ANTIGEN-INDUCED LOCOMOTOR RESPONSES IN LYMPHOCYTES*

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Chemotaxis is a reaction by which the direction of locomotion of cells is determined by chemical substances in their environment (1). As such, it is probably an important mechanism for homing of cells onto targets at inflammatory sites and possibly in other situations. Chemical substances also influence the locomotion of cells in other ways than by orientation of their direction of locomotion. For example, they can determine the rate of locomotion of cells, a reaction known as chemokinesis. To clarify the nomenclature of the chemically determined reactions of leukocytes, it has been proposed by an international group of workers in the field of cell locomotion that the terms "chemotaxis" and "chemokinesis" should be used precisely, as defined above (2). Previously, the term chemotaxis had been used imprecisely to cover any chemically stimulated locomotion of leukocytes, e.g., into filters. The distinction between chemotaxis and chemokinesis is not a trivial one. Chemotactic reactions are likely to lead to accumulation of cells at the gradient source. Chemokinetic reactions increase cell movement without providing a mechanism to retain cells at the gradient source. The difference is of obvious importance in inflammation.

It is now clear that chemical substances determine the locomotion of lymphocytes as well as of phagocytic cells. Schreiner and Unanue (3) showed that binding of anti-immunoglobulin to lymphocytes bearing surface immunoglobulin caused an increase in rate of locomotion of the cells without influencing their direction, i.e., it caused a chemokinetic reaction. The first demonstration of chemotaxis of lymphocytes, as properly defined, was from our laboratory (4). The direction as well as the rate of locomotion of cultured human lymphocytes was shown to be determined by substances, e.g., casein or activated plasma, that had earlier been shown to be chemotactic for phagocytes. Lymphocytes show chemotactic reactions most obviously after they have been activated, e.g., by a period of culture in vitro (5), and it is possible that activation in vivo has a similar effect. Locomotion and chemotaxis of lymphocytes are stimulated by a wide variety of substances, not only the chemotactic factors mentioned above, but also polyclonal activators such as lectins, provided these are used as attractants at submitogenic doses. Both phytohemagglutinin (PHA)1 and a staphylococcal protein A, but not concanavalin A (Con A), added at very low doses to the lower compartment of a Boyden chamber, enhance locomotion of PHA-transformed human lymphoblasts. This was shown to be chemotactic locomotion toward both PHA (5) and protein A (unpublished studies).

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1 Abbreviations used in this paper: BSA, bovine serum albumin; Con A, concanavalin A; FITC, fluorescein-isothiocyanate; HSA, human serum albumin; PHA, phytohemagglutinin.
If chemotactic reactions can be induced in lymphocytes by some polyclonal activators, it is possible that antigen can stimulate similar reactions in cells bearing appropriate receptors. If this were so, it would encourage lymphocytes to migrate to sites of antigen deposition and thus facilitate antigen recognition. It therefore seemed worthwhile to test the hypothesis that antigen would be chemotactic for primed lymphocytes, and experiments that explore that possibility are described here. It is obviously most important in such experiments to distinguish between chemotaxis and chemokinesis. This has been achieved here by using the leading front method of measuring chemotaxis in filters (6) and by a "checkerboard" assay. In this assay, a series of tests is set up in which the concentrations of attractant above and below the filter are varied, and the influence both of the concentration gradient of the attractant and of its absolute concentration can be studied. The assay is central to the design of the experiments in this paper, and the distinction between chemotaxis and chemokinesis is, as will be apparent, of prime importance in understanding the activation of lymphocyte locomotion.

**Materials and Methods**

**Proteins.** The following proteins were used as antigens: human serum albumin (HSA, Behringwerke, Marburg-Lahn, West Germany), bovine serum albumin (BSA, Sigma Chemical Co., St. Louis, Mo.), ovalbumin (Calbiochem, San Diego, Calif.). Other proteins used as controls were egg white lysozyme (Koch-Light Laboratories Ltd., Colnbrook, England), horse heart myoglobin (B.D.H., Poole, Dorset, England), gelatin (Plasmagel, Neuilly, France), and human IgG prepared by separation of human plasma on QAE-Sephadex A50 (Pharmacia Fine Chemicals, Piscataway, N.J.).

**Immunization of Mice.** Adult male CBA mice were divided into groups of 50 animals, each group being primed by intraperitoneal injection of 0.5 mg of one of the above antigens and 2 × 10⁹ killed *Bordetella pertussis*. At a suitable time 28 or more days after priming, the animals were challenged with antigen (0.5 mg in 0.2 ml) in saline given subcutaneously into both flanks. The mice were killed at various time intervals thereafter, and lymphocytes prepared as detailed below. With each group, a group of unimmunized mice was also killed, and lymphocytes from these were used as controls. In preliminary experiments (Table I), a control group of mice was prepared by injection of *Bordetella pertussis* without antigen at the time the other groups of animals were primed. This group of unprimed controls was challenged with the appropriate antigen in the same way as the test groups. While lymphocytes were collected, sera were taken from all mice to check for serological evidence of an immune response to the appropriate antigen by gel precipitation.

**Preparation of Lymphocytes.** Lymphocyte suspensions for study in vitro were prepared from the inguinal lymph nodes, draining the site of antigen challenge. Mice were killed at various times between 3 and 10 days after challenge, the inguinal lymph nodes were dissected free, the lymphocytes teased out, washed three times, and suspended in Gey's solution. In this way, between 40 and 100 × 10⁹ lymphocytes could be obtained from a group of six to eight mice. The viability of the cells was high (over 70% in all experiments). The cells were washed three times to remove protein and diluted in Gey's solution at a concentration of 2.5 × 10⁹ cells/ml for use in the filter assay.

**Assays of Chemotaxis.** Chemotaxis was assayed by the micropore filter technique. The method was basically that described by Wilkinson (7) with adaptations for the study of lymphocytes. The filters used were cellulose nitrate filters of 8-μm pore size (Sartorius, Göttingen, Germany). Cells were placed above the filters in Gey's solution. The effect of adding protein to this solution is discussed in Results. The chambers were incubated at 37°C for 3 h, and the filters were then detached, stained, and mounted. Cell locomotion was measured by using the leading front technique of Zigmond and Hirsch (6).

**Distinction of Chemotaxis from Chemokinesis.** The effect of the absolute concentration of the attractant and of the concentration gradient on locomotion of lymphocytes in filters was studied by the checkerboard assay first described by Zigmond and Hirsch (6). A series of chambers was set up...
in each of which the concentration of the attractant either above the filter or below the filter or both was different from that in the other chambers. Thus, in certain chambers, the gradient across the filter was positive—i.e., concentration increasing across the filter from bottom to top—in others the gradient was negative, and in others there was no gradient but the absolute concentration of the attractant in which the cells were moving varied from chamber to chamber. Details of the concentrations used are shown in the tables. From the distance attained by the leading front of cells at various absolute concentrations, it was possible to calculate how far cells moving in any given gradient should have migrated into the filter when, insensitive to the gradient, they had responded to the absolute concentration at the different points reached in the filter. The necessary calculations are detailed in an appendix to the paper of Zigmond and Hirsch (6) and were programmed into a Hewlett-Packard (Hewlett-Packard Co., Palo Alto, Calif.) desk calculator. By this means, the effect of the gradient on the distance achieved by the leading front could readily be perceived because the distances migrated by gradient-responsive cells were higher in positive gradients, and lower in negative gradients than the calculated figures. The checkerboard assay has now been used for this purpose by a number of workers (4, 6, 8, and 9) and appears to be the most exact method presently available for the demonstration of the effect of gradients on cell locomotion in filter assays.

Estimation of Proportion of the Total Cell Population which Migrated into the Filter. The number of cells in a defined constant area of the field was counted under a × 40 flat-field objective both on top of the filter and at 10-μm intervals into the filter. The percentage of cells moving was

\[
\frac{\text{no. of cells within filter}}{\text{no. of cells within and on top of filter}} \times 100
\]

Membrane Fluorescence. Lymphocyte suspensions were prepared as detailed above from lymph nodes taken from HSA-immunized mice 6 days after challenge. A control suspension of lymphocytes was prepared from the nodes of ovalbumin-immunized mice. These cells were placed in a solution of fluorescein-isothiocyanate (FITC)-labeled HSA—1 mg/ml—(fluorescein-protein ratio 1.3:1.0) for 60 min, then washed, resuspended in unlabeled HSA, placed on a warm microscope stage at 37°C in a slide-and-coverglass preparation, and observed directly under a fluorescence microscope. The object of the experiment was to determine what proportion of cells took up FITC-HSA and whether uptake was associated with locomotion. The reason for washing and resuspension in unlabeled HSA was to remove background fluorescence which made it difficult to see the cells under dark-ground illumination. The presence of unlabeled HSA at the second stage was to allow the HSA-responsive cells to continue to show locomotor forms. 200 cells from HSA-immunized mice and 200 from ovalbumin-immunized mice were examined, and the proportion showing fluorescence, or a locomotor morphology, judged by elongation and the presence of a posterior uropod or a leading edge and by movements or translocation during the period of observation, was determined.

Results

Morphology of Cells Migrating into Filters. In previous studies, we have reported the chemotactic activity of lymphoblasts, either from human cell clones in continuous culture (4) or normal human blood lymphocytes transformed with polyclonal activators (5). In the present study many of the cells found in the lymph nodes of immunized mice were in blast form; 3 days after challenge, many of the cells moving into filters were large lymphocytes. Thereafter, the number of large lymphocytes in the population decreased, and at 9 and 10 days after challenge, the migrating population consisted mainly of small cells. These small lymphocytes migrated more actively than did small lymphocytes from a control unimmunized population. They did not fall through filters of 8-μm pore size. In negative control tests with no attractant present, they showed little migration into the filter (Table I).

A comparison of the migration of lymphocytes taken at different times after challenge from unprimed and from HSA-primed mice into filters in response to a
TABLE I

Migration of Antigen-Primed Lymphocytes Toward Antigen Below a Filter. Comparison of HSA-Primed Lymphocytes with Unprimed Cells Taken at Various Times after Antigenic Challenge

| Lymphocytes 2.5 x 10^9 cells/chamber, no added protein | Days after challenge | Gey's solution/(negative control) | Endotoxin-activated plasma, 10% (positive control) | HSA, 1 mg/ml | Ovalbumin, 1 mg/ml | Lysozyme, 1 mg/ml |
|-------------------------------------------------------|----------------------|---------------------------------|-----------------------------------------------|-------------|------------------|------------------|
| Unprimed                                              | 3                    | 15                              | 51                                           | 23          | 15               | 17               |
| HSA-primed                                            | 3                    | 18                              | 49                                           | 52          | 21               | 20               |
| Unprimed                                              | 6                    | 17                              | 37                                           | 19          | 18               | 19               |
| HSA-primed                                            | 6                    | 19                              | 30                                           | 46          | 19               | 18               |
| Unprimed                                              | 9                    | 13                              | 37                                           | 23          | 21               | 20               |
| HSA-primed                                            | 9                    | 14                              | 46                                           | 42          | 22               | 20               |

Figures are means of the distances migrated in 10 microscope fields per test (SEM < 5% of mean in all cases). Unprimed controls were given Bordetella pertussis only, at the time the other animals were HSA-primed. Unprimed and HSA-primed animals were given a challenge dose of HSA (see Materials and Methods).

nonspecific stimulus (endotoxin-activated plasma), the antigen HSA, and two unrelated proteins, ovalbumin and lysozyme, is set out in Table I. Both populations of lymphocytes responded to the plasma attractant. The HSA-primed cells moved further into the filter toward HSA than the unprimed cells. This does not prove, however, that HSA was acting as a chemotactic factor for these cells. The action of HSA could have been chemokinetic, and this possibility is examined in the next section. The cells showed no more migration toward ovalbumin or lysozyme than cells in the negative control test without protein. Note that cells taken 3 days after antigen challenge moved a little further into the filter than cells taken at later times, but the differences were slight.

Chemokinetic Effect of Serum Albumin on Lymphocytes from Unprimed Mice. It was noticed in preliminary experiments that lymphocytes taken from unimmunized mice and suspended in media containing HSA or BSA, at concentrations of 500 μg/ml or more, migrated further into filters toward attractants than cells suspended in albumin-free media, and also that the unstimulated migration of HSA-suspended cells was higher than that of protein-free cells in negative control tests where no attractant was added below the filter. This was also evident when cells were used that had been inadequately washed (less than three times) so that traces of serum albumin remained in the suspending medium. We, therefore, tested the effect of serum albumin (HSA) on the locomotion of lymphocytes from unprimed mice in a checkerboard assay (Table IIa). Essentially, this test showed that lymphocytes were responsive to the absolute concentration of HSA and migrated further into the filter when this concentration was raised in the absence of a concentration gradient (figures on diagonal from upper left to lower right). Table IIa shows that this was the case over the HSA concentration, range 50–650 μg/ml. At higher concentrations,
Table II
Checkerboard Assay to Study the Effect of Varying the Absolute Concentration and Concentration Gradient of HSA on the Migration of Unimmunized Mouse Lymph Node Lymphocytes into Filters, and the Same Assay in which the Figures Represent the Proportion of the Total Cell Population that Moved into the Filter

| HSA concentration below filter, µg/ml | 0  | 50 | 250 | 450 | 650 |
|--------------------------------------|----|----|-----|-----|-----|
| (a) Lymphocyte migration, µm in 3 h into 8-µm pore-size filters |
| HSA above filter, µg/ml |
| 0  | 24 | 23 | 18(24) | 25(24) | 30(25) |
| 50 | 25(27) | 28 | 34(32) |
| 250 | 37(38) | 42 |  |
| 450 | 49(46) | 53 |  |
| 650 |  |
| (b) Percent lymphocyte in the filter |
| HSA above filter, µg/ml |
| 0  | 9  | 20 | 12  | 18  | 14  |
| 50 | 15 | 12 | 23  |
| 250 | 11 | 31 |
| 450 | 30 | 21 |
| 650 | 26 |

Figures in parentheses represent migration calculated as detailed by Zigmond and Hirsch (1973) on the basis that cells are responsive only to the absolute concentration of HSA and not to concentration differences. Figures without parentheses represent the result actually obtained (mean of 10 readings). For interpretation of these results, see text.

this dose-response curve flattened out and no increased response was seen when the HSA concentration was taken higher than 1 mg/ml. Furthermore, the gradient had virtually no effect on this migration. Cells migrating in positive gradients (above the diagonal) or in negative gradients (below the diagonal) showed migration close to the figure expected by calculation on the basis of chemokinesis alone (figures in parentheses). A repeat of this experiment gave a closely similar result. Thus, the results in Table II suggest that HSA had a chemokinetic effect but no chemotactic effect on unprimed lymphocytes, and similar results were obtained with BSA. The chemokinetic effect of proteins on lymphocytes was confined to serum albumins among those we have studied. Lymphocytes suspended in ovalbumin, gelatin, lysozyme, IgG, or myoglobin without a chemotactic stimulus migrated about the same distance into the filter as those in Gey's solution without protein. These experiments raised the possibility that the result of Table I, where HSA-primed lymphocytes migrated toward HSA, could equally have been the result either of chemotaxis or of chemokinesis alone; thus, this result required amplification by testing the behavior of primed lymphocytes in a checkerboard assay.

Migration of Primed Lymphocytes to Serum Albumin. Table III shows a checkerboard assay in which lymphocytes from BSA-primed mice migrated in various concentrations and concentration gradients of BSA. The figures on the diagonal from upper left to lower right represent migration in various absolute concentrations of BSA in the absence of a gradient. It is obvious that the rate of
TABLE III
Checkerboard Assay to Study the Migration of BSA-Primed Lymphocytes in the Presence of Various Absolute Concentrations and Concentration Gradients of BSA

| BSA below filter, µg/ml | 0   | 200 | 400 | 600 |
|-------------------------|-----|-----|-----|-----|
| Lymphocyte migration, µm in 3 h into 8-µm pore size filters |
| BSA above filter, µg/ml | 0   | 200 | 400 | 600 |
| 0                      | 17  | 35(25) | 58(32) | 69(40) |
| 200                    | 46(55) | 83 | 82(60) |   |
| 400                    | 55(59) | 56 |   |   |
| 600                    | 48(54) | 41(53) |   |   |

Lymphocytes taken 3 days after challenge with BSA. For explanation, see Table IIa and text.

 locomotion was influenced by the BSA concentration, i.e., that the cells showed a chemokinetic reaction to the BSA. However, if the behavior of cells moving in concentration gradients is examined, it is evident that cells moving in a positive BSA gradient (above the diagonal) were penetrating further into the filter than would be expected from figures in parentheses based on response to the absolute concentration alone. By the same token, cells moving in a negative gradient were not penetrating so far as would be expected. The gradient of BSA, as well as the absolute concentration of BSA, determined the distance reached by the cells. This is consistent with the view that they showed both chemotaxis and chemokinesis in response to BSA.

Similar results were obtained when HSA-primed lymphocytes were tested for locomotor behavior in the presence of HSA. In two such checkerboard experiments, evidence for both chemotaxis and chemokinesis of HSA-primed cells to HSA on the pattern shown in Table III was found. Moreover, both BSA-primed and HSA-primed cells responded to both HSA and BSA in the same way, suggesting antigenic cross-reactivity between the two proteins. Evidence for such cross-reactivity was obtained serologically in gel precipitin tests using the sera of HSA-immunized and BSA-immunized mice. Sera taken 3 days after challenge showed no precipitation with antigen, although at this time, the lymph node lymphocytes were responding chemotactically to antigen. 7 days after challenge and, thereafter, sera from HSA-immunized mice gave a precipitin reaction with HSA that showed a reaction of identity with BSA. Similarly, sera from BSA-immunized mice gave reactions of identity with BSA and HSA. Note that the concentrations at which serum albumins exerted chemotactic effects on primed lymphocytes were fairly high, between 200 and 600 µg/ml (3 × 10⁻⁶ M–10⁻⁵ M). Lymphocytes from the same animals failed to show any chemotactic or chemokinetic response to ovalbumin.

Migration of Primed Lymphocytes in the Presence of Antigens Other Than Serum Albumins. The results presented above suggest that serum albumin has a chemokinetic effect on all lymphocytes, as well as a chemotactic effect confined to serum albumin-primed lymphocytes. It is possible that the chemotactic responses of primed lymphocytes to antigens with such dual effects are not typical of their responses to nonchemokinetic antigens. Screening tests showed that ovalbumin had no chemokinetic effect on the locomotion of unprimed
TABLE IV

Checkerboard Assay to Show the Migration of Ovalbumin-Primed Lymphocytes in the Presence of Various Absolute Concentrations and Concentration Gradients of Ovalbumin and in the Absence of HSA

| Ovalbumin below filter, ng/ml | 0 | 10 | 100 | 1000 |
|-----------------------------|---|----|-----|------|
| Ovalbumin above filters, ng/ml |   |    |     |      |
| 0                           | 19| 23(19)| 25(16)| 17(23) |
| 10                          | 18(14)| 14| 23(23)|        |
| 100                         | 25(25)| 26|      |       |
| 1,000                       | 17(20)| 14(20)|     | 19    |

Lymphocytes taken 7 days after challenge with ovalbumin. For explanation, see Table IIa and text.

lymphocytes, and we therefore studied this protein in more detail. Ovalbumin-sensitized cells were tested in two control checkerboard experiments against HSA as an attractant. In both experiments, HSA showed a chemokinetic effect of the type shown in Table IIa but no chemotactic effect. This made it legitimate to use HSA as a chemokinetic support medium for experiments on the chemotactic effect of ovalbumin on ovalbumin-sensitized cells.

In a preliminary experiment, ovalbumin-sensitized cells (in 1 mg/ml HSA) were allowed to migrate toward ovalbumin below the filter in a series of chambers at doses varying from 1 ng/ml to 1 mg/ml. A dose-response curve was obtained in which the distance that the leading front reached (36 μm in 3 h in the absence of ovalbumin) rose to a peak at 55 μm in 3 h in response to 1 μg/ml ovalbumin below the filter, thereafter declining to 41 μm in 3 h to 1 mg/ml ovalbumin. Ovalbumin was, therefore, studied in checkerboard experiments covering the ascending slope of this dose-response curve. In the first such experiment (Table IV), ovalbumin-primed cells were allowed to migrate toward ovalbumin in the absence of added protein. A very poor response was seen with little migration into the filter (maximum 28 μm in 3 h) and poor evidence of chemotaxis. The cells seemed to require a chemokinetic medium in order to respond chemotactically to antigen. The next experiment was, therefore, done studying the migration of ovalbumin-primed cells in a medium containing 500 μg/ml HSA in a series of chambers containing various concentrations and concentration gradients of ovalbumin. The results are shown in Table Va. The figures along the diagonal from upper left to lower right show little evidence for a chemokinetic effect of ovalbumin. However, the cells migrating in a positive gradient of ovalbumin (above the diagonal) migrated further than would be expected on the basis of a response to absolute concentration alone, which suggests that the gradient influenced their migration, i.e., that they showed a chemotactic reaction. This is less evident in the negative gradients below the diagonal, but there the chemokinetic effect of the HSA in which the cells were resuspended may have predominated over the negative "trapping" chemotactic effect of the ovalbumin above the filter. The chemotactic concentrations of ovalbumin were very much lower than those of serum albumins and were in the range of 100–1,000 ng/ml, i.e., 2.5 × 10⁻⁸–2.5 × 10⁻⁹ M ovalbumin. In controls for
TABLE V
Checkerboard Assay to Show the Migration of Ovalbumin-Primed Lymphocytes, Suspended in HSA (500 μg/ml), in the Presence of Various Absolute Concentrations and Concentration Gradients of Ovalbumin, and the Same Assay in which the Figures Represent the Proportion of the Total Cell Population that Moved into the Filter

| Ovalbumin below filter, ng/ml | 0     | 10    | 100   | 1,000 |
|------------------------------|-------|-------|-------|-------|
| (a) Lymphocyte migration, μm in 3 h into 8-μm pore size filters | 34    | 30(34) | 41(38) | 47(36) |
| Ovalbumin above filter, ng/ml | 33(32) | 32    | 37    | 39    |
| 0                            | 44(36) | 37(39) | 37(39) | 39    |
| 10                           |       |       |       |       |
| 100                          |       |       |       |       |
| 1,000                        |       |       |       |       |

(b) Percent lymphocytes within the filter

| Ovalbumin above filter, ng/ml | 0     | 10    | 100   | 1,000 |
|------------------------------|-------|-------|-------|-------|
| 0                            | 14    | 13    | 27    | 27    |
| 10                           | 11    | 11    | 21    |       |
| 100                          | 24    | 24    | 21    |       |
| 1,000                        | 24    | 24    | 19    |       |

Lymphocytes taken 10 days after challenge with ovalbumin. For explanation, see Table IIa and text.

these experiments, lymphocytes from unimmunized mice suspended in HSA showed no chemotactic reaction to ovalbumin.

Proportions of Cells Migrating under Different Conditions. The number of cells migrating into filters was estimated as a proportion of the total population to study the effect of antigen in recruiting migrating cells. The percentage of cells migrating into the same filters used for checkerboard assays was estimated. Tables IIb and Vb show the results of two such studies. In Table IIb unprimed lymphocytes migrating in various concentrations of HSA were studied. This was a test in which HSA had a chemokinetic effect on locomotion, but in which no chemotactic factor was present. There was a tendency for a larger proportion of the cell population to move into the filter at high HSA concentrations than at low HSA concentrations, suggesting that chemokinetic substances recruit cells to migrate into the filter. Table Vb shows a study of ovalbumin-primed lymphocytes, suspended in HSA, in the presence of varying concentrations of ovalbumin. In this assay, the ovalbumin was showing a chemotactic effect, seen in Table Va. It is evident from Table Vb that, at high ovalbumin concentrations (100–1,000 ng/ml) above or below the filter, a larger proportion of the lymphocyte population (>20%) was induced to migrate than was the case at low ovalbumin concentrations (0–10 ng/ml). Thus, antigen, acting as a chemotactic factor, recruits cells to migrate into the filter.

The percentage of cells responding to chemotactic factors was also studied by using an HSA-primed and an ovalbumin-primed population and studying their uptake of FITC-labeled HSA and their morphology on a slide-and-coverglass preparation. A larger proportion of the HSA-primed than of the ovalbumin-primed population showed membrane fluorescence with HSA (Table VI), and
there was an association between oriented, locomotor morphology, with a leading edge and posterior uropod, and the uptake of FITC-HSA. Most of the fluorescent cells were oriented and most of the unlabeled cells were rounded and not oriented, suggesting that cells that migrate into filters toward antigen are induced to do so after antigen-binding to a cell membrane receptor.

Discussion
The results presented here point to the following conclusions. Primed lymphocytes show a locomotor response to the priming antigen which is likely to be chemotactic because the response is sensitive to the gradient of antigen. To show this response optimally, the cells have to be stimulated chemokinetically, i.e., their rate of locomotion must be increased. We have shown that serum albumin has a nonspecific chemokineti effect on all lymphocytes whether from immunized or nonimmunized animals. Inasmuch as it has also been shown to be chemokinetic for neutrophils (10), this is probably a general effect of serum albumin on motile blood cells. It would, therefore, seem reasonable to suggest that its nonspecific stimulant effect also facilitates lymphocyte movement in vivo because serum albumin is the major protein in the biological fluids in which lymphocytes normally exist in the body.

These results pose some critical questions about the methods employed to measure chemotaxis. Clearly it is inadequate simply to place sensitized cells on top of a filter and the sensitizing antigen below, if the migration observed is to be interpreted as chemotactic. If the antigen were serum albumin, the migration could equally well be chemokinetic, and if the antigen were other than a serum albumin, chemotactic effects might not be manifested unless chemokinetic serum albumin were added. Therefore, a more sophisticated assay is required. The checkerboard assay of Zigmond and Hirsch (6) seems to offer much the best method for analyzing the effects of attractant gradients on the movement of cells in filters as well as the effects of absolute concentration, and, therefore, offers a means to distinguish chemotaxis from chemokinesis. Even more convincing would be a visual assay in which the rate and direction of locomotion of cells and their turning behavior in relation to a gradient source could be measured directly. Unfortunately, presently available methods of this type depend on diffusion of the attractant from a solid source, e.g., a clump of microorganisms or aggregated protein, which is not ideal for the study of the effects of soluble antigens on cell behavior.

In our original study of lymphocyte locomotion (4), we reported that lymphoblasts showed chemotactic reactions, but we did not study the behavior of small
lymphocytes. In the present experiments, in which lymphocytes were studied at different times between 3 and 10 days after challenge with antigen, we observed that cells of various sizes migrated into the filters. A higher proportion of these cells were large lymphocytes at 3 days than they were at 10 days, but lymphocytes do not require to be in blast form to show chemokinetic and chemotactic responses, and small lymphocytes behave in a similar manner.

The present study confirms our earlier findings (4, 5) that the filter assay is a suitable method for the measurement of lymphocyte locomotion. For a long time it was thought that this was not the case, and that lymphocytes either did not migrate or fell through filters. By the use of mouse lymph node cells as studied here, or cultured human lymphocytes (5, and O'Neill and Parrott, manuscript in preparation), we have obtained cell populations that migrate into filters in a reproducible manner and whose response to chemotactic factors can be distinguished from chemokinetic reactions. However, the precise conditions under which lymphocytes show locomotor reactions have not been properly defined, and it is possible that some lymphocyte populations lack the capacity to show such reactions.

Directed locomotion of lymphocytes can be initiated by signals of widely varying types. At present we have evidence that lymphocytes show chemotactic reactions toward relatively nonspecific factors such as casein and denatured serum albumin, and that these reactions can be abolished by treating the cells with lipid-specific membrane-modifying agents (5). This chemotaxis is similar to that of neutrophils and monocytes and may serve the same function of attracting cells into areas of inflammation. Lymphocytes also show chemotactic reactions to such polyvalent ligands as PHA and staphylococcal protein A. These reactions are abolished by treating the cell surface with proteases but not with lipid-specific agents, and are less apparent in phagocytic cells (5). Here we show chemotaxis of lymphocytes to a more restricted stimulus, i.e., to antigen. Normally, phagocytes do not respond chemotactically to antigen, but, if neutrophils or macrophages are coated with appropriate antibody, they also can show locomotor responses to antigen (11, 12).

Although lymphocytes from immunized mice show chemotactic reactions to the immunizing antigen, we have not established the specificity of these reactions, and our findings are reminiscent of the difficulties that have arisen in attempts to detect any specificity in lymphocyte migration toward antigen in vivo. HSA-sensitized lymphocytes migrate as well toward BSA as toward HSA and vice versa, which suggests that the cell-membrane interaction that initiates such reactions may not be of a highly restricted specificity and may allow recognition of cross-reacting antigens. However, this would need study with highly defined antigens, e.g., haptens of known structure, to establish the degree of specificity involved.

Summary

The effect of protein antigens on the locomotion of lymphocytes from the lymph nodes draining the site of antigenic challenge in immunized mice, and from the same nodes in control mice, was studied in filters using a checkerboard assay in which the absolute concentration and the concentration gradient of attractant was varied in a series of chambers. Serum albumin (HSA or BSA)
was chemokinetic for unimmunized lymphocytes inasmuch as the distance migrated into filters by cells in its presence varied with the absolute concentration of albumin, but not with the concentration gradient, indicating an influence of the serum albumin on the rate but not on the direction of locomotion. Ovalbumin and nonalbumin proteins did not show this effect. Using the same assay, the migration of primed lymphocytes in the presence of the priming antigen was shown to be influenced by the antigen gradient in a way that suggested a positive chemotactic response of the lymphocytes to antigen. This response was only shown clearly when the cells were in a chemokinetic medium containing serum albumin.

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