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Electrophoretic Mobilities of Antigen-Stimulated Lymph Node Cells

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Summary. The effect of immunization against a bacterial antigen on electrophoretic mobility of lymph node cells has been studied.

Incubation of non-stimulated lymph node cells with antigens altered their electrophoretic mobility. However, on washing the antigen away, the original mobility was regained.

Antigen-stimulated lymph node cells had a lower electrophoretic mobility. It was further irreversibly reduced on incubation with the same antigen. This suggests the possibility of formation of firm antigen–antibody complexes at the cell surface.

Implications of these findings and feasibility of using the technique of cell electrophoresis for the study of immune response at the cellular level is discussed.

INTRODUCTION

In recent years it has been demonstrated that cells in suspension migrate in a characteristic and reproducible pattern when placed in an electric field. This property has been developed into a refined technique of cell electrophoresis and has been applied in a variety of investigations, including serology (Forrester, Macpherson and Ambrose, 1962; Forrester, Ambrose and Stoker, 1964; Ruhenstroth-Bauer, Straub, Sachtleben and Fuhrmann, 1961; Stein, Seaman and Heard, 1962). The characteristic mobility of the cells is dependent on the charge group densities on the cell membrane, and any change in these charged groups results in an alteration in the mobility pattern of the cells. Thus the specificity of the electrophoretic mobility has become a valuable investigational tool in the study of alterations in cell membranes during biological processes.

There has been a growing interest in studies related to the elaboration of antibodies by single cells, and techniques have been developed to demonstrate intra- and pericellular antibodies at the single cell level (Reiss, Mertens and Ehrich, 1950; Mäkelä and Nossal, 1961). The demonstration of bacterial immobilization by lymph node cells immunized against the specific bacterial antigen (Nossal and Lederberg, 1958; Nossal, 1958) directed our attention to the possibility of the cell surface carrying antibodies and thus modifying the charge density of the cell membrane. The present studies were undertaken to determine the change in the surface and hence the electrophoretic mobility pattern of lymph node cells from animals immunized against bacterial antigens (hereinafter referred to as 'antigens' for brevity).

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MATERIALS AND METHODS

(i) *Effect of TAB antigen on unsensitized lymph node cells*

Cells from axillary lymph nodes of 6-week-old Wistar rats were teased out in balanced physiological saline containing 0·13 M sodium chloride and 0·01 M potassium chloride. The cells were washed twice and re-suspended in the same medium. Two aliquots of the suspension were incubated with a 1:1000 dilution of a commercial preparation of TAB antigen* for 30 minutes at 37°. One of these was later washed twice with balanced saline to remove the added antigen and the cells re-suspended in the original medium. The third aliquot was incubated without the addition of the antigen. The electrophoretic mobilities of all three aliquots were measured under identical conditions. The percentage of plasma cells in the suspension was determined microscopically.

TAB and *Vibrio cholerae* were chosen for this work because they were known to be strong antigens and it was most important for this study of the surface properties of sensitized lymphocytes to obtain the maximum possible stimulation. This might have been difficult with chemically defined antigens, which would have had other advantages. The electrical mobilities of the TAB and *Vibrio cholerae* preparations themselves could not be accurately determined, though both are known to be negatively charged. The commercial preparations contain both intact bacteria and soluble antigens.

(ii) *Effect of* Vibrio cholerae *antigen on unsensitized cells*

The above studies were repeated using 8-week-old animals and a 1:1000 dilution of a commercial preparation of *Vibrio cholerae* antigen† during incubation of the cells.

(iii) *Effect of TAB antigen on cells sensitized against TAB*

A batch of animals (6 weeks old) was immunized with the TAB antigen and killed during the period of maximum secondary response. Lymph node cell suspensions were prepared and studied as in Experiment (i).

(iv) *Effect of* Vibrio cholerae *antigen on cells sensitized against* Vibrio cholerae

A batch of animals (8 weeks old) was immunized with the *Vibrio cholerae* antigen and killed during the period of maximum secondary response. Lymph node cell suspensions were prepared and studied as in Experiment (ii).

(v) *Comparison between electrophoretic behaviour and antibody titre*

The results of the earlier experiments indicated an alteration in the mobility pattern of immunized lymph node cells. In order to gain further information the following experiment was undertaken. A batch of animals was immunized with TAB antigen. Starting from the day following the booster injection, animals were killed over a period of 9 days at regular intervals and the electrophoretic mobilities of the lymph node cells were determined. Antibody titres were measured in all the animals on the day of slaughter.

The antigens used are special medical preparations intended to give high antibody titres and were chosen for this particular property. As mentioned in the footnotes, they contain whole organisms besides soluble antigens.

All the measurements of electrophoretic mobilities were made using a cylindrical

* Vaccine supplied by Glaxo Laboratories (India) Pvt. Ltd, containing *Salmonella typhi* (1000 million) *Salmonella paratyphi* A (750 million) and *Salmonella paratyphi* B (500 million) per ml.

† Vaccine supplied by Bengal Immunity Co. Ltd, containing 8×10^9 organisms/ml (Inaba and Ogawa 1:1).

microelectrophoresis apparatus based on the design of Bangham, Flemens, Heard and Seaman (1958) and modified by Ambrose and Ryder (1960). The measurements were done in balanced physiological saline, using the microinjection technique. Migration times of at least thirty cells were observed for each mobility measurement, using a stop watch with an accuracy of ±0·01 seconds. The polarity of the electrodes was reversed after each observation. In general, the observations were extended to give a migration time close to 8 seconds.

RESULTS

A series of observations carried out under Experiments (i–v) are presented in Figs. 1 and 2. The circles represent the mean mobilities and the limits on either side of it extend

TABLE 1

	Incubated without antigen	Incubated with antigen	Incubated with antigen and washed
(A) Non-stimulated (6 weeks old)	1·65 ±0·067	1·23 ±0·026	1·68 ±0·060
(B) TAB-stimulated	1·416±0·021	—	0·990±0·035
(C) Non-stimulated (8 weeks old)	1·429±0·104	1·838±0·138	1·489±0·060
(D) *Vibrio cholerae*-stimulated	1·314±0·038	—	1·029±0·032

(A) and (B), antigen used TAB; (C) and (D), antigen used *Vibrio cholerae.*

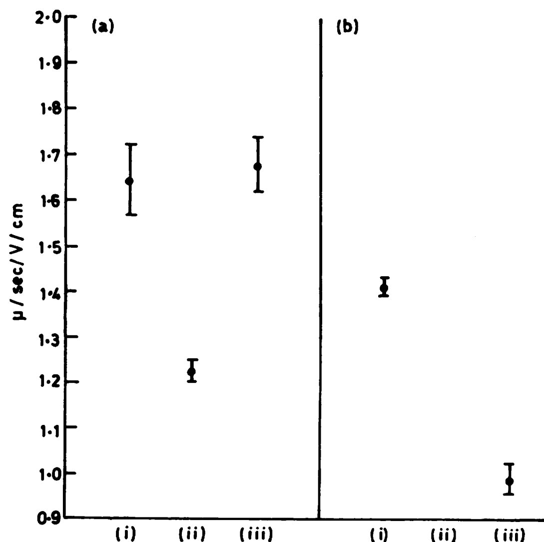

FIG. 1. Electrophoretic mobilities of (a) non-stimulated and (b) TAB-stimulated lymph node cells after the following *in vitro* treatments: (i) incubated without antigen, (ii) incubated with antigen and unwashed, and (iii) incubated with antigen and washed.

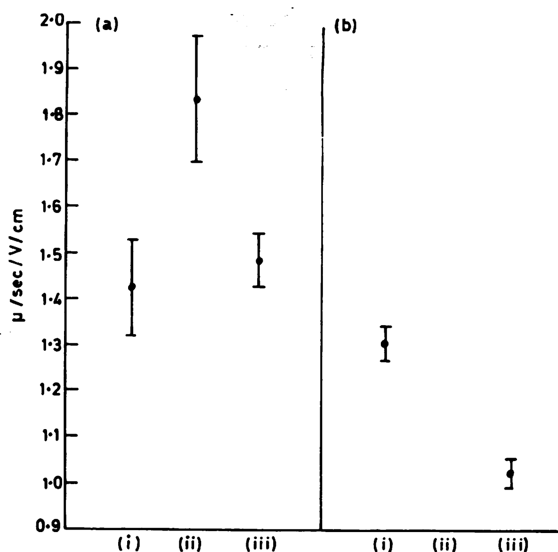

FIG. 2. Electrophoretic mobilities of (a) non-stimulated and (b) *Vibrio cholerae*-stimulated lymph node cells after the following *in vitro* treatments: (i) incubated without antigen, (ii) incubated with antigen and unwashed, and (iii) incubated with antigen and washed.

over twice the standard error. The mobilities are given in μ/sec/V/cm. The numerical values for mobilities in the different experiments are presented in Table 1.

When the sensitized cells treated with antigen were examined under a high power microscope they presented a star shaped appearance, suggesting that bacteria were adhering to the surface. In order to avoid introducing a bias in the measurements no attempt was made to identify the specific cell types affected, particularly as these are difficult to identify in the apparatus, but independent plasma cell counts were made on all samples. These ranged from 25 to 30 per cent maximum, depending on the day of secondary response. There appears to be a close correspondence between the percentage of plasma cells in the suspension and the percentage of immunized cells with an electrophoretic value lower than the minimum value observed in lymph node cells from non-immunized animals.

DISCUSSION

It has been known that the erythrocytes behave like an homogeneous population in respect to their electrophoretic mobility. The cells from the lymph nodes, however, behave like an heterogeneous population. This is not surprising since the cell population is a mixture of large, medium and small lymphocytes and plasma cells. The electrophoretic mobility of the cells is dependent on the charge densities at the cell membrane and is generally negative in sign. It has been shown that charge densities on the cell membrane alone can influence the mobility pattern (Seaman and Swank, 1963). When an antigen is added to a suspension of lymph node cells which have not been immunized against the antigen, the mobility of the cells is altered. This change is reversible in as much as the original mobilities can be restored by washing away the antigen (Table 1). This pattern of response has been shown in both situations where a TAB or a *Vibrio cholerae* antigen has been used. It appears that the antigen forms a loose coat around the cells and the net

surface charge is entirely that of the antigen. In the case of TAB-coated cells the negative charge is lowered, which in turn reduces the mobility. The *Vibrio cholerae* antigen-coated cells presumably have an excess of negative charge which results in an increase in mobility. It is likely that the carboxyl groups of the uronic acid present in appreciable amounts in *Vibrio cholerae* antigen (Shrivastava, 1961) contribute to the excess negative charge.

In contrast, lymph node cells behave differently during the peak secondary response of immunized animals. In general, these cells have a lower mobility than the corresponding controls, suggesting a reduction in the charge densities of the cell membrane. This assumption gains strength when the electrophoretic mobilities are determined after incubation with the corresponding antigen. In both cases where TAB or *Vibrio cholerae* antigen was used, a reduction in the mobility values was observed. The differences were statistically significant. In addition, such immunized cells incubated with the specific antigens do not recover their original mobilities, even on repeated washings. This indicates that firm antigen–antibody complexes are formed at the cell surface leading to an irreversible change in the charge densities at the cell membrane. It is a clear indication that the cell membrane carries the antibodies and the interaction of antigen with the antibody occurs at the cell membrane. It appears that the products of the antigen–antibody interactions are specific, when the net reduction in the mobilities in the two groups is considered. The reduction in the mobility of cells immunized against TAB, after incubation with the antigen, is 0·43 μ/sec/V/cm. The corresponding value for the *Vibrio cholerae* antigen-treated cells is 0·29 μ/sec/V/cm. The difference between the two values is statistically significant, indicating that the nature of the antigen–antibody complexes in the two cases is not identical.

It might be argued at this point that the antigens used are not very well defined chemically, and, in fact, may be a mixture of a variety of soluble antigens as well as whole organisms. However, as stated earlier, our object in undertaking this work has been to demonstrate changes in the surface of lymph node cells due to antigenic stimulation. It is precisely for this reason that antigens were chosen which would produce the maximum possible alteration in the cell surface. Pure antigens were therefore avoided as such a strong response would have been more difficult to achieve with them.

The observation that the immunized cells have a reduced mobility would lead to the suspicion that all the cells produce or carry antibodies. However, it was seen that, on an average, about 30 per cent of the immunized cells had a mobility lower than the minimum value observed in the corresponding control animals. These figures agree with the independent estimate of the percentage of plasma cells in various samples. Further, our preliminary studies indicate that the decrease in the medianal mobility values has an inverse semi-logarithmic relationship to the increase in antibody titres.

From the foregoing it is clear that the behaviour of electrophoretic mobilities of lymph node cells reflects truly the antigen–antibody responses at the cellular level. The technique of microelectrophoresis thus provides yet another approach to problems in immunology, in addition to antibody adsorption by cells carrying antigens as already described (Sachtleben, 1965; Forrester, Dumonde and Ambrose, 1965).

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