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Auto-antibody dependent activation of the autologous classical complement pathway by guinea-pig red cells treated with influenza virus or neuraminidase: *in vitro* and *in vivo* study

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Summary. Guinea-pig erythrocytes that had been exposed to influenza A virus or *Vibrio cholerae* neuraminidase activated the classical complement pathway in autologous serum. Because all viral particles were eluted from the treated cells, activation was not dependent on anti-viral antibodies or on the particles themselves. After a threshold of 45–55% desialation, had been reached, the relative capacity of treated cells to activate complement increased very rapidly with desialation. Desialation unmasked sites on which natural auto-antibodies of the IgM class were fixed. Antibody fixation on the membrane led to C3b deposition on the cell membrane and activation of the classical complement sequence then cell lysis. The relevance of *in vitro* lysis of desialated cells to *in vivo* clearance of these cells is not certain because C4-deficient guinea-pigs were able to eliminate desialated cells from the blood stream as efficiently as did normal guinea-pigs. Nevertheless, membrane desialation occuring during myxovirus infection could lead to autoimmunity and tissue changes, as well as to recovery by eliminating virus-modified cells.

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INTRODUCTION

Influenza infection is a common viral disease and it has been speculated that immunological mechanisms could be partly responsible for tissue alterations observed in the course of the disease (Oldstone & Dixon, 1971). These immunological mechanisms involve antibodies fixed on antigenic sites exhibited by infected cells and lead to complement-mediated cell lysis (Perrin *et al.*, 1976; Verbonitz *et al.*, 1978). Complement components are present in bronchoalveolar secretions (Robertson *et al.*, 1976) and their activity in airway secretions is frequently increased during respiratory infections (Rossen *et al.*, 1965; Loos & Brunner, 1979). The role of the complement system during viral respiratory infections is far from clear; sometimes it is phlogistic by potentiating immunopathological injury (Johnson & Ward, 1974) or by releasing active mediators and attracting reactive cells (Busse *et al.*, 1979; Desai *et al.*, 1979; McCarthy & Henson, 1979; Shaw *et al.*, 1980); Stimler, Hugli & Bloor, 1980; Larsen, Mitchell & Henson, 1980; Ogawa *et al.*, 1981). On the other hand, it protects the host by limiting viral replication and viraemia (Hicks *et al.*, 1978; Hirsch, Winkelstein & Griffin, 1980).

During influenza infection, the complement system can, theoretically, be activated by several mechanisms. Viral particles themselves could activate the alterna-

tive pathway (Hirsch, Griffin, & Winkelstein, 1981; McSharry, Pickering & Caliguiri, 1981); cells infected with budding viruses have been shown to activate complement (McConnell *et al.*, 1981; Okada & Okada, 1981; Smith *et al.*, 1981); hydrolytic enzymes released by reactive cells recruited in the airways during the infection can cleave C3 and C5; lastly, viral-induced desialation of the cells leads to activation of the alternative pathway in a heterologous system using guinea-pig red cells and human serum (Lambré & Thibon, 1980; Lambré *et al.*, 1982).

In this report we demonstrate that, after treatment with influenza virus or with neuraminidase purified from the virus or from *Vibrio cholerae*, guinea-pig red cells were lysed in autologous serum through activation of the classical complement pathway. Viral or enzymatic treatment unmasked antigenic sites on the cell membrane leading to the fixation of natural auto-antibodies of the IgM class followed by immune elimination as shown by *in vitro* lysis and *in vivo* clearance from the blood stream. *In vitro* lysis did not seem fully relevant to *in vivo* clearance, since C4-deficient guinea-pigs cleared enzyme-treated cells as well as normal animals.

These results demonstrate that besides the well known role of the alternative pathway, the classical complement pathway can participate in the immediate defence mechanism against a viral infection providing that the infection leads to a modification of the host-cell surface allowing the fixation of natural auto-antibodies. However if the virus unmasked large numbers of antigenic sites, numerous cells should be lysed. The physiological mechanism of removal of aged erythrocytes, which are characterized by a decrease in their membrane sialic acid content, could be exaggerated by the action of virus.

MATERIALS AND METHODS

Buffers

Veronal-buffered saline (VBS), VBS containing 0·15 mM calcium and 0·5 mM magnesium (VBS^{++}), VBS^{++} containing 0·1% gelatin (GVB^{++}), GVB containing 0·04 M ethylenediamine tetracetic acid (GVB-EDTA; Nelson *et al.*, 1966) and GVB containing 2 mM magnesium and 8 mM ethyleneglyed tetracetic acid (GVB-Mg-EGTA) (Nyedegger, Fearon & Austen, 1978) were prepared as described.

Virus and viral treatment of erythrocytes

Human influenza A Hong-Kong/1/68 (H3N2) virus was grown in allantoic cavities of 11-day-old embryonated chicken eggs for 48 hr. Infective allantoic fluids were clarified by centrifugation at 5000 *g* for 15 min at 4°. The supernatant was centrifuged at 6500 *g* for 15 min at 4° and pelleted virus was further purified by ultracentrifugation on a 30–60% linear sucrose gradient or by absorption-elution on formalinized chicken red cells (Lambré & Kasturi, 1979). Purified viruses were resuspended in phosphate-buffered saline (PBS). Viral concentration was measured using a haemagglutination test (Hirst 1942) and expressed as haemagglutinating units (HAU) per millilitre.

Guinea-pig erythrocytes (Egp) were collected in Alsever's solution then washed twice in VBS. For viral treatment of cells, 0·5 ml of packed Egp was incubated with purified virus in 5 ml of 0·025 M Tris HCl, 0·138 M NaCl, 5 mM dextrose, 0·9 mM $CaCl_2$, 0·4 mM $MgCl_2$, 5 mM KCl, 0·2 mM Na_2HPO_4, pH 6·9, for 1 hr at +4° so as to allow viruses to stick to the cell membrane, and then centrifuged at 1800 *g* for 15 min at 4°. There was no residual haemagglutinating activity in the supernatant when Egp were treated with up to 15,000 HAU/ml of packed cells. Elution of the virus from Egp was achieved by incubating the cells at 37° for 30 min in 5 ml of the Tris buffer. After centrifugation at 1800 *g* for 15 min at 25°, viral elution was assessed by measuring the haemagglutinating activity in the supernatant. The procedure was repeated until no residual haemagglutinating activity could be detected in the supernatants as previously observed (Lambré *et al.*, 1982) no residual viral particles could be seen on the cells by electron microscopic examination at this stage and no alterations of the shape of the cells could be observed. Virus-treated Egp (Egp.V) were then washed twice in VBS^{++} and used immediately or stored in GVB-EDTA at 4° for no longer than 2 days. Control erythrocytes were incubated in the same conditions without virus.

Neuraminidase treatment of erythrocytes

Viral neuraminidase was purified from influenza virus A virus 3QB (H3N2) Hong Kong variant after treating the purified virus with sarcosyl (Stanley *et al.*, 1973) or bromelaine (Brand & Skehel, 1972) as described (Cabezas *et al.*, 1980). The preparation was pure as assessed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl-sulphate (Weber & Osborn, 1969) and was free of detectable protease activity. Neuraminidase from *Vibrio cholerae* was

purchased from Boehringer AG, Marburg Lahn (West Germany).

For treatment with sialidase, Egp were washed in 0·05 M Na acetate, O,13 M NaCl buffer pH 6·5, centrifuged at 1800 *g* for 5 min, resuspended to 1×10^9/ml in the same buffer containing 1000 U/ml of viral neuraminidase or 0·5 to 50 U/ml of neuraminidase from *Vibrio cholerae* (1 U releases 1 nmol of N-acetyl neuraminic acid from fetuin per min at 37°). After incubation for 45 min at 37°, neuraminidase-treated erythrocytes (Egp-Nase) were centrifuged at 1800 *g* for 10 min at +4°, washed twice in VBS^{++} and resuspended in this buffer. Control Egp were incubated in the same conditions without sialidase.

Sialic acid content in Egp membranes

For determination of sialic acid associated with membranes of untreated Egp or of Egp that had been treated with virus or sialidases, stroma from 1×10^9 erythrocytes was hydrolyzed in 0·1 M HCl at 80° for 60 min and free sialic acid was assayed by the thiobarbituric acid method (Warren, 1959).

Activation of autologous classical complement pathway by treated Egp

Activation of the classical complement pathway was measured by direct lysis of Egp in dilutions of autologous serum in VBS^{++}. Egp (2×10^7 in 0·2 ml of VBS^{++}) were added to 0·2 ml of autologous guinea-pig serum. The reaction mixtures were incubated for 45 min at 37° with agitation; 2·5 ml of ice-cold 0·15 M NaCl were added to each sample, and the percentage of lysis of Egp was determined. The reagent blank showed no lysis; 100% lysis was obtained by addition of 2·5 ml distilled water to 2×10^7 Egp. Prior to the test, guinea-pig serum was assayed using an enzyme-linked assay (ELISA; Lambré & Kasturi, 1979) in order to verify the absence of anti-influenza antibodies.

Antiglobulin test

Antiglobulin tests using goat anti-guinea-pig IgG (1+2), and sheep anti-guinea-pig IgG 1, IgM or C3 serum purchased from Nordic Laboratories (Tilburg, the Netherlands) were performed as follows.

Egp (1×10^9) were treated with 50 U of *Vibrio cholerae* neuraminidase in order to obtain a total desialation of the cells. Egp were adjusted to 1×10^8/ml in VBS, then 0·5 ml of 1/20 or 1/100 diluted autologous serum was added to $2{\cdot}5 \times 10^8$ treated Egp and allowed to stand at room temperature for 1 hr with constant gentle shaking. The cells were then washed twice in VBS before using in the antiglobulin tests. Prior to the test, goat and sheep antisera were absorbed with neuraminidase-treated Egp (0·5 ml of heat-inactivated antiserum diluted 1/10 in VBS with 1×10^9 treated Egp) for 1 hr at 2°.

Erythrocytes labelling and in vivo *survival*

After treatment with neuraminidase from *Vibrio Cholerae* (50 U) 1×10^9 erythrocytes were labelled with 0·5 μCi ^{51}Cr ($[Na^{51}]CrO_4$, 250 μCi/μg chromium, Amersham, UK) then re-inoculated intravenously in the autologous donor. Blood samples were collected at 0 min, 15 min, 45 min, 2 hr and 24 hr. Radioactivity was determined in the whole blood or separately in the plasma and in blood cells and also in spleen and liver homogenates.

RESULTS

Activation of the autologous classical complement pathway by red cells treated with influenza virus or neuraminidase

Egp-Nase and Egp-V that had been treated with 15,000 viral HAU were examined for their capacity to activate the autologous classical complement pathway as assessed by the direct lysis assay described in 'Materials and Methods'. No lysis of Egp-V or Egp-Nase was observed when the cells were incubated in autologous serum diluted in GVB-Mg-EGTA whereas 100% lysis was observed when the serum had been diluted in VBS^{++} (Fig. 1). It is noteworthy that lysis of Egp-V was variable from one individual to another, in some cases no lysis occured. This phenomenon was not due to the presence of anti-influenza specific antibodies in the serum since the absence of antiviral antibodies in the serum had been previously verified with an ELISA.

When Egp-Nase were incubated with the autologous serum that had been previously treated to 50° for 20 min in order to inactivate B, a decrease in the haemolytic activity was observed (Fig. 2). However it must be mentioned that heating the serum to 50° for 20 min will also slightly inactivate C2. A complete loss of the haemolytic activity was obtained by heating the serum at 56° for 30 min (Fig. 2).

When Egp-Nase and serum came from a C4-deficient guinea-pig, no lysis was observed, but lysis was obtained when pure human C4 (400 μg/ml of serum) was added to the incubation mixture (Fig. 2).

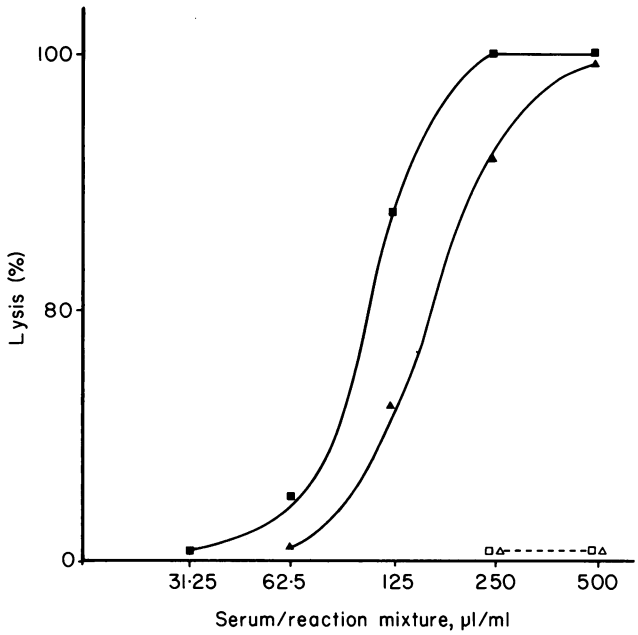

Figure 1. Lysis of guinea-pig erythrocytes treated with 50 U of *Vibrio cholerae* neuraminidase (squares) or with 15000 Haemagglutinating Units of influenza virus (triangles) in autologous serum diluted in VBS^{++} (closed symbols) or in GVB-Mg-EGTA (open symbols).

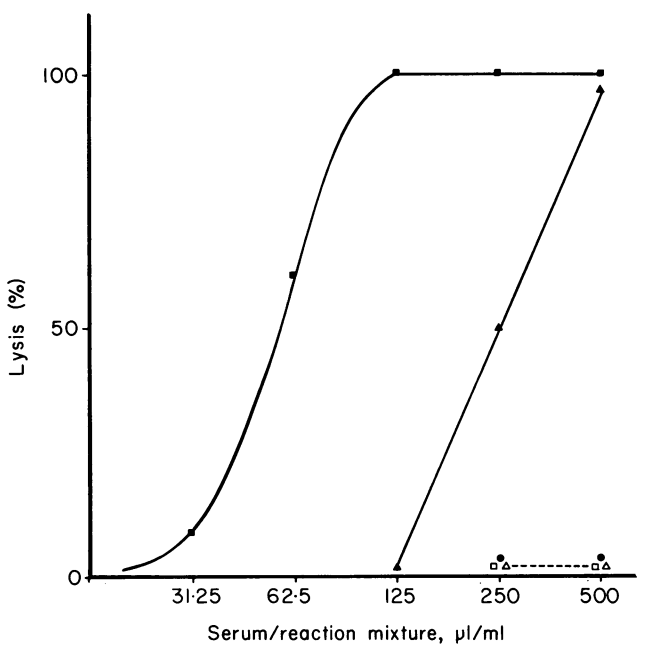

Figure 2. Lysis of guinea-pig erythrocytes treated with 50 U of *Vibrio cholerae* neuraminidase, in autologous serum: heated to 50° (▲), heated to 56° (△) or in pre-absorbed serum (●); in autologous C4-deficient serum (□) and after addition of pure C4 (■).

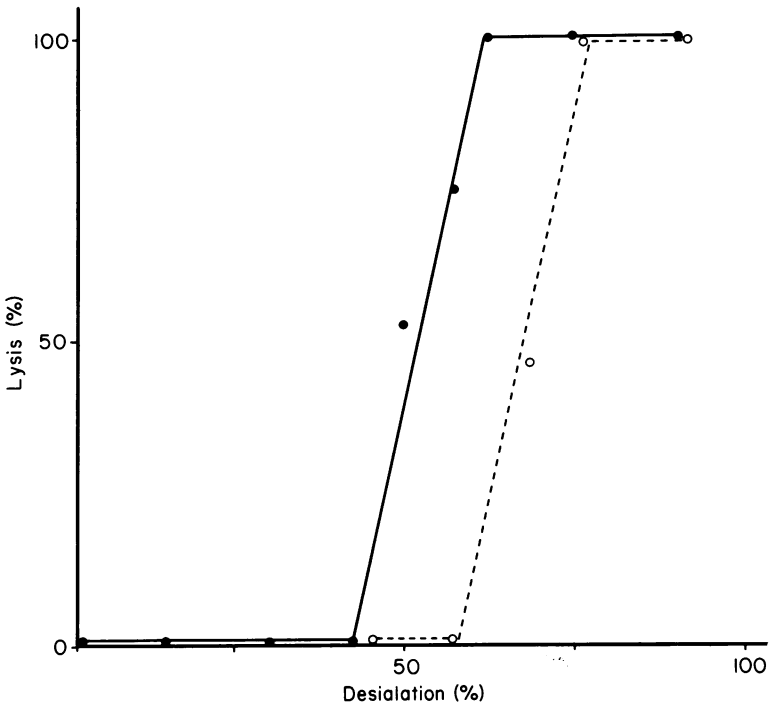

Figure 3. Correlation between lysis in pure autologous serum and the amount of sialic acid released from the cells after treatment with incremental amounts of sialidase from *Vibrio cholerae*, in two different animals.

Thus Egp-V and Egp-Nase are lysed by the autologous serum through activation of the classical complement pathway. This activation was not found if autologous sera were absorbed on treated Egp, prior to the haemolytic tests (Fig. 2).

The dose–response effect of treatment of Egp with bacterial neuraminidase on the capacity of the cells to activate the classical complement pathway was examined with incremental amounts of enzyme ranging from 0·5 to 50 units. The relative activating capacity of Egp-Nase, as assessed by the extent of lysis of the cells in autologous serum diluted 1/2 in VBS^{++}, showed a threshold of 45–55% desialation had to be reached for the haemolytic activity to appear (Fig. 3). The threshold value varied between individuals.

Fixation of antibodies on the membrane of Egp treated with neuraminidase

Because activation of the complement system occurred through the classical pathway and since preabsorption of the serum with treated Egp resulted in a loss of the haemolytic activity of the serum, the fixation of auto-antibodies on the cell membrane was examined by using the antiglobulin tests (Table 1). The ability of treated cells to fix C3 was also examined (Table 2). The results show that natural auto-antibodies of the IgM class bind to the membranes of treated Egp during incubation in autologous serum and that this leads to C3 deposition on the membrane. The IgM-dependent complement activation proceeded through the classical pathway since no C3 deposition occured in VBS-EDTA or GVB-Mg-EGTA.

Survival of treated erythrocytes *in vivo*

The clearance of enzyme-treated ^{51}Cr-labelled erythrocytes was measured in normal guinea-pigs up to 6 days after intravenous injection. The survival of untreated ^{51}Cr-labelled erythrocytes was normal, whereas treated red cells were cleared from the blood stream within 20 min (Fig. 4). No radioactivity was found in the plasma. Treated erythrocytes from a C4-deficient guinea-pig were cleared as efficiently as in normal animals.

In both cases, most of the radioactivity was found in the liver (over 85%) and some in the spleen (5–10%).

Table 1. Antiglobulin-test on neuraminidase treated or untreated guinea-pig erythrocytes incubated in autologous serum

Antiserum	Anti-IgG (1+2)	Anti-IgG 1	Anti-IgM	Anti-albumin
Untreated Egp+autologous serum	neg	neg	neg	neg
Egp-Nase+buffer	neg	neg	neg	neg
Egp-Nase+autologous serum	±*	neg	+++*	neg
Egp-Nase+absorbed autologous serum†	neg	neg	neg	neg

*After incubation with autologous serum diluted 1/20, the reaction was positive up to a dilution of 1/160 with anti-IgG (1+2) and up to 1/5120 with anti-IgM.

†The autologous serum had been absorbed with neuraminidase-treated erythrocytes for 1 hr at 2°.

Table 2. C3 deposition on the membrane of neuraminidase-treated cells during incubation with autologous serum diluted 1/300

Egp-NASE incubated in	Autologous serum in			
	Buffer	VBS^{++}	GVB-Mg-EGTA	EDTA	Preabsorbed autologous serum
Anti-C3	neg	+++*	neg	neg	neg

* The reaction was positive up to a dilution of 1/5120 with anti-C3 antiserum.

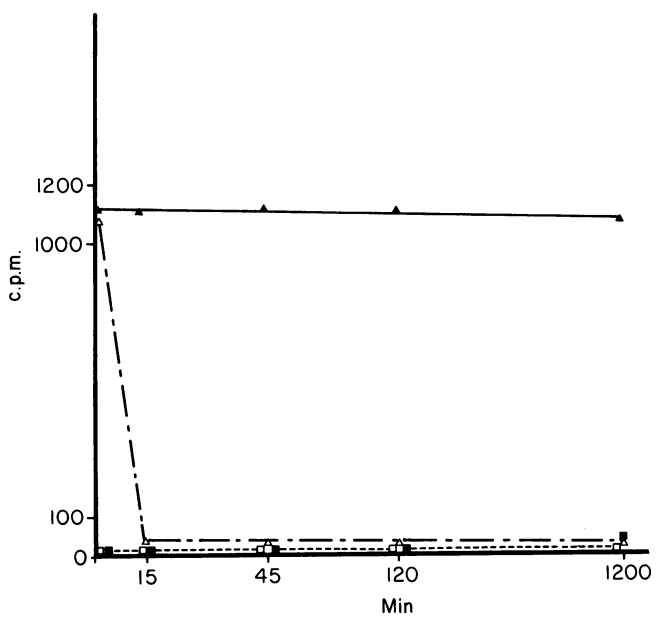

Figure 4. Survival of ^{51}Cr-labelled erythrocytes with (open symbols) or without (closed symbols) prior treatment with sialidase. Radioactivity (c.p.m./ml) in erythrocytes (triangles) or plasma (squares). Semilogarithmic plot.

DISCUSSION

Guinea-pig erythrocytes treated with influenza virus or with neuraminidase from *Vibrio cholerae* are lysed during incubation in autologous serum at 37° in the presence of Ca^{++} and Mg^{++}. In contrast, they are not lysed in the absence of both ions or of the single Ca^{++}, or if the serum had been previously heated to 56°. These results suggested an activation of the classical complement pathway that was confirmed by the observation that a C4-deficient serum was not able to lyse the autologous treated red cells unless pure C4 was added.

In contrast, treated Egp were not lysed in autologous serum preabsorbed with treated cells, indicating that activation of the classical complement pathway resulted from a fixation of antibodies on the cell membrane during incubation.

Activation of the classical pathway by virus-treated cells was not dependent on the presence of viral particles bound to the erythrocyte membrane, since no residual particles could be seen upon scanning electron microscopic examination of the Egp-V after viral elution (Lambré *et al.*, 1982). Furthermore, guinea-pig sera were examined by ELISA (Lambré & Kasturi, 1979) for the presence of anti-influenza antibodies, but none were found.

It has previously been shown that in a heterologous system, treated Egp activates the alternative pathway of human complement, in an antibody-independent way (Lambré & Thibon, 1980), we have now demonstrated that Egp activate the autologous classical complement pathway through an antibody-dependent reaction.

The ability of neuraminidase-treated Egp to activate the classical pathway presented a threshold in that 45–55% desialation was necessary before any activation was seen. With further desialation, activity increased very rapidly and reached a plateau when total desialation was obtained. The presence of a threshold in the relationship between the ability of the cells to activate complement in autologous serum and the extent of desialation is a second difference from the reaction in heterologous serum where a direct correlation without any threshold was observed between desialation and activating capacity.

These findings suggested that activation of the classical pathway by Egp-V or Egp-Nase was dependent on virus- or enzymatic-induced desialation of the cells and on the subsequent fixation of naturally occurring auto-antibodies on antigenic sites unmasked by the treatment. Furthermore, the presence of a threshold in the relative desialation that had to be reached for the activating capacity to occur could explain why in some cases we did not observe any lysis of virus-treated cells. In fact while bacterial sialidase removed up to 100% of the thiobarbituric assay titrable sialic acid from Egp membranes because it cleaves 2-3, 2-6 and 2-8 linkages of N-acetylneuraminic acid to carbohydrates, viral sialidase, because its specificity is restricted to 2-3 and 2-8 linkages (Rosenberg & Schengrund, 1976), removed only approximately 50% of the sialic acid from Egp membranes (Lambré *et al.*, 1982). Thus it is possible that in some individuals the relative desialation obtained after viral treatment was below the threshold value, making the lytic reaction impossible.

In vivo experiments have shown that in the guinea-pig, as in numerous animal species already studied (dogs, rats, rabbits, humans) neuraminidase-induced (Cattegno, Bladier & Cornillot, 1974; Durocher, Payne & Conrad, 1975; Bell *et al.*, 1977) or virus-induced (Stewart, Petenyi & Rose, 1954; Gardner, Wright & Williams, 1961) desialation, considerably shortened the red cells' half-life. It was interesting to notice that C4-deficient guinea-pigs were as able as normal guinea-pigs to clear treated red cells very rapidly from the bloodstream indicating that intravascular lysis was not the main mechanism responsible for *in vivo* clearance of the desialated cells. The fact that the radioactivity was found mainly in the liver could indicate that treated cells were recognized by Kuppfer cells via their Fc receptor and thus trapped in the liver. We demonstrated that IgG and mainly IgM were fixed on the membrane of desialated cells after incubation in the autologous serum. This fixation of IgM seems similar to the fixation of T-agglutinin on the Thomsen-antigen unmasked on human red cells by sialidase treatment (Vaith & Uhlenbruck, 1978), and fixation of IgG on desialated human red cells has also been described (Kay, 1978).

Desialation of the membrane induces a reaction of the host leading to the fixation of auto-antibodies on the membrane, then lysis via an activation of the autologous classical complement pathway *in vitro*, and to *in vivo* clearance from the bloodstream. Thus desialation represents an alteration of the cell membrane leading to the elimination by the host of this cell after it has been exposed to myxoviruses.

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REFERENCES

BELL W.C., LEVY G.N., WILLIAMS R. & AMINOFF D. (1977) Effect of galactose oxidase, with and without prior sialidase treatment, on the viability of erythrocytes in circulation. *Proc. natn. Acad. Sci. U.S.A.* **74,** 4205.

BRAND C.M. & SKEHEL J.J. (1972) Crystalline antigen from the influenza virus envelop. *Nature New Biology*, **238,** 145.

BUSSE W.W., COOPER W., WARSHAUER D.M., DICK E.L., WALLOW I.H.L. & ALBRECHT R. (1979) Impairment of isoproterenol, H2 histamine, and prostaglandin E1, response of human granulocytes after incubation *in vitro* with live influenza vaccines. *Am. Rev. respir. Dis.* **119,** 561.

CABEZAS J.A., CALVO P., EID P., MARTIN J., PEREZ N., REGLERO A. & HANNOUN C. (1980) Neuraminidase from influenza A virus (H3N2) specificity towards several substrates and procedure of activity determination. *Biochim. biophys. Acta*, **616,** 228.

CATTEGNO L., BLADIER D.Q. & CORNILLOT P. (1974) The role of sialic acid in the determination of survival of rabbit erythrocytes in the circulation. *Carbohyd. Res.* **34,** 361.

DESAI U., KREUTZER D.L., SHOWELL H., ARROYAVE C.V. & WARD P.A. (1979) Acute inflammatory pulmonary reactions induced by chemotactic factors. *Am. J. Pathol.* **96,** 71.

DUROCHER J.R., PAYNE R.C. & CONRAD M.E. (1975) Role of sialic acid in erythrocyte survival. *Blood*, **45,** 11.

GARDNER E. JR, WRIGHT C.S. & WILLIAMS B.Z. (1961) The survival of virus-treated erythrocytes in normal and splenectomized rabbits. *J. Lab. clin. Med.* **58,** 743.

HICKS J.T., ENNIS F.A., KIM E. & VERBONITZ M. (1978) The importance of an intact complement pathway in recovery from a primary viral infection: influenza in decomplemented and in C5-deficient mice. *J. Immunol.* **121,** 1437.

HIRSCH R.L., WINKELSTEIN J.A. & GRIFFIN D.E. (1980) The role of complement in viral infections. III. Activation of the classical and alternative complement pathways by Sindbis virus. *J. Immunol.* **124,** 2507.

HIRSCH R.L., GRIFFIN D.E. & WINKELSTEIN J.A. (1981) Host modification of Sindbis virus sialic acid content influences alternative complement pathway activation and virus clearance. *J. Immunol.* **127,** 1740.

HIRST G.K. (1942) The quantitative determination of influenza virus and antibodies by mean of red cell agglutination. *J. exp. Med.* **75,** 49.

JOHNSON D.J. & WARD P.A. (1974) Acute immunologic pulmonary alveolitis. *J. clin. Invest.* **54,** 349.

KAY M.M.B. (1978) Role of the physiologic autoantibody in the removal of senescent human red cells. *J. supramol. Struct.* **9,** 555.

LAMBRÉ C.R. & KASTURI K. (1979) A microplate immunoassay for anti-influenza antibodies. *J. immunol. Meth.* **26,** 61.

LAMBRÉ C.R. & KASTURI K. (1979) A microplate immunoassay for anti influenza antibodies. *J. immunol. Meth.* **26,** 61.

LAMBRÉ C.R., KAZATCHKINE M.D., MAILLET F. & THIBON M. (1982) Guinea-pig erythrocytes, after their contact with influenza virus, acquire the ability to activate the human alternative complement pathway through virus induced desialation of the cells. *J. Immunol.* **128,** 629.

LAMBRÉ C.R. & THIBON M. (1980) Ability to activate the alternative complement pathway acquired by human and guinea-pig erythrocytes after contact with influenza virus. *Ann. Immunol. (Institut Pasteur)*, **131C,** 213.

LARSEN G.L., MITCHELL B.C. & HENSON P.M. (1981) The pulmonary response of C5 sufficient and deficient mice to immune complexes. *Am. Rev. respir. Dis.* **123,** 434.

LOOS M. & BRUNNER H. (1979) Complement components (C1, C2, C3, C4) in bronchial secretions after intranasal infection of guinea pigs with Mycoplasma pneumoniae: dissociation of unspecific and specific defence mechanisms. *Infect. Immun.* **25,** 585.

MCCARTHY K. & HENSON P. (1979) Induction of lysosomal enzyme secretion by alveolar macrophages in response to the purified complement fragments C5a and C5a des-arg. *J. Immunol.* **123,** 2511.

MCCONNELL I., GORMAN N.T., RANIWALLA J. & POWELL P. (1981) Activation of the alternative complement pathway by normal and transformed cells in homologous and heterologous sera. *Eur. J. Immunol.* **11,** 126.

MCSHARRY J.J., PICKERING R.J. & CALIGUIRI L. (1981) Activation of the alternative complement pathway by enveloped viruses containing limited amounts of sialic acid. *Virology*, **114,** 507.

NELSON R.A., JENSEN J., GIGLI I. & TAMURA N. (1966) Methods for the separation, purification and measurement of the nine components of hemolytic complement of guinea pig serum. *Immunochemistry*, **3,** 111.

NYDEGGER U.E., FEARON D.T. & AUSTEN K.F. (1978) The modulation of the alternative pathway of complement in C2-deficient human serum by changes in concentration of the component and control proteins. *J. Immunol.* **120,** 1204.

OGAWA H., KUNKEL S.L., FANTONE J.C. & WARD P.A. (1981) Comparative study of eosinophil and neutrophil chemotaxis and enzyme release. *Am. J. Pathol.* **105,** 149.

OKADA H. & OKADA N. (1981) Sendai virus infected cells are readily cytolised by guinea pig complement without antibody. *Immunology*, **43,** 337.

OLDSTONE M.B.A. & DIXON F.J. (1971) Acute viral infection: tissue injury mediated by anti-viral antibody through a complement effector system. *J. Immunol.* **107,** 1274.

PERRIN L.H., JOSEPH B.S., COOPER N.R. & OLDSTONE M.B.A. (1976) Mechanism of injury of virus infected cells by antiviral antibody and complement: participation of IgG, $F(ab')_2$, and the alternative complement pathway. *J. exp. Med.* **143,** 1027.

ROBERTSON J., CALDWELL J.R., CASTLE J.R. & WALDMAN R.H. (1976) Evidence for the presence of components of the alternative (properdin) pathway of complement activation in respiratory secretions. *J. Immunol.* **117,** 900.

ROSENBERG A. & SCHENGRUND C.L. (1976) Sialidases. In: *Biological Roles of Sialic Acid*, (eds A. Rosenberg and C. L. Schengrund), p. 295. Plenum Press, New York.

ROSSEN R.D., BUTLER W.T., CATE T.R., SZWED C.F. & COUCH R.B. (1965) Protein composition of nasal secretion during respiratory virus infection. *Proc. Soc. exp. Biol. Med.* **119,** 1169.

SHAW J.O., HENSON P.M., HENSON J. & WEBSTER R.O. (1980) Lung inflammation induced by complement derived chemotactic fragments in the alveolus. *Lab. Invest.* **42,** 547.

SMITH T.F., MCINTOSH K., FISHAUT M. & HENSON P.M. (1981) Activation of complement by cells infected with respiratory syncytial virus. *Infect. Immun.* **33,** 43.

STANLEY P., CROOK N.E., STREADER L.G. & DAVIDSON B.E. (1973) The polypeptides of influenza virus. *Virology*, **56,** 640.

STEWART W.B., PETENYI C.W. & ROSE H.M. (1955) The survival time of canine erythrocytes modified by influenza virus. *Blood*, **10,** 228.

STIMLER N.P., HUGLI T.E. & BLOOR C.M. (1980) Pulmonary injury induced by C3a and C5a anaphylatoxins. *Am. J. Pathol.* **100,** 327.

VAITH P. & UHLENBRUCK G. (1978) The Thomsen agglutination phenomenom: a discovery revised 50 years later. *Z. Imm-Forsch*, **154,** 1.

VERBONITZ M.W., ENNIS F.A., HICKS J.T. & ALBRECHT P. (1978) Hemagglutinin-specific complement dependent cytolytic antibody response to influenza infection. *J. exp. Med.* **147,** 265.

WARREN L. (1959) The thiobarbituric acid assay of sialic acid. *J. biol. Chem.* **234,** 19741.

WEBER K. & OSBORN M. (1969) The reliability of molecular weight determination by dodecyl-sulfate polyacrylamide gel electrophoresis. *J. biol. Chem.* **244,** 4406.