concentrations of β1H used for the sial-

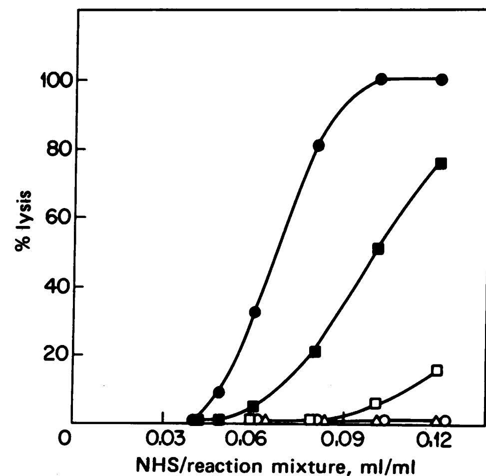

FIG. 2. Lysis of untreated sheep erythrocytes (○) and erythrocytes pretreated with 0.188 (△), 0.375 (□), 0.75 (■), and 1.5 μmol (●) of $NaIO_4$ per 10^{10} erythrocytes, after incubation with various concentrations of normal human serum (NHS) diluted in GVB-MgEDTA.

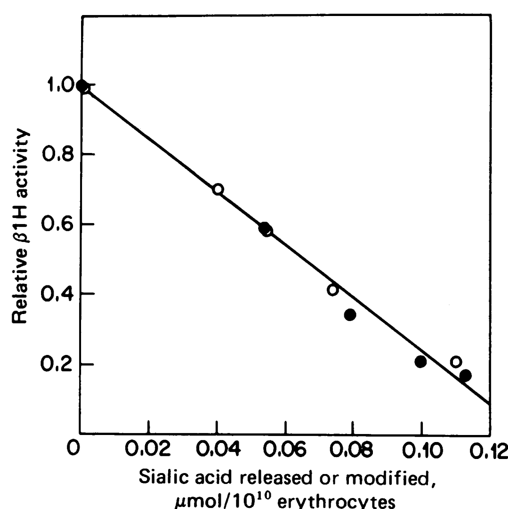

FIG. 3. Relationship between the amount of sialic acid removed by sialidase (●) or modified by $NaIO_4$ (○) per 10^{10} sheep erythrocytes and the relative capacity of β1H to dissociate Bb from properdin-stabilized C3b,Bb sites subsequently formed on the altered cells.

idase-treated erythrocytes. There was direct relationship between the number of $NaIO_4$-modified sialic acid residues per erythrocyte and the concentration of β1H necessary for dissociation of Bb such that 5 times more β1H was required for erythrocyte membranes bearing the fewest intact sialic acid residues (Table 2).

In order to compare the effects of enzymatic removal of sialic acid residues and chemical modification on subsequent β1H dissociation of convertase sites, the relative activities of β1H were plotted as a function of the amount of sialic acid modified or removed (Fig. 3). The dose-response curves of the two treatments were superimposable, indicating that removal of the two terminal exocyclic carbon atoms from membrane sialic acid with $NaIO_4$ impairs β1H interaction with cell-bound convertase to the same extent as removal of the entire carbohydrate moiety. A 1% decrease in intact membrane sialic acid was associated with a 1% decrease in relative β1H activity.

The relative capacities of C3bINA and β1H to inactivate C3b bound to untreated erythrocytes and to erythrocyte membranes treated with the highest concentration of $NaIO_4$ were assessed with the same concentrations of control proteins as used for the sialidase-treated erythrocytes. Whereas normal erythrocyte membranes supported 76% inactivation of bound C3b by C3bINA and β1H, erythrocyte membranes with modified sialic acid residues permitted only 24% inactivation of bound C3b.

DISCUSSION

Sheep erythrocyte membranes acquired the capacity to activate the human alternative complement pathway in normal human serum after their sialic acid residues had been removed or converted to the heptulosonic acid derivative (Figs. 1 and 2). Studies with isolated proteins of the alternative pathway demonstrated that the altered erythrocyte membranes resembled natural activators, such as zymosan (9), rabbit erythrocytes (10), and *E. coli* (25), in protecting bound C3b from inactivation by C3bINA and β1H and bound C3b,Bb from decay-dissociation by β1H (Tables 1 and 2). The observation that membrane sialic acid modulates the interaction of β1H with membrane-bound C3b provides a biochemical basis for the capacity of this pathway to discriminate between activating and nonactivating particle surfaces.

The capacity of sheep erythrocytes to activate the alternative pathway after $NaIO_4$ treatment is attributed to modification of membrane sialic acid residues because only this carbohydrate of sialoglycoproteins is oxidized under the conditions utilized in this study (42–44). The two terminal carbon atoms of the polyhydroxylated side chain are released as formaldehyde and formic acid, converting the sialic acid to its heptulosonic acid derivative. Measurement of the formaldehyde released from sheep erythrocytes permitted quantification of the number of sialic acid residues modified in this manner and led to the demonstration that removal of C8 and C9 carbon atoms of the polyhydroxylated side chain was functionally equivalent to removal of the entire carbohydrate with sialidase (Fig. 3). This observation indicates that membrane sialic acid is required for effective interaction of β1H with membrane-bound C3b so as to dissociate Bb and that secondary effects of sialic acid removal, such as decreasing the net negative charge of the membrane or exposing penultimate galactose residues on glycoproteins, are not the basis for the reduced activity of β1H. This conclusion is consistent with the linear inverse relationship between the amount of membrane sialic acid residues removed or modified and relative β1H activity (Fig. 3). Because sheep erythrocyte membranes contain 0.12–0.13 μmol of sialic acid per 10^{10} erythrocytes, a 1% decrease in intact sialic acid residues is associated with approximately a 1% decrease in relative β1H activity (Fig. 3).

The extent of lysis of sialidase- and $NaIO_4$-treated sheep erythrocytes in normal human serum chelated with EGTA was dependent on the number of sialic acid residues removed or modified (Figs. 1 and 2; Tables 1 and 2). Modification of the membrane so as to reduce β1H-dependent dissociation of C3b,Bb sites by 40–50% was necessary to achieve minimal lysis of the erythrocytes. This finding suggests that particle-dependent transition from low-grade fluid phase C3 cleavage in whole serum to surface-dependent amplified C3b generation and deposition represents a threshold in a continuum of relative control protein function. These limited reductions in the capacity of β1H to dissociate membrane-bound C3b,Bb that are below the threshold for lysis by an exclusive alternative pathway activation mechanism could nevertheless enhance amplification of C3 cleavage that was initiated with C3b deposited by the interaction of antibody and the classical pathway proteins. Thus, membrane sialic acid may also modulate recognition of particles by the humoral adaptive immune response.

The greater lysis of $NaIO_4$-treated erythrocytes than of sialidase-treated erythrocytes in human serum at comparable reductions of β1H activity (Figs. 1 and 2; Tables 1 and 2) may reflect different susceptibilities of the altered cells to lysis by the complement C5–C9 cytolytic complex. This may relate to the finding that $NaIO_4$-treated cells require less B than do sialidase-treated cells to generate hemolytically active sites when C3b is already bound to the cells.

Participation of membrane sialic acid in many biologic reactions has been described (45) and several reactions may relate to its role in modulation of the alternative pathway. Senescent human erythrocytes have diminished amounts of sialic acid residues (46), and sialidase-treated erythrocytes of several mammalian species are rapidly removed from the circulation by sequestration in the liver (27). A possible mechanism for recognition and clearance of aging or altered erythrocytes could be membrane deposition of C3b that is facilitated by impaired β1H activity. A variant cell line of mouse L cells that was resistant to the cytotoxic action of antibody and complement was found to have twice the membrane sialic acid content as the sensitive parent cell line (47). As cytotoxicity was restored after treatment with sialidase, resistance of the variant may have

been secondary to augmented β1H activity by the additional sialic acid residues. Thus, regulation of the amplification convertase of complement by modulation of β1H activity with membrane sialic acid may enhance or impair recognition of altered host cells.

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1. Müller-Eberhard, H. J. (1975) *Annu. Rev. Biochem.* **44,** 697–724.
2. Porter, R. R. (1977) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36,** 2191–2196.
3. Pillemer, L., Blum, L., Lepow, I. H., Ross, O. A., Todd, E. W. & Wardlaw, A. C. (1954) *Science* **120,** 279–285.
4. Gewurz, H., Shin, H. S. & Mergenhagen, S. E. (1968) *J. Exp. Med.* **128,** 1049–1057.
5. Platts-Mills, T. A. E. & Ishizaka, K. (1974) *J. Immunol.* **113,** 348–358.
6. Theofilopoulos, A. N. & Perrin, L. H. (1977) *Science* **195,** 878–880.
7. Fearon, D. T. & Austen, K. F. (1975) *J. Immunol.* **115,** 1357–1361.
8. Fearon, D. T. & Austen, K. F. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3220–3225.
9. Fearon, D. T. & Austen, K. F. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 1683–1687.
10. Fearon, D. T. & Austen, K. F. (1977) *J. Exp. Med.* **146,** 22–33.
11. Müller-Eberhard, H. J. & Götze, O. (1972) *J. Exp. Med.* **135,** 1003–1008.
12. Hunsicker, L. G., Ruddy, S. & Austen, K. F. (1973) *J. Immunol.* **110,** 128–138.
13. Fearon, D. T., Austen, K. F. & Ruddy, S. (1974) *J. Exp. Med.* **139,** 355–366.
14. Fearon, D. T., Austen, K. F. & Ruddy, S. (1973) *J. Exp. Med.* **138,** 1305–1313.
15. Daha, M. R., Fearon, D. T. & Austen, K. F. (1976) *J. Immunol.* **116,** 1–7.
16. Vogt, W., Dames, W., Schmidt, G. & Dieminger, L. (1976) *J. Immunol.* **116,** 1753 (abstr).
17. Pensky, J., Hinz, C. F., Jr., Todd, E. W., Wedgwood, R. J., Boyer, J. T. & Lepow, I. H. (1968) *J. Immunol.* **100,** 142–158.
18. Minta, J. O. & Lepow, I. H. (1974) *Immunochemistry* **11,** 361–368.
19. Fearon, D. T. & Austen, K. F. (1975) *J. Exp. Med.* **142,** 856–863.
20. Schreiber, R. D., Medicus, R. G., Götze, O. & Müller-Eberhard, H. J. (1975) *J. Exp. Med.* **142,** 760–772.
21. Chapitis, J. & Lepow, I. H. (1976) *J. Exp. Med.* **143,** 241–257.
22. Whaley, K. & Ruddy, S. (1976) *Science* **193,** 1011–1013.
23. Weiler, J. M., Daha, M. R., Austen, K. F. & Fearon, D. T. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3268–3272.
24. Whaley, K. & Ruddy, S. (1976) *J. Exp. Med.* **144,** 1147–1163.
25. Fearon, D. T. (1978) *J. Immunol.* (abstr.), in press.
26. Pangburn, M. K., Schreiber, R. D. & Müller-Eberhard, H. J. (1977) *J. Exp. Med.* **146,** 257–270.
27. Aminoff, D., Bell, W. C., Fulton, I. & Ingebrigtsen, N. (1976) *Am. J. Hematol.* **1,** 419–432.
28. Dalmasso, A. P. & Müller-Eberhand, H. J. (1964) *Proc. Soc. Exp. Biol. Med.* **117,** 643–650.
29. Yachnin, S., Laforet, M. T. & Gardner, F. H. (1961) *Blood* **17,** 83–96.
30. Arguilla, E. R., Hamlin, J., Hamashige, S. & Miller, H. (1964) *J. Exp. Med.* **120,** 841–856.
31. Lauf, P. K. (1975) *J. Exp. Med.* **142,** 974–988.
32. Hughes, R. C., Palmer, P. D. & Sanford, B. H. (1973) *J. Immunol.* **111,** 1071–1080.
33. Boyle, M. D., Ohanian, S. H. & Borsos, T. (1976) *J. Immunol.* **116,** 661–668.
34. Tack, B. F. & Prahl, J. W. (1976) *Biochemistry* **15,** 4513–4521.
35. Fearon, D. T. (1977) *J. Immunol.* **119,** 1248–1252.
36. Weber, K. & Osborne, M. (1969) *J. Biol. Chem.* **244,** 4406–4412.
37. Thorell, J. I. & Larsson, I. (1974) *Immunochemistry* **11,** 203–206.
38. Warren, L. (1959) *J. Biol. Chem.* **234,** 1971–1975.
39. Nelson, R. A., Jr., Jensen, J., Gigli, I. & Tamura, N. (1966) *Immunochemistry* **3,** 111–135.
40. MacFayden, D. A. (1945) *J. Biol. Chem.* **158,** 107–133.
41. Bryant, R. E. & Jenkins, D. E., Jr. (1968) *J. Immunol.* **101,** 664–668.
42. van Lenten, L. & Ashwell, G. (1971) *J. Biol. Chem.* **246,** 1889–1894.
43. Liao, T. H., Gallop, P. M. & Blumenfeld, O. O. (1973) *J. Biol. Chem.* **248,** 8247–8253.
44. Blumenfeld, O. O., Gallop, P. M. & Liao, T. H. (1972) *Biochem. Biophys. Res. Commun.* **48,** 242–251.
45. Jeanloz, R. W. & Codington, J. F. (1976) in *Biological Roles of Sialic Acid*, eds. Rosenberg, A. & Shengrund, C. L. (Plenum, New York), pp. 201–238.
46. Baxter, A. & Beeley, J. G. (1975) *Biochem. Soc. Trans.* **3,** 134–136.
47. Shearer, W. T., Gottlieb, C. & Kornfeld, S. (1977) *J. Immunol.* **119,** 614–617.