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In vitro Studies on Antibody Production by Lymph Node Cells Using Cell Electrophoresis

K. SUNDARAM, G. P. PHONDKE AND P. SUNDARESAN

Biology and Medical Groups, Bhabha Atomic Research Centre, Bombay 8, India

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Summary. Investigations were undertaken on rat lymph node cell suspensions using cell electrophoretic techniques: (a) to examine the specificity of antigen–antibody binding, (b) to determine any relationship between the electrophoretic mobility pattern and antibody production, and (c) to estimate the potentiality of a single cell to produce antibody to more than one antigen at the same time. The results, in brief, indicate that a drastic reduction in the mean mobility is caused when the immunized cells are treated with the same antigen used for immunization. These interactions have antigenic specificity. In addition, it has been shown that not all cells in a lymph node produce antibodies and the percentage of cells that produce antibodies changes from day to day. The maximum response occurs between the 4th and 5th day of the second antigenic administration. Studies with simultaneous administration of two bacterial antigens support the concept that by and large a lymph node cell produces specific antibodies to only one antigen at a time.

INTRODUCTION

In our earlier communication (Sundaram, Phondke and Ambrose, 1967), lymph node cells immunized against a bacterial antigen were shown to exhibit a consistent and reproducible decrease in the electrophoretic mobility. It was also demonstrated that, on pre-treatment with the antigen, this change was enhanced and was irreversible even after repeated washings of the cell suspension. It is recognized that any modification in the charge group densities at the cell surface brings about a change in the electrophoretic mobility (Forrester, Ambrose and Stoker, 1964). It was, therefore, suspected that in the presence of the specific antigen, immunized lymph node cells may form firm antigen–antibody complexes at the cell surface, leading to an alteration of the surface charge densities and to a corresponding change in the mobility pattern. Forrester, Dumonde and Ambrose (1965) have independently observed similar modifications of ascites tumour cells exposed to antibodies and complement. 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.

The present investigations are undertaken: (i) to examine the specificity of antigen–antibody binding, (ii) to determine any relationship between the electrophoretic mobility pattern and antibody production, and (iii) to estimate the potentiality of a single cell to produce antibody of more than one type at the same time.

MATERIALS AND METHODS

Cells from axillary lymph nodes of 6–8-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. The electrophoretic mobility of these cells was determined in a cylindrical microelectrophoresis apparatus based on the design of Bangham, Flemens, Heard and Seman (1958).

Experiment 1

A batch of eight animals was immunized with daily injections of 0·2 ml of TAB antigen* for 3 days followed by another injection of 0·2 ml of TAB after an interval of 15 days in both the front footpads. The animals were killed after 5 days following the last injection and the axillary lymph node cell suspensions were prepared separately from each animal. The cell suspensions were divided into three aliquots. The electrophoretic mobility of the lymph node cells in the first aliquot was determined without any further treatment. A 1:1000 dilution of TAB antigen was added to aliquot 2 and 1:1000 dilution of Vibrio Cholera antigen (VC)† was added to aliquot 3. After incubation at room temperature for 1 hour, the cell suspensions in aliquots 2 and 3 were washed three times with physiological saline and the electrophoretic mobilities were determined.

Experiment 2

A batch of eight animals was immunized with VC antigen. The immunization schedule and killing times were similar to Experiment 1. However, aliquot 2 was incubated with 1:1000 dilution of VC antigen and aliquot 3 with 1:1000 dilution of TAB antigen. The cells were washed three times in physiological saline and the electrophoretic mobilities of the cell suspensions in each of the aliquots were analysed.

Experiment 3

A batch of fifteen animals was immunized with TAB antigen. Beginning from the 4th day, three animals were killed on the 4th, 5th, 6th, 8th and 9th day after the last injection. The cell suspension from each animal was divided into two aliquots. One aliquot was incubated with TAB and the other with VC antigen as in Experiment 1. All the cell suspensions were washed three times with physiological saline prior to determining the electrophoretic mobilities. The antibody titre of the serum was measured in all the animals on the day of killing.

Experiment 4

The design of experiments was similar to Experiment 3 but VC antigen was used for immunization.

Experiment 5

A batch of twelve animals was immunized simultaneously with TAB and VC antigens and three animals were killed on the 5th, 6th, 7th and 10th day after the last injection. The cell suspension from each animal was divided into three aliquots. Aliquot 1 was

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

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

incubated with TAB antigen, aliquot 2 with VC antigen and aliquot 3 with a mixture of TAB and VC antigens. The cell suspensions were washed thoroughly before the electrophoretic mobilities were determined.

RESULTS

The results of Experiments 1 and 2 are presented in Fig. 1. The values represent the mean and the 95 per cent confidence limits of the mean. It is observed that in both the

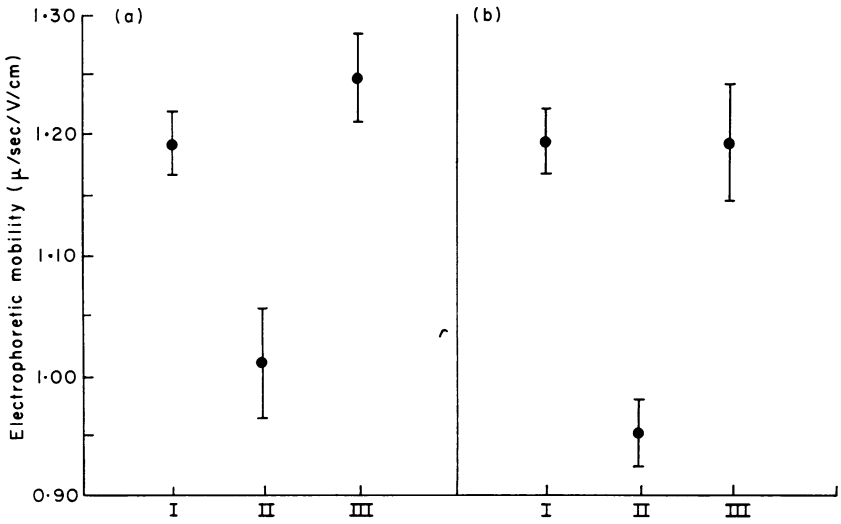

FIG. 1. Mean and 95 per cent confidence limits of the mean electrophoretic mobility values of immunized lymph node cells both before and after incubation with the specific (right) antigen and non-specific (wrong) antigen. (a) TAB stimulated, (b) VC stimulated. I = control; II = right antigen; III = wrong antigen.

situations, a drastic reduction in mobility occurs only when the immunized cells are incubated with the appropriate antigen. These results indicate that the reduction in mobility is specific and is a consequence of the fixing of the antigen by the cells that carry the specific antibody. Our previous studies (Sundaram *et al.*, 1967) have shown that under similar experimental conditions, non-immunized lymph node cells do not bind either TAB or VC antigens.

The results of Experiments 3 and 4 are presented in Table 1. It will be observed that in the TAB system, the mean mobility values show a progressive reduction from the 4th day after the last injection and gradually tend to approach the control value after the 9th day. In the VC system the maximum depression occurs between the 5th and 6th days. In addition, it is observed that the reduction in mobility is brought about only when these cells are incubated with the specific antigen used for immunization.

Fig. 2 represents the relationship of the decrease in electrophoretic mobility, the antibody titres and the computed percentage of antibody containing cells on various days after antigen administration. For purposes of computing the percentage of antibody containing cells on a given day of response, the individual values in the sample are compared with the mean value of unstimulated lymph node cells. All values which fall within two

TABLE 1

ELECTROPHORETIC MOBILITY VALUES EXPRESSED IN μ/sec/V/cm OF ANTIGEN STIMULATED LYMPH NODE CELLS INCUBATED WITH SPECIFIC ANTIGENS (ALL THE MOBILITY VALUES CARRY A NEGATIVE SIGN)

Stimulation	Incubation	4th day	5th day	6th day	8th day	9th day
TAB	TAB	1·116 ±0·028	1·011 ±0·045	1·029 ±0·027	1·040 ±0·049	1·179 ±0·048
	VC	1·335 ±0·029	1·245 ±0·042	1·208 ±0·054	1·193 ±0·032	1·170 ±0·045
VC	VC	1·124 ±0·028	0·952 ±0·026	0·974 ±0·038	1·131 ±0·032	1·129 ±0·026
	TAB	1·273 ±0·026	1·191 ±0·034	1·190 ±0·023	1·213 ±0·040	1·233 ±0·039

Control = 1·29 ± 0·026.

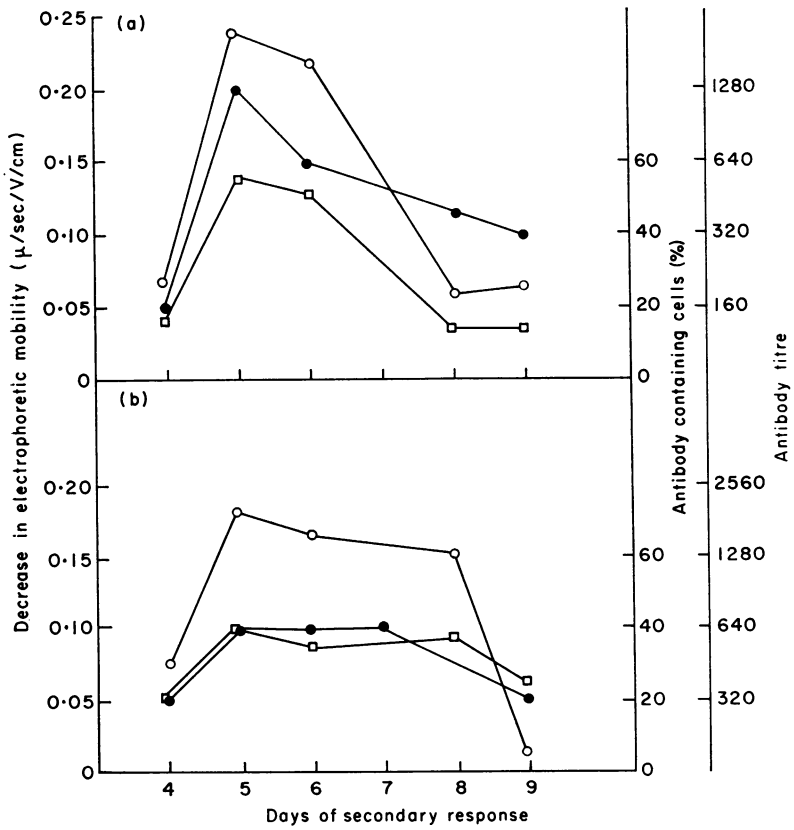

FIG. 2. Pattern of response of the decrement in the mean electrophoretic mobility (○) and its relationship to the percentage of antibody producing cells (□) and antibody titres (●) on various days of secondary response. (a) VC stimulated, (b) TAB stimulated.

standard deviations of this value are classified as non-antibody containing cells and those cells with mobility values outside these limits are regarded as antibody containing cells.

The results of Experiment 5 are presented in Table 2. It is observed that the individual mean values of the TAB and VC systems on different days of secondary response show a pattern of change similar to those seen in Experiments 3 and 4. The objective of this experiment was to examine whether a single cell produces more than one antibody at the same time. The mean mobility values of lymph node cells from animals immunized,

TABLE 2

MEAN ELECTROPHORETIC MOBILITIES OF LYMPH NODE CELLS STIMULATED SIMULTANEOUSLY WITH TWO ANTIGENS (ALL THE MOBILITY VALUES CARRY A NEGATIVE SIGN)

Days of secondary response	Antigen used for incubation		
	TAB	VC	TAB and VC
5	1·044 ± 0·036	0·993 ± 0·045	1·028 ± 0·035
6	1·017 ± 0·028	1·030 ± 0·05	1·022 ± 0·04
7	1·040 ± 0·043	1·134 ± 0·047	1·054 ± 0·032
10	1·183 ± 0·046	1·315 ± 0·043	1·311 ± 0·04

Control = 1·307 ± 0·03.

simultaneously, with TAB and VC antigens fall between the values independently determined with TAB and VC antigens. These results indicate that the antibody sites in a cell bind only one specific antigen. On the other hand, if the large majority of the cells were capable of binding both the antigens then the mean mobility value of such cell populations would be expected to be far more drastically reduced than the values observed in the experiment.

DISCUSSION

Earlier studies (Sundaram *et al.*, 1967) have shown that the electrophoretic mobility of lymph node cells is not altered permanently unless firm antigen–antibody complexes are formed at the cell surface. The presence of an antigen in the suspending medium of lymph node cells from non-immunized animals does change the mobility which is, however, restored by removal of the antigen from the medium. In the latter situation, the modification in the mobility is brought about by a change in the electrolyte concentration of the suspending medium and perhaps also by an alteration in the resultant charge density at the cell surface by the presence of the antigen. The studies of Morgan (1949) with bacteria and those of Möller and Möller (1962) with mammalian cells have indicated the role of surface components as carriers of antigenic determinants. Further, the presence of antibodies at the cell surface has been demonstrated by the bacterial immobilization studies of Nossal and Lederberg (1958) and Nossal (1958). In the present investigations it was observed that a significant and permanent reduction in the mobility occurs on incubation of TAB stimulated cells with TAB antigen. A similar response was elicited when VC stimulated cells were incubated with VC antigen (Fig. 1). These observations are in conformity with those of Forrester *et al.* (1965) and serve to establish the importance of cell surface components in immune reactions.

A significant feature of these interactions is their specificity. Interactions that result in a change in the mobility take place only when the antigen used for incubation is the same as that used for immunization. Fig. 1 clearly indicates that incubation of TAB stimulated cells with VC antigen or vice versa does not affect the original mobility. Such specificity of interaction has been observed independently by Mäkelä and Nossal (1961) and Mäkelä (1964) in different systems and with different techniques. Further, this specificity is maintained throughout the secondary response (Table 1).

A reduction in the mean mobility of immunized lymph node cells on incubation with the specific antigen, thus, gives an indication of the presence of antibody-carrying cells in that population. In addition the degree of reduction in the mean mobility value can be used as a measure of serum antibody levels (Fig. 2). In the present study a close correspondence was observed between the serum antibody levels and the reduction in the mean mobility. Such a relationship is observed for both TAB and VC antigens.

The changes in the mean mobility values of cells carrying antibodies could be due to either a change in the resultant surface charge densities of the individual cells or an absolute increase in the proportion of antibody carrying cells in the population. The former situation could result by passive adherence of circulating antibodies to the cell surface or by an increase in the number of antibody sites and, hence, an increase in the number of antigen–antibody complexes per cell. The observations of Mäkelä (1964) that passive immunization does not alter immune reactions at the cell surface either qualitatively or quantitatively excludes the first possibility. In regard to the number of antibody sites per cell, it is obvious that any change in their number should effect a corresponding modification of the true mean mobility of the antibody producing cells.

In our earlier studies (Sundaram *et al.*, 1967), it has been shown that a population of lymph node cells from an immunized animal is a heterogeneous mixture of normal (non-producers) and antibody producing cells in different stages of development. Independently determined values of normal cells was utilized to segregate the population of cells into two groups, one consisting of non-producers and the other of antibody producing cells. Analysis of the present data showed that the proportion of antibody producing cells varies from day to day whereas the true mean mobility of these antibody producing cells both in TAB and VC systems remain relatively constant. The consistency in the true mean mobility of the antibody producing cells substantiates the suggestion of Mäkelä (1964) that there are few antibody sites in a cell and in most cases only one. The observed variations in the proportion of antibody producing cells are in agreement with the findings of Balfour, Cooper and Alpen (1965). This pattern of response reflects the kinetics of antibody producing cells in the lymph nodes. The slightly higher values obtained by us could be due to the inclusion of antibody producing cells in all stages of development and to the higher sensitivity of our system.

The data presented in Table 2 relate to experiments in which two antigens (TAB and VC) were used simultaneously to immunize the animals. Reduction in mobility was observed when these cells were incubated separately with either of the antigens. This indicates that the population consists of cells producing antibodies to both the antigens. A reduction in mobility was also observed when the cells were incubated with a mixture of both the antigens. However, the mean mobility value of these cells always fell in between those of TAB incubated and VC incubated cells but nearer to the lower value. This observation supports the concept that by and large a cell produces specific antibody to one antigen only at a time.

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