Immunology, 1965, **8**, 37.

The Effects of Antibodies on Cells

II. CHANGES IN THE ELECTROPHORETIC MOBILITY OF ASCITES TUMOUR CELLS TREATED WITH ANTIBODIES AND COMPLEMENT

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(*Received* 9*th December* 1963)

Summary. This communication describes the use of micro-electrophoresis in studying the changes in ascites tumour cells exposed to antibodies and complement.

Treatment of the cells with rabbit antibody led to a change in electrophoretic mobility consistent with a surface adsorption of γ-globulin. The addition of complement led to a reduction in this electrophoretic effect of antibody. Treatment of the cells with neuraminidase, which produced a marked fall in their electrophoretic mobility, did not alter the effect of rabbit antibody and complement on the cells.

Incubation with iso-antibody, in the presence or absence of complement, did not alter the mobility of the ascites cells measured at pH 7·0.

The addition of 0·01 M calcium chloride to the electrophoresis medium produced a fall in mobility of the cells exposed to either antibody preparation in the presence of complement. Therefore, although iso-antibody and complement did not produce direct changes in cell mobility, changes in the cell surface could be detected by electrophoresis in the presence of calcium ions.

The possibility is discussed that, during immune cytolysis, unmasking of phosphate groups of phospholipids might take place in the cell surface.

INTRODUCTION

Studies on the serological behaviour of bacteria (Morgan, 1949) and dissociated mammalian cells (Moller and Moller, 1962) have drawn attention to the importance of surface components as carriers of antigenic determinants. Though some antigens are undoubtedly intracellular, it is likely that antigenic properties of the surface may determine the susceptibility of a cell to immune conditions, whether mediated by humoral or by cellular mechanisms.

For particles of the size of living cells suspended in salt solutions, the mobility in an electric field depends only upon the nature of the surface and not upon the size, shape or orientation of the particle (Abramson, Moyer and Gorin, 1942). Measurement of the electrophoretic mobility of cells treated with antibody and complement may thus provide information concerning the nature of immune reactions at the cell surface; this method of observation does not subject the cell to further alteration. Recent studies by Sachtleben (1964) have shown that incubation of human erythrocytes with ABO antisera results in a reduction in the electrophoretic mobility of the cells.

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As described in a previous communication, histochemical techniques revealed that exposure of ascites tumour cells to antisera resulted in unmasking of cytoplasmic phospholipid; if complement was added, this change also appeared to involve the cell surface (Dumonde, Bitensky, Cunningham and Chayen, 1965). If these reactions involved charged groups in the cell surface, then they might produce an alteration in the electrophoretic mobility of these cells. This communication describes the use of cell electrophoresis to reveal changes in the surface of ascites tumour cells exposed to antibody, in the presence or absence of complement.

MATERIALS AND METHODS

Cells and Antisera

EL4 ascites tumour cells were maintained and used for the preparation of iso-immune and immune rabbit sera by the methods described in the previous paper (Dumonde *et al.*, 1965).

Immune rabbit γ-globulin was prepared from pooled sera by precipitation at pH 6·4 in a large volume of 19·5 per cent w/v ammonium sulphate containing 2·9 per cent w/v sodium chloride at 0–4° (Friedman, 1958). The precipitated globulin was taken up in a small volume of saline and dialysed for 2 days in the cold against buffered Ringer–glucose solution. The protein concentration was adjusted to 10 mg./ml. (biuret) and aliquots of the fraction were stored at −18°. As judged by paper electrophoresis, more than 90 per cent of the protein present was γ-globulin.

Incubation Conditions for Ascites Cells

For harvesting and washing cells prior to use, and for diluting antisera and complement, a tris-buffered Ringer–glucose solution (glucose 0·01 M : tris–HCl buffer, pH 7·3, 25 mM) was regularly used. EL4 cells were washed twice in buffer and diluted to a cell density of 2×10^7 cells/ml. The percentage of viable cells before incubation was determined for each batch by counting cells diluted in 1 : 2000 Trypan Blue. Only fresh cell suspensions containing less than 5 per cent of damaged cells were used in the experiments. Determinations of electrophoretic mobility were carried out on cells that had previously been washed in the saline media used for electrophoresis.

By the use of standard conditions of incubation of cells with antisera it was possible to compare the sensitivities of serological and electrokinetic methods in detecting absorbed antibody. Volumes of 0·25 ml. of serial dilutions of sera or immune γ-globulin were added to 1 ml. aliquots of cell suspension containing 2×10^7 cells/ml. and the mixtures incubated for 15 minutes at 37°. The cells were washed twice with 10 ml. volumes of buffer or electrophoresis medium and resuspended to a volume of 1 ml. Viable cell counts were immediately done on 0·1 ml. aliquots of these suspensions. The proportion of cells stainable by Trypan Blue was usually less than 10 per cent; suspensions containing more than 10 per cent of damaged cells were discarded.

In some experiments the ascites cells were treated with neuraminidase prior to cell electrophoresis or prior to incubation with antisera. This enzyme was very kindly supplied by Dr. G. L. Ada as a highly purified freeze-dried preparation. Suspensions containing 2×10^7 cells were incubated with 300 units of enzyme* in a volume of 1·5 ml. at pH 6·2, in the presence of 1 mM $CaCl_2$ and 0·05 M glucose, at 37° for 30 minutes. The cells were

* Units of neuraminidase defined in French and Ada (1959).

then washed twice in buffer or medium before further use. By Trypan Blue testing, incubation with this enzyme was found not to impair the viability of the ascites cells. In control experiments the neuraminidase preparation was inactivated by heating to 100° for 2 hours at pH 6·0.

Cytotoxicity and Immunofluorescence Studies

Cytotoxic effects were studied by adding 0·25 ml. fresh complement (half-strength guinea-pig serum) to 1 ml. aliquots of cells that had been treated with serial dilutions of antisera and washed in buffer. After incubation at 37° for a further 30 minutes, a second viable-cell count was performed. An increase in the proportion of dead cells to more than 75 per cent of the total (a 7- to 10-fold increase) was regarded as evidence of adsorption of antibody on the cell surface. In this way the highest dilution of antibody was determined that would sensitize the cells to the subsequent action of complement. In control experiments complement was inactivated at 56° for 30 minutes.

For immunofluorescence, smears were made of washed suspensions of cells that had been similarly exposed to various dilutions of antisera. These smears were allowed to dry in air for 24 hours at room temperature and were then stained with the appropriate anti-globulin reagent conjugated with fluorescein isothiocyanate. Preparations of stained smears were examined in a fluorescence microscope as previously described (Dumonde *et al.*, 1965). In additional experiments similar suspensions of cells were incubated for 15 minutes at 37° together with antibody and complement; they were then washed twice with buffer and smears of cells prepared for immunofluorescence staining.

The results of these serological studies were expressed as 'titres' of antibody, that is, the highest dilutions of sera or immune γ-globulin which would promote the cytotoxic or immunofluorescent reactions.

Cell Electrophoresis

Effects of normal and immune sera on ascites cells were studied by observing the electrophoretic mobility of individual cells in suspension. By incubating cell suspensions with dilutions of antisera or immune globulin, it was possible to determine the highest dilution of antibody that would still produce a detectable change in cell mobility. To study the action of complement on the cell surface, 1 ml. aliquots of cell suspension were incubated for 15 minutes at 37° with 0·125 ml. each of antiserum and complement and 0·25 ml. of buffer solution. Control experiments included incubation of cells with complement alone, with immune sera and heat-inactivated complement, or with normal sera and active complement. In all experiments, treated cells were washed twice with 10 ml. of the appropriate electrophoresis medium before making measurements.

Electrophoretic measurements were made in a cylindrical chamber of the type described by Bangham, Flemans, Heard and Seaman (1958) employing grey platinum electrodes. The basic medium used had a constant ionic strength, $I = 0{\cdot}15$, comprising 0·144 M NaCl and 0·006 M KCl. This salt solution was made up in glass-distilled water from analytical grade reagents and its pH was adjusted with 0·15 M HCl or NaOH to give a series of media of pH from 4·0 to 10·0. Washed cells were resuspended in the media at concentrations of 2×10^6 cells/ml. and equilibrated at 25° before placing in the electrophoresis chamber. By reversing the direction of the electric field, measurements were made on twenty cells moving in alternate directions. From these data a mean mobility and standard deviation were calculated. The standard deviation for these cell suspensions was never greater than

0·06 μ/sec./volt/cm. and was generally 0·04 or 0·05. These values have not been put in the tables for the sake of clarity but they were constant throughout. The results of measurements of treated cells made at different pH values were conveniently expressed as pH–mobility curves.

In order to provide evidence that the observed effects of antisera were due to absorption of antibody onto the cell surface, the electrophoretic behaviour of the immune rabbit γ-globulin was studied following adsorption on silica particles. In this way the pH–mobility curve obtained for particle-adsorbed γ-globulin could be compared with that obtained by electrophoresis of cells similarly exposed to the immune γ-globulin. For this purpose the particle Gasil 35, supplied by Joseph Crosfield and Sons, Ltd., was found to be suitable both on account of its size (about 5 μ diameter) and the neutral pH of an aqueous suspension. Gasil particles were washed 8 times in the electrophoresis medium at pH 7·0 and resuspended to a density of 15 mg./ml. Volumes of 1 ml. of this suspension were incubated with 0·25 ml. amounts of the immune γ-globulin at 25° for 30 minutes; the particles were then washed twice in the electrophoresis media at pH values from 4·0 to 10·0, and resuspended at a suitable concentration for electrophoretic measurements over this range of hydrogen ion concentration. In further experiments, both cells and particles were exposed at pH 7·0 to serial dilutions of the immune globulin, washed at pH 7·0 and examined in the electrophoresis chamber. Under these conditions it was possible to determine the extent to which the effect on the mobilities of cells and particles could be reduced by diluting the γ-globulin. The results with ascites cells were also compared with the sensitivity of the serological methods in the detection of antibody adsorbed onto cell surfaces from serial dilutions of immune sera and immune γ-globulin.

As described in the preceding paper (Dumonde *et al.*, 1965) exposure of ascites cells to antibody and complement resulted in an increased availability of surface material that gave a histochemical reaction for phospholipid. If phosphate groups of phospholipid were unmasked in the cell surface, the addition of calcium ions might be expected to alter the surface charge to an extent that should be detectable by electrokinetic measurements. It was found that the electrophoretic mobilities of two particulate preparations of synthetic phospholipids, di-palmitoyl lecithin (DPL) and di-palmitoyl phosphatidyl ethanolamine (DPPE), were markedly reduced by the addition of millimolar concentrations of $CaCl_2$ (see Results and Fig. 6), a concentration of 0·01 M $CaCl_2$ at pH 7·0 having an almost maximal effect. With these Ca ion concentrations, the mobilities of mouse and human erythrocytes were, however, unaltered, their surface negative charge being due predominantly to carboxyl groups of sialic acid residues which do not enter into specific gegenion association with calcium at this concentration. Accordingly, ascites cells which had been treated with antibody and complement were electrophoresed at pH 7·0 in a medium containing 0·01 M $CaCl_2$, keeping the total ionic strength of the medium constant at 0·15.

RESULTS

The results of cytotoxicity and immunofluorescence studies carried out with rabbit antibody are presented in Table 1. The absorption of antibody by normal EL4 cells, from dilutions of immune sera or immune γ-globulin, was detected as readily by the cytotoxic effect of added complement as by fluorescent antiglobulin reagent. By both these criteria, the serological methods could detect absorption of antibody from antisera diluted

to 1:128. Furthermore, the titre obtained with the immune γ-globulin fraction (at a starting concentration of 10 mg. of protein per ml.) did not differ significantly from that of the whole immune serum.

TABLE 1

CYTOTOXIC AND IMMUNOFLUORESCENCE TITRES OF RABBIT SERA INCUBATED WITH NORMAL AND NEURAMINIDASE-TREATED EL4 ASCITES CELLS

	Cytotoxic titres			*Immunofluorescence titres*		
	Normal serum	*Immune serum*	*Immune γ-globulin**	*Normal serum*	*Immune serum*	*Immune γ-globulin**
Untreated cells	1/2	1/128	1/128	1/4	1/128	1/256
Cells + active neuraminidase	1/2	1/64	1/128	1/4	1/64	1/128
Cells + heat-inactivated neuraminidase	1/2	1/128	1/128	1/4	1/64	1/128

* Undiluted γ-globulin = 10 mg. of protein/ml.

Treatment of EL4 ascites cells with neuraminidase did not affect the ability of the cells to absorb rabbit antibody from serial dilutions of immune sera or immune globulin, as detected by immunofluorescence. In addition, the enzyme treatment did not alter the susceptibility of the EL4 cells to the cytotoxic action of rabbit antibody and complement (Table 1).

In the absence of complement, only the cell surfaces were stained for bound antibody by the immunofluorescent method (Fig. 1a). However, after 15 minutes' incubation with

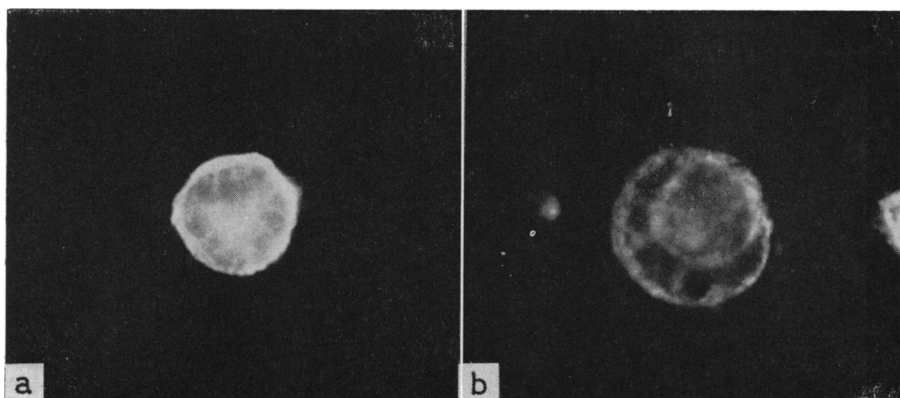

FIG. 1. Immunofluorescent reactions of EL4 ascites cells exposed to rabbit antibody and complement in suspension. ×1200. (a) EL4 cells with antibody; no complement. (b) EL4 cells incubated with antibody and complement.

antibody and complement, the surface fluorescence of many of the cells appeared to be reduced, while the interior of the cells stained brightly (Fig. 1b). It appeared that swelling of the ascites cells was accompanied by entry of antibody into their cytoplasm.

Lower titres were obtained with iso-antisera prepared against EL4 cells in BALB/c mice. The cytotoxic titres of the isoantisera were usually 1:32 whilst immunofluorescence reactions could be observed with sera diluted to 1:16.

Table 2 shows that exposure of EL4 cells to immune rabbit serum resulted in a marked decrease in their electrophoretic mobility, this change being more pronounced in acid conditions. The results are presented graphically in Fig. 2 as a pH–mobility curve, and

TABLE 2

EFFECT OF pH ON THE ELECTROPHORETIC MOBILITY OF EL4 ASCITES CELLS EXPOSED TO NORMAL AND IMMUNE RABBIT SERA IN THE ABSENCE OF COMPLEMENT

Incubation condition	*Mobility of cells (μ/sec./volt/cm.) at pH of electrophoresis*				
	4·0	5·0	7·0	9·0	10·0
Cells only; no serum	−1·07	−1·11	−1·12	−1·13	−1·24
Cells+normal serum	−1·10	−1·12	−1·13	−1·14	−1·16
Cells+immune serum	−0·32	−0·45	−0·62	−0·99	−1·04
Cells+immune γ-globulin	−0·33	−0·45	−0·62	−0·91	—

it is also shown that exposure of EL4 cells to normal rabbit serum did not result in any alteration in electrophoretic mobility. The results in Table 2 show that this effect of immune rabbit serum could be entirely reproduced by exposure of the cells to immune γ-globulin.

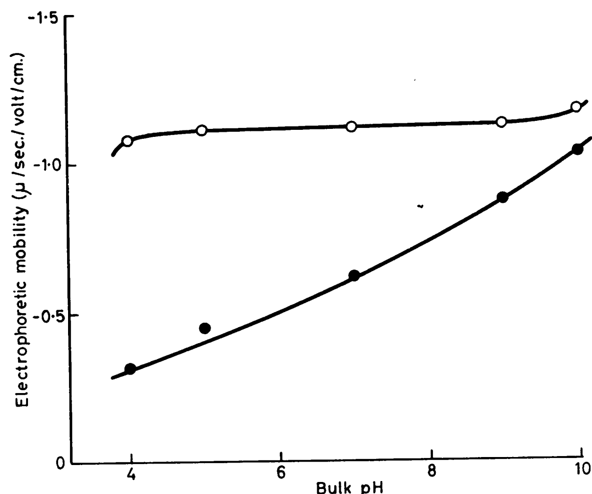

FIG. 2. pH–mobility curves of normal EL4 cells (○) and of cells exposed to rabbit antiserum in the absence of complement (●).

Fig. 3 shows the results of electrophoresing bare and γ-globulin-coated silica particles. The isoelectric point of the adsorbed γ-globulin preparation was found to lie between pH 4·5 and 4·7. This value, which is lower than that for γ-globulin in free solution, may have resulted either from changes in the protein charge consequent on adsorption to a polar surface (Abramson *et al.*, 1942) or from traces of α- and β-globulins present in the protein fraction. Incubation of the particles with the γ-globulin fraction resulted in an alteration of their electrophoretic behaviour which obscured the large change in mobility that occurred between pH 6·0 and 8·0 for uncoated silica particles. It was notable that the pH–mobility curve of the coated silica particles bore a qualitative resemblance to the curve obtained for ascites cells treated with rabbit antibody.

In Fig. 4 it is shown that the effect of the immune globulin on both cells and particles at pH 7·0 could be similarly reduced by diluting the γ-globulin. Under the conditions of these incubations, this reduction of effect on both cells and particles occurred over a similarly narrow range of globulin dilutions. The highest dilution of immune globulin at

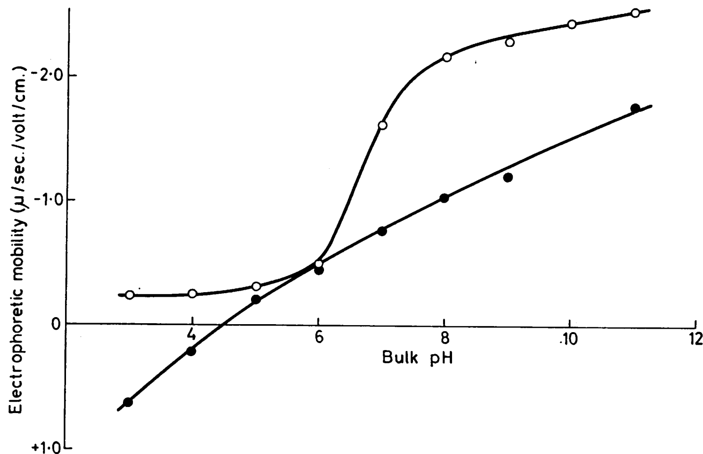

FIG. 3. pH–mobility curves of silica particles before (○) and after coating with the immune rabbit γ-globulin (●).

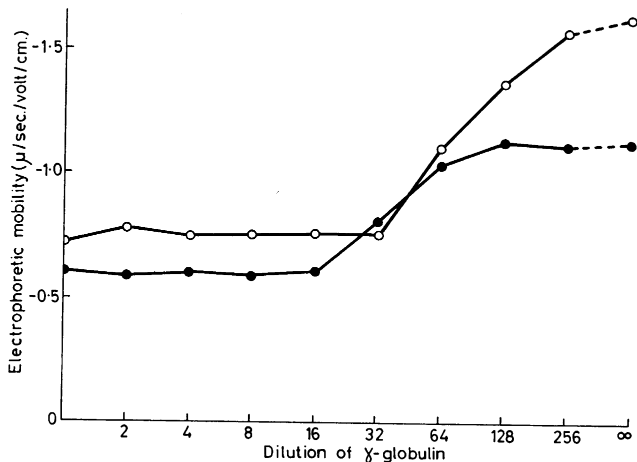

FIG. 4. Electrophoretic mobilities at pH 7·0 of EL4 cells (●) and silica particles (○) exposed to similar dilutions of immune γ-globulin.

which an effect on cell mobility could just be detected was 1:64. Comparison of this result with those in Table 1 reveals that the electrophoretic method was as sensitive as the serological methods in detecting rabbit antibody adsorbed on to ascites cells.

It was concluded from these studies that the effect of immune rabbit sera on the electrophoretic mobility of EL4 ascites cells could be adequately explained by the adsorption of γ-globulin onto the cell surface. Five washings in the suspending medium failed to reverse or to reduce this effect on the cell mobility. At decreased concentrations of

γ-globulin, the magnitude of the effect on cell mobility was related to the concentration of γ-globulin presented to the cell and presumably depended on the concentration of γ-globulin molecules adsorbed at the cell surface.

In Fig. 5 the pH–mobility curve for cells incubated with normal rabbit serum is compared with the curves for cells treated with immune globulin, in the presence or absence of complement. Heat-inactivated complement produced no effect on the cell mobility. The results of these studies show that the presence of active complement reduced the magnitude of the change in mobility that had been caused by the interaction of rabbit

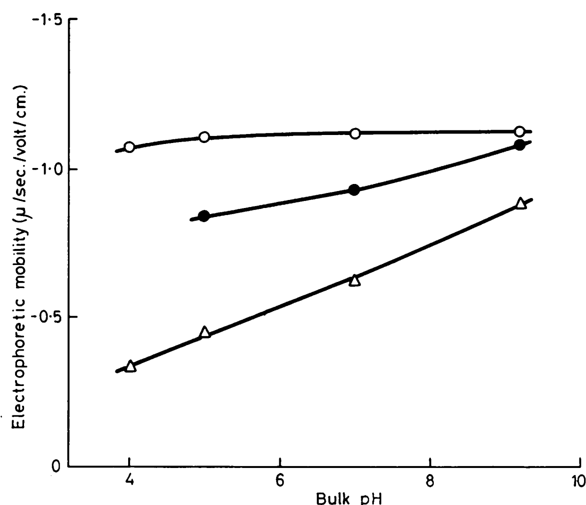

FIG. 5. pH–mobility curves of EL4 cells exposed to normal rabbit serum (○), immune rabbit γ-globulin (△), and immune γ-globulin together with active complement (●).

antibody with the cell surface. The immunofluorescence findings (Fig. 1b) indicated that under these conditions antibody had entered the cells. If the γ-globulin concentration at the cell surface was a major factor in determining the electrophoretic mobility of antibody-treated cells, then loss of antibody from the cell surface might have partly accounted for the observed effects of added complement.

The addition of 0·01 M $CaCl_2$ to the electrophoresis medium at pH 7·0 caused a marked reduction in the mobility of cells that had been exposed to rabbit antibody and complement (Table 3). No such effect of calcium was observed when complement alone had been used; normal sera and complement also gave no effect. A similar concentration of $CaCl_2$ markedly reduced the effective charge of phospholipid particles in suspension (Fig. 6), this being observed both with phosphatides of choline and ethanolamine. It was of interest that similar concentrations of $CaCl_2$ had no effect on the electrophoretic mobility of human erythrocytes (Fig. 6). The dominant ionogenic species at the surface of the human erythrocyte is the carboxyl group of a bound N-acetylated neuraminic acid (Cook, Heard and Seaman, 1961); the present experiments indicate that there was very little interaction with calcium, at the concentrations employed.

The effect of added calcium on the mobility of phospholipid particles was presumably due to ionic interaction between calcium and phospholipid phosphate. The results of these experiments suggested that an action of complement involved unmasking of

phosphate groups of phospholipid in the surface of EL4 ascites cells treated with rabbit antibody.

In the iso-immune system it was found that incubation of EL4 cells with iso-antisera at pH 7·0 produced no change in electrophoretic mobility (Table 3), though the serological results indicated that iso-antibody was indeed present on the cell surface. The addition

TABLE 3

EFFECT OF ADDED CALCIUM IONS (0·01 M) ON THE ELECTROPHORETIC MOBILITY OF EL4 CELLS TREATED WITH ANTISERA IN THE PRESENCE AND ABSENCE OF ACTIVE COMPLEMENT

Incubation condition	*Electrophoretic mobility at pH 7·0*		*Change in mobility with Ca^{++}*
	No added Ca	*Added Ca (0·01 M)*	
Normal cells: no serum	−1·12	−1·11	nil
Cells + rabbit antiserum + heat-inactivated complement	−0·62	−0·58	nil
Cells + rabbit antiserum + active complement	−0·93	−0·53	0·40
Cells + iso-antiserum + heat-inactivated complement	−1·11	−1·11	nil
Cells + iso-antiserum + active complement	−1·11	−0·90	0·21

of complement to the system likewise produced no change in mobility, despite adequate evidence of a cytotoxic effect. However, the addition of 0·01 M $CaCl_2$ to the electrophoresis medium induced a significant reduction in the mobility of cells treated with iso-antibody and complement (Table 3). The mobility fell from −1·11 to −0·90, this change (0·21 μ/sec./volt/cm.) being about one half of that observed in the hetero-immune system (0·40 μ/sec./volt/cm.). It appeared that although the concentration of iso-antibody

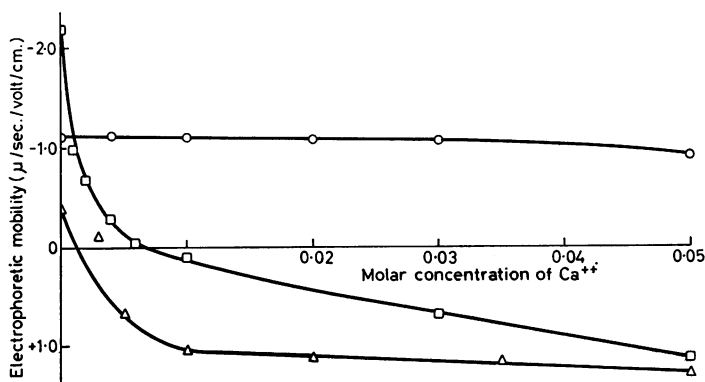

FIG. 6. Effect of increasing the ionic concentration of calcium on the electrophoretic mobility of human erythrocytes (○) and of phospholipid particles in suspension: di-palmitoyl lecithin (□); di-palmitoyl phosphatidyl ethanolamine (△).

molecules was relatively low at the cell surface, the cytotoxic action of added complement involved a similar unmasking of phosphate groups, as had been suggested by the studies with rabbit antibody and complement.

Incubation of EL4 cells with neuraminidase resulted in a marked decrease in their electrophoretic mobility measured at pH 7·0, from −1·12 to −0·71. However, the pH-mobility curve of these cells revealed no change in mobility over the range pH 4 to 9;

in addition, the presence of 0·01 M $CaCl_2$ in the medium at pH 7·0 was without effect on the mobility. From these experiments it seemed likely that apart from the loss of the outermost sialic acid residues of the cell, the surface of neuraminidase-treated cells was not further damaged. The results in Table 1 show that this effect of neuraminidase did not alter the ability of the EL4 cells to react with the rabbit antibody. It was also found that incubation of the neuraminidase preparation at 100° for 2 hours, at pH 6·0, was sufficient to nullify its effect on the surface charge of the ascites cells.

DISCUSSION

These experimental findings show that the incubation of ascites tumour cells with antibody and complement results in cell surface changes which can be detected by the technique of cell micro-electrophoresis.

All mammalian cells that have so far been examined have been found to carry a net negative charge under physiological conditions. This electrical charge has been variously attributed to phosphate groups of membrane phospholipids and to carboxyl groups arising from acidic proteins at the cell surface (Bangham, Pethica and Seaman, 1958). However, several authors have recently demonstrated that sialic acid groups play an important part in determining the surface charge of many mammalian cells (Eylar, Madoff, Brody and Oncley, 1962; Ruhenstroth-Bauer, Fuhrmann, Kubler, Rueff and Munk, 1962). In the case of the human erythrocyte its mode of linkage in the surface ultrastructure has been partly elucidated (Cook, 1962). Their contribution has been demonstrated in a variety of mouse ascites tumour cells and for hamster fibroblasts in culture (Forrester, 1964).

The electrical properties of the cell surface depend not only on the magnitude of the net surface charge but also on the relative contributions that different types of charge group make to the total. Thus the effects of added materials on the electrophoretic behaviour of any cell type may be due to alterations in the relative contributions of the groups already present in the cell surface, as well as to the electrical properties of the added material itself. In media of physiological ionic strength, significant contributions to the surface charge will be made by groups situated within an outer zone of the cell some 10 Å in depth (Heard and Seaman, 1960). Cell electrophoresis may therefore be expected to yield information concerning this outer zone.

The presence of charged sialic acid groups in the surface of EL4 ascites cells was shown by the effect of treatment with neuraminidase in reducing the electrophoretic mobility of the cells by 35 per cent. The addition of $CaCl_2$ (0·01 M) to the electrophoresis medium was without effect on the mobility of normal and of neuraminidase-treated cells. This suggested that phosphate groups of membrane phospholipids were completely masked by other surface materials in these cells. The residual charge after treatment with neuraminidase may be partly due to sialic acid groups in a linkage with their parent macromolecules which is not susceptible to hydrolysis by the enzyme (see Klenk and Gielen, 1963).

Incubation with rabbit antisera led to the adsorption of antibody on the cells, which were then sensitive to the cytotoxic effect of added complement; the immunofluorescent technique showed that in the absence of complement the antibody was localized at the cell surface and did not appear to enter the intact cell. The effect of rabbit antisera on cell mobility appeared to be largely determined by the γ-globulin on the surface, for similar variations of mobility with pH were obtained with rabbit γ-globulin adsorbed onto silica particles. However, although the added protein was having a major influence on the

surface charge of these cells, their pH–mobility curve did not entirely coincide with that for γ-globulin adsorbed on silica particles. It appeared that some of the fixed negative charge of the cell surface was still influencing the electrophoretic mobility of the treated cells. The presence of 0·01 M calcium ion in the electrophoresis medium had no effect on the mobility of these treated cells and it appeared that whatever membrane rearrangements or dislocations had taken place subsequent to reaction with the antisera, they had not resulted in phosphate groups becoming involved in the generation of the zeta-potential.

The addition of complement to cells incubated with rabbit antibody led to a reduction in the change in electrophoretic mobility caused by antibody alone. In addition the immunofluorescent technique revealed that during immune swelling of the cells, antibody had entered their cytoplasm. The reduction by complement of the effect of antibody on mobility may thus have been partly due to a fall in the surface concentration of γ-globulin on the swollen cells. However, a further effect of complement was demonstrated by electrophoresis of these cells in the presence of 0·01 M $CaCl_2$ at pH 7·0; this produced a fall in mobility of about 0·40 μ/sec./volt/cm. indicating that calcium was being strongly bound at the surface of shear. Similar changes were observed when electrophoresing suspensions of synthetic phospholipids in calcium-containing media (Fig. 6) and this suggested that the combined action of antibody and complement on the cell led to an unmasking of phosphate groups of membrane phospholipids so that they were now capable of influencing the surface charge.

It was of interest that cells which had been treated with neuraminidase were unaltered in their serological reactivity towards rabbit antibody and complement, though this enzyme treatment led to a marked reduction in net surface charge. It appeared that the outermost sialic acid residues on the ascites cell were not exclusively involved in the attachment of rabbit antibody to the cell surface.

Incubation of cells with iso-antisera led to uptake of iso-antibody by ascites cells, which could be demonstrated by immunofluorescence and by the sensitivity of the treated cells to the cytotoxic action of complement. However, no alteration of the electrophoretic mobility of these cells was observed; the addition of complement, though producing immune lysis, likewise caused no detectable change in mobility. Compared with rabbit antisera, the inability of iso-antisera to effect a change in cell mobility may have been partly due to a lower concentration of reactive sites at the cell surface, or to the lower serological titres of the iso-antisera. The charge on mammalian cells is rather sparse; in the case of the EL4 ascites cells it is about one electronic charge per 1000 $Å^2$. It is therefore quite possible for molecules of antibody to react with regions of the cell surface which do not carry the charge. A similar suggestion has been made by Sachtleben (1964) to explain the inability of Rh antisera to affect the electrophoretic mobility of human erythrocytes.

However, when calcium ion (0·01 M) was added to the electrophoresis medium at pH 7·0 the mobility of cells treated with iso-antibody and complement fell by 0·21 μ/sec./volt/cm. It thus appears that a similar reaction to complement was involved in both hetero-immune and iso-immune cell lysis; these findings agree with the histochemical observations previously reported (Dumonde *et al.*, 1965). It is concluded that immune cytolysis could result in a rearrangement of charged groups in the cell surface without any gross effect on the magnitude of the net surface charge.

The findings reported in this communication extend current interest in the surfaces of cells participating in immune reactions and demonstrate the usefulness of cell electrophoresis in the investigation of these events.

ACKNOWLEDGMENTS

We are greatly indebted to Professor A. Haddow, Professor E. G. L. Bywaters and Dr. L. E. Glynn for their encouragement and advice. We are grateful to Dr. G. L. Ada of the Walter and Eliza Hall Institute, Melbourne, for a gift of crystalline neuraminidase, and to Dr. J. R. Batchelor and the late Dr. P. Gorer for providing us with mice and tumour. Mrs. C. M. Walter gave valuable technical assistance.

This investigation has been supported by grants to the Chester Beatty Research Institute from the Medical Research Council, the British Empire Cancer Campaign, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

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