Cell Transport in the Millipore Filter Assay

During inflammation, leukocytes cross the blood vessel wall and migrate to the inflammatory site in response to gradients of diffusible chemical attractants produced there. This directed migration response to a chemical gradient, termed chemotaxis, can be studied experimentally in the Millipore filter assay. We have applied a mathematical model to analyze cell population migration in the assay in terms of two parameters analogous to molecular transport coefficients. The random motility coefficient, μ, reflects the cell response to uniform concentrations of chemical attractant, while the chemotaxis coefficient, χ, reflects the response to a concentration gradient. We have measured μ and χ by comparing theoretical cell density profiles to those measured in the assay. Both parameters vary as a function of the attractant concentration; μ ranges from 10^{-10}-10^{-9} cm^2/s and χ ranges from 10-100 cm^2/s·M for the attractant tested. These values agree with ones predicted *from a priori* theoretical relationships for μ and χ. Quantitation of the Millipore filter assay provides a framework for the quantitation of analogous cell transport systems such as a composite assay simulating cell migration across the vessel wall.

Helen M. Buettner
Douglas A. Lauffenburger
Department of Chemical Engineering

Sally H. Zigmond
Department of Biology
University of Pennsylvania
Philadelphia, PA 19104

Introduction

The ability of living cells to transport themselves across barriers *in vivo* plays an important role in the inflammatory response. Moving under their own power, white blood cells called leukocytes cross the blood vessel wall and migrate into the tissue in a seemingly purposeful manner to reach inflammatory sites in the body that are considerably removed (many cell diameters) from their entry point. The speed and precision with which these cells localize at distant sites are thought to depend critically upon cues that they receive from their environment in the form of chemical gradients. Neutrophil leukocytes can accumulate in substantial numbers within 30 minutes at localized sites of tissue infection or irritation without general systemic infiltration into tissue (Wilkinson, 1982).

Cell movement in response to chemical attractants can occur in two distinct ways:

1) Through randomly directed movements, termed random motility

2) Through movements biased in the direction of a gradient, termed chemotaxis.

The attractant mediates the movement by binding to receptors on the cell surface. In uniform concentrations of attractant, ligand binds to receptor and the motile machinery of the cell is activated, but without a directional signal, only the random component of movement is effected. A gradient in the environment of the cell, however, is translated into a gradient in the number of bound receptors across its surface, imparting a sense of direction to the cell and resulting in chemotactic behavior as well. Clearly, cells undergoing chemotaxis would seem to be at an advantage in reaching their target efficiently; but it is also possible for cells moving randomly to accumulate at a given point along a gradient if the degree of random motility varies with concentration, a frequently observed effect known as chemokinesis. In either case, the presence of a gradient is an essential feature for the net accumulation of cells at a given point. To understand the relative importance of each effect on the migration of leukocytes to an inflammatory site and the significance of chemical mediation compared to other forms of directional influence (such as adhesive gradients), it is desirable to obtain quantitative measures of chemotaxis and chemokinesis.

Because *in vivo* systems present a problem in their complexity, as well as the inaccessibility to physical measurement of many of the sites where these responses presumably occur, *in vitro* assays have been developed to isolate and study cell movement in response to potential environmental stimuli encountered *in vivo*. One of the most widely used assays is the Millipore filter assay introduced by Boyden (1962). The assay is conducted in a chamber, illustrated in Figure 1, that closely resembles a dia-

Correspondence concerning this paper should be addressed to H. M. Buettner who is currently at the Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104-6084.

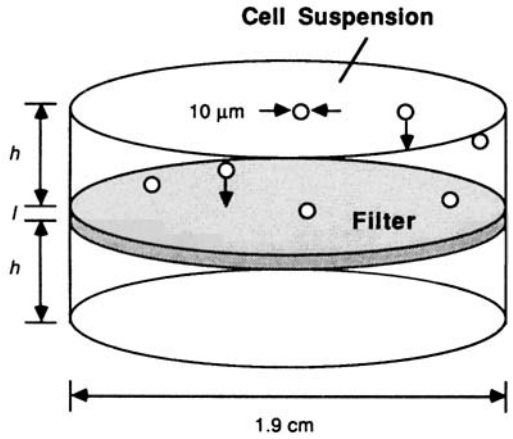

Figure 1. Basic features of the Millipore filter assay.
Filter thickness and compartment heights were $l = 0.015$ cm and $h = 0.3$ cm, respectively.

phragm diffusion cell for measuring diffusion coefficients of molecular species: a thin, porous membrane separates two fluid-filled compartments between which transfer of the species of interest can occur. In this case, however, the species of interest comprises cells rather than molecules. The assay chambers are scaled down considerably to conserve cells and biological reagents, and consequently, the compartments are left unstirred to avoid rapid decay of the gradient and shearing of the cells. In normal use, the assay for chemotaxis is performed by placing cells in the upper compartment, a chemical attractant in the lower and allowing migration to proceed for a specified time before interrupting the assay, removing, clearing and fixing the filter and examining it under the microscope. Chemokinesis is assayed by placing equal concentrations of attractant in both compartments.

The analogy to diffusion is reinforced by the remarkably diffusion-like profiles in cell density through the thickness of the filter seen under the microscope. This analogy has long been ignored, however, in usual attempts to quantitate migration in the filter assay. Commonly, quantities such as the distance moved by the furthest cells or the number of cells that have passed some arbitrary point in the filter are selected as measures of the migration that has occurred. Although much less time-consuming to obtain than a density profile, these measures reflect physical characteristics of the assay, obscuring the intrinsic cell behavior being evaluated. Recently, Rothman and Lauffenburger (1983) applied a more fundamental approach in quantitating the under-agarose assay. This approach is based on a general expression for the flux of moving cells proposed by Alt (1980) and cast in the following phenomenological form by Lauffenburger (1983)

$$J_c = -\mu(a)\nabla c + \left[\chi(a) - \frac{1}{2}\frac{d\mu}{da}\right] c\nabla a, \qquad (1)$$

where c is the cell density and a is the attractant concentration. The random motility coefficient μ and the chemotaxis coefficient χ characterize cell movement in the same way that the diffusion coefficient characterizes the diffusion of a molecular species. The first term on the righthand side of Eq. 1 represents the dispersion of a cell population in the absence of an attractant gradient. The remaining term represents the net "drift" of a cell population in the presence of an attractant gradient. This drift may be due to true chemotaxis (preferential directional orientation in a concentration gradient), described by the parameter χ, or it may be due to chemokinesis (variation of cell speed with concentration) in a gradient, described by the variation of the parameter μ with attractant concentration a (Lauffenburger, 1983). The results of Rothman and Lauffenburger provided a straightforward method for quantitative determination of the relative contributions of chemotaxis and chemokinesis in the under-agarose assay through the measurement of the parameters μ and χ (Lauffenburger et al., 1983; Tranquillo et al., 1988). In this paper, we follow the same approach to analyze the Millipore filter assay. In particular, we determine the random motility coefficient, μ, and the chemotaxis coefficient, χ.

Experimental Methods

Circular stainless steel Sykes-Moore culture chambers (Bellco Glass, Inc., Vineland, NJ) with 150 μm thick, 3.0 μm pore size, nitrocellulose Millipore filters (Millipore Corp., Bedford, MA) separating the bottom and top compartments were used to obtain migration profiles. The height of each compartment was 0.3 cm. Migration was studied in response to formyl-norleucyl-leucyl-phenylalanine (FNLLP), a synthetic analog of the naturally occurring bacterial factor formylmethionyl-leucyl-phenylalanine (Marasco et al., 1984) with a diffusion coefficient of $D = 1 \times 10^{-5}$ cm^2/s (Stickle et al., 1984). At the start of an experiment, 0.8 mL of Hanks balanced salt solution containing the desired concentration of attractant was injected into the bottom compartment through injection ports on the side of the chamber, while 1.6×10^6 leukocytes suspended in 0.8 mL of the same medium with either the same (chemokinesis assay) or a different (chemotaxis assay) concentration of the attractant were placed onto the filter in the upper compartment. The cells were obtained from rabbit peritoneal exudate withdrawn 4 hours following an intraperitoneal injection of shellfish glycogen in sterile saline solution to stimulate movement of leukocytes into the peritoneal cavity. The peritoneal exudate thus obtained consists of >95% polymorphonuclear leukocytes (PMN), with the remainder composed of larger and slower-moving white blood cell types (Wilkinson, 1982). The chambers were incubated at 37°C for a set period of time ranging from 30–60 minutes, and then the filters were removed and fixed in formaldehyde to halt the migration. After staining the filters, they were dehydrated in ethanol and placed in xylene to render them transparent for viewing under the microscope. The number of cells per area was measured by counting the cells in focus at 10 μm intervals through the thickness of the filter, with the counting area defined by a reticule placed in the eyepiece. Ten to 25 profiles were measured per filter, and the results averaged to obtain a mean cell density profile in the filter.

Mathematical Model

The general cell flux expression given by Eq. 1 was applied to describe the movement of cells through the filter, yielding the following conservation equation for cell density in the filter:

$$\frac{\partial c}{\partial t} = -\nabla \cdot J_c. \qquad (2)$$

The attractant profile was modeled by Fick's law with no-flux boundary conditions at either end of the chemotaxis chamber, as described by Lauffenburger and Zigmond (1981):

$$a(x,t) = a_1 + \frac{(a_2 - a_1)}{2} \sum_{n=-\infty}^{\infty} \left\{ \text{erf}\left[\frac{-h + 4nh + x}{2\sqrt{DT}}\right] + \text{erf}\left[\frac{3h - 4nh - x}{2\sqrt{Dt}}\right] \right\}, \quad (3)$$

where a_1 and a_2 are the initial concentration in the upper and lower well, respectively, h is the well height, and D is the diffusion coefficient.

As indicated by Eq. 1, the coefficients μ and χ will depend, in general, upon the attractant concentration. For particular application to the Millipore filter assay, however, the following simplifications are possible:

1) μ is constant across the thickness of the filter

$$\mu(a) = \mu(\bar{a}),$$

where $\bar{a}$ is the attractant concentration at the midpoint of the filter. Although our results clearly demonstrate a functional dependence of μ on a when sampled over a broad range of concentrations, the thin filter intercepts such a small portion of the attractant profile that the difference in a from one side of the filter to the other is quite small. To show that μ is essentially constant across the filter for any gradient of interest, μ was determined as a function of distance into the filter by first finding the attractant concentration profile in the filter from Eq. 3 and then interpolating the experimental random motility data in Figure 6 measured in the absence of a gradient, as discussed below. The resulting values for $\mu(x)$, reported in Figure 3 relative to the midpoint value, $\mu(\bar{a})$, indicate that the value of μ at the edges of the filter is within 5% of the midpoint value 5 minutes after the start of the experiment even for the steepest gradients. Hence, we approximate μ in the filter by the value obtained at the midpoint concentration, $\bar{a}$, which remains constant during the course of the experiment at the average of the initial concentrations in the two compartments of the assay chamber.

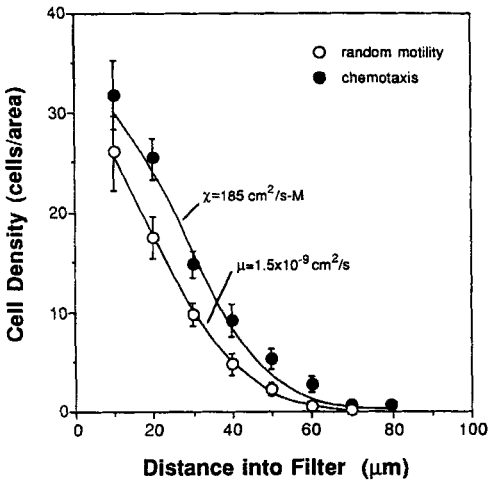

Figure 2. Typical cell density profiles measured in the filter for a uniform attractant concentration of 10^{-9} M (random motility), and an initial gradient between 10^{-9} M in the bottom compartment and no attractant in the top compartment (chemotaxis).

Migration was allowed to proceed for 30 minutes.

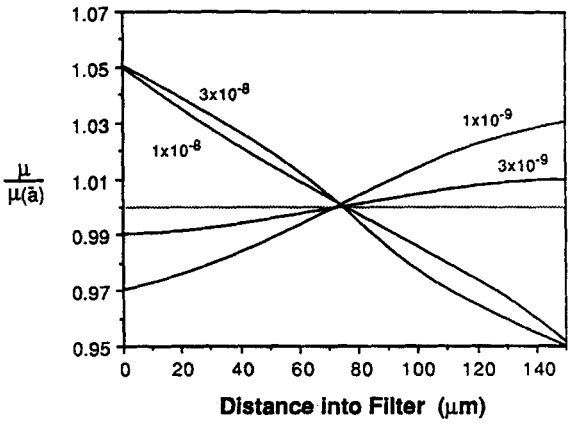

Figure 3. Random motility coefficient as a function of position in the filter at 5 min into the experiment for different attractant concentrations.

$\mu(\bar{a})$ is the random motility coefficient at the midpoint of the filter.

2) χ is constant across the thickness of the filter

$$\chi(a) = \chi(\bar{a}).$$

Since we have little knowledge of the functional form of $\chi(a)$, this can only be postulated *a priori* and then verified later by self-consistency checks with our results. $\chi(a)$ cannot be measured independently of the gradient as μ can be, and a theoretical relationship for $\chi(a)$ is not known with certainty. The best relationship we currently have is based upon the equilibrium binding of attractant to cell surface receptors

$$\chi = \chi_o \frac{R_T(a) K_d}{(K_d + a)^2} v(a), \quad (4)$$

where K_d is the dissociation constant of binding, $R_T(a)$ is the total number of receptors on the surface of the cell available for binding at a given attractant concentration, $v(a)$ is the cell speed and χ_o is a constant. This form was suggested initially by the empirical observations of Zigmond (1977) indicating that the fraction of cells oriented up an attractant gradient was proportional to receptor occupancy on the cell surface. The mathematical derivation of Eq. 4, provided by Tranquillo et al. (1988), begins by assuming that the chemotactic flux is proportional to the gradient in bound receptors across the cell surface:

$$\chi \nabla a \propto \nabla R_b. \quad (5)$$

Since the number of bound receptors, R_b, is given by

$$R_b = \frac{R_T a}{K_d + a}, \quad (6)$$

the gradient in bound receptors can be expressed as:

$$\nabla R_b = \frac{R_T K_d}{(K_d + a)^2} \nabla a. \tag{7}$$

Substituting Eq. 7 into Eq. 5 yields the form shown in Eq. 4. $R_T(a)$ and K_d are known for the attractant FNLLP, and $v(a)$ can be determined from measurements of $\mu(a)$ by Eq. 11 below. Thus, it is possible to find the ratio $\chi/\chi(\bar{a})$, shown plotted in Figure 4. These results indicate that for even the steepest initial gradient, the value of χ at the edge of the filter is within 12% of the midpoint value 5 min into the experiment. Since we presently do not have enough data to provide significant insight into the deviations from this prediction that may occur, we assume for these preliminary measurements that χ remains constant at the midpoint value $\bar{a}$.

In addition, based on the geometry of the assay, we assume:

3) cells are distributed uniformly parallel to the surface of the filter so that net movement occurs only in the x direction, i.e., through the thickness of the filter.

Applying these simplifications, yields the following form of Eq. 2

$$\frac{\partial c}{\partial t} = \mu(\bar{a}) \frac{\partial^2 c}{\partial x^2} - \chi(\bar{a}) \frac{\partial}{\partial x}\left(c \frac{\partial a}{\partial x}\right), \tag{8}$$

which can be solved for the cell density profile in the filter subject to the boundary and initial conditions (Buettner, 1987):

$$V \frac{\partial c}{\partial t} = A\left(\mu(\bar{a}) \frac{\partial c}{\partial x} - \chi(\bar{a})\, c \frac{\partial a}{\partial x}\right) \qquad x = 0, \tag{9a}$$

$$c \to 0 \qquad x \to \infty, \tag{9b}$$

$$c = c_0 \qquad t = 0. \tag{9c}$$

A is the cross-sectional area of the filter perpendicular to the x direction and $V \cdot c(0, t)$ is the number of cells resting on the top surface of the filter at any time. This formulation of the boundary and initial conditions assumes that the cells, placed on the filter in suspension, settle rapidly to the surface of the filter so that all of the cells are initially present at $x = 0$. Supporting this assumption, experiments to determine the fraction of cells settled out of an equivalent suspension (with respect to density, volume and height) in a Sykes-Moore chamber with the filter removed showed that essentially all of the cells reached the transparent floor of the chamber within the first 5 minutes of the experiment (Buettner, 1987). At the density of cells used in these experiments (1.6×10^6 total cells per chamber), calculations show that all of the 10 μm diameter cells easily fit within a single monolayer on the surface of the filter. Therefore, an alternate definition of V is that of the thin disk of cross-sectional area A and height equal to the height of one cell monolayer, 10 μm, in which the cells resting on the filter are contained, Figure 5. The boundary condition given by Eq. 9a was selected on the basis of a comparison of several different boundary conditions at $x = 0$, including one assuming a resistance to cells entering the filter. The best description of the data was obtained using Eq. 9a, which assumes that the depletion of cells from the surface is governed only by the rate of cell flux into the filter.

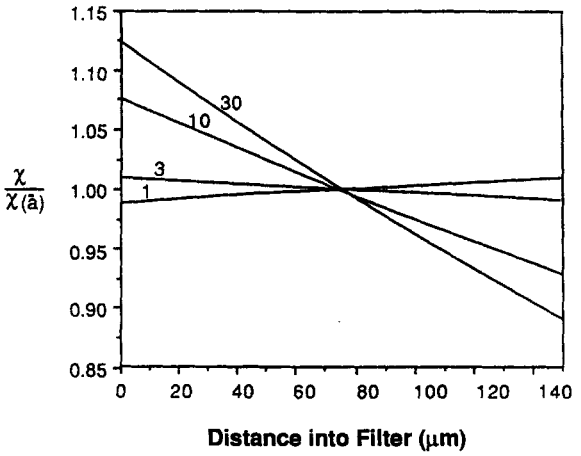

Figure 4. Chemotaxis coefficient as a function of position in the filter at 5 min into the experiment, relative to the value at the midpoint, $\chi(\bar{a})$.

The numbers on the curves indicate the initial attractant concentration in the lower well in units of 10^{-9} M. The initial concentration in the upper well is zero.

In the case of a uniform attractant concentration throughout the chamber, the second term on the right hand side of Eq. 8 is identically equal to zero and the model can be solved analytically using Laplace transforms to yield a theoretical cell density profile of the form

$$c(x, t) = c_0 e^{x/L} e^{\mu t/L^2} \operatorname{erfc}\left(\sqrt{\mu t}/L + x/2\sqrt{\mu t}\right), \tag{10}$$

where $L = V/A$. This solution was fit to experimental random motility profiles using a simplex optimization routine to obtain best-fit values of μ for each attractant concentration measured. The full chemotaxis equation was solved using the Crank-Nicholson finite difference method. The value of μ at the midpoint of the filter was interpolated from previously determined values of $\mu(a)$; hence, χ was the only unknown to be fit. The calculation was sufficiently rapid that upper and lower bounds on χ were determined initially by trial and error and then substituted into a straightforward iterative procedure employing a single outer loop to find the best-fitting value of χ. In both cases, the best fit was defined as the minimum value of

$$d^2 = \sum_{i=1}^{k} (n_{m_i} - n_i)^2,$$

where n_{m_i} and n_i represent the theoretical and experimental cell density, respectively, in the ith focal plane. k is the number of focal planes, located at successive 10 μm intervals from the top of the filter, in which the density was counted. A percent error in

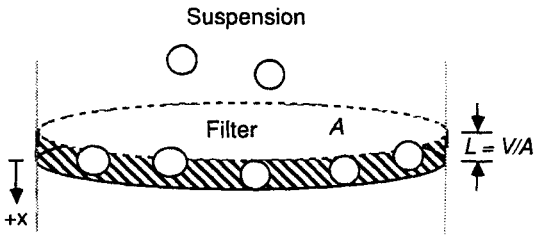

Figure 5. Relevant geometry in Eq. 9a.

total cells counted was also calculated:

$$\delta = \frac{\sqrt{d^2}}{\sum_{i=1}^{k} n_i} \times 100\%.$$

Experimental Results

Values of $\mu(\bar{a})$ and $\chi(\bar{a})$ were obtained from cell density profiles such as those shown in Figure 2, taken over a range of attractant concentrations. The value of $\mu(\bar{a})$ was first determined by fitting experimental random motility profiles to Eq. 10. $\mu(\bar{a})$ was then substituted into Eq. 8 to find $\chi(\bar{a})$ from the corresponding chemotaxis profile. The error, δ, was typically $<5\%$ and in all cases was $\leq 10\%$. In general, the value of δ was relatively insensitive to changes in μ or χ of $<10\%$. Therefore, the values reported for any given experiment should be taken as $\pm 10\%$. While the separation between random motility and chemotaxis profiles was never large, the chemotaxis profile was consistently more developed than the random motility profile at concentrations where a measurable response occurred, and the separation was statistically significant as observed by the nonoverlapping error bars. Typical concentration-dependent values of μ and χ are plotted in Figures 6 and 7, respectively. Reproducibility of these measurements was $\pm 60\%$ or better, mimicking the variation in cell speed observed for different individuals, even within a single population of cells (Wilkinson, 1982). Despite this large reproducibility error in μ and χ, their dependence on concentration was apparent.

Since the population of cells used in these experiments is essentially homogeneous (>95% PMN's), the values of μ and χ obtained are reasonably assumed to represent a uniform characteristic of the population rather than an average of several subpopulation characteristics. Past and current studies on cell types such as rabbit and human PMN's (Tranquillo et al., 1988), alveolar macrophages (Glasgow et al., in press) and capillary endothelium (Rupnick et al., 1988) using the under-agarose assay suggest that the values of μ and χ do vary, however, not only between cell type, but also between species. Thus, different cell types will presumably migrate with somewhat different characteristics in the Millipore assay, and values of μ and χ for each cell type should be measured independently. Differences also exist in the values of μ and χ measured for the same cell

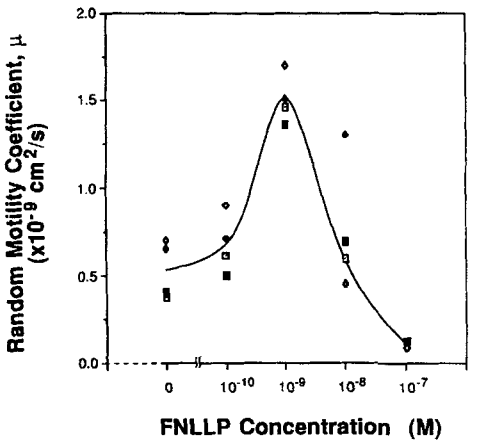

Figure 6. Measured values of μ as a function of the attractant concentration.

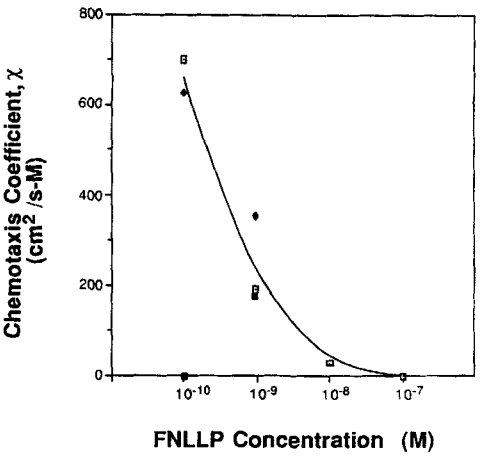

Figure 7. Measured values of χ as a function of the attractant concentration.

type in different assay systems, as discussed below, a fact that may help to provide future insight into biophysical mechanisms of cell migration.

Although a significant difference exists between the experimental random motility and chemotaxis profiles measured for a given average concentration, the small separation between the curves may reflect a more rapid decay of the chemotactic gradient than is optimal for detecting a chemotactic response. Zigmond (1977) has shown that the limit at which a cell can detect a gradient is approximately a 1% increase in concentration across the 10 μm diameter of the cell at concentrations near the K_d of binding. (At concentrations away from the K_d, a larger gradient is required.) The fractional increase in concentration across the cell is related to the concentration gradient according to the expression

$$f = \frac{1}{a}\frac{\partial a}{\partial x} \times 10\mu m,$$

where a is measured at the midpoint of the cell and the quantity $(1/a)\ (\partial a/\partial x)$, is referred to as the specific gradient. The specific gradient is largest at $x = 0$, so f calculated at $x = 0$ represents the maximum increase the cell will see at any point in time. Figure 8 shows that the percent increase across the cell drops rapidly to around 1% for a diffusion coefficient of 10^{-5} cm^2/s. Thus, the attractant gradient is very near the limit of detection by the cell for the majority of the experiment with the probable consequence that the measurable chemotactic response is much smaller than it might be in a more slowly decaying gradient. In principle, this hypothesis could be tested using a chemotactic factor to which the cells are similarly sensitive, but which exhibits a lower diffusion coefficient. At present, however, no obvious candidates exist.

Theoretical Relationships for μ and χ

As phenomenological parameters, μ and χ represent a useful way of characterizing normal and abnormal motility responses of cell populations to a variety of biologically significant attractants and in assays of different composition and architecture. The potential of μ and χ is enhanced significantly, however, through the following relationships to fundamental quantities of individual cell movement, derived by Lauffenburger (1983) and

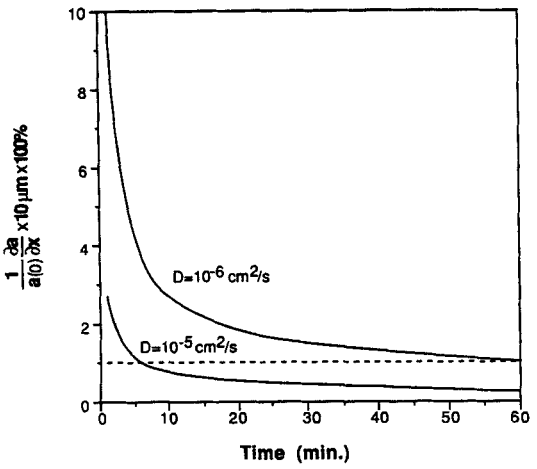

Figure 8. Percent change in attractant concentration across a 10 μm diameter cell at the top edge of the filter.

Tranquillo et al. (1988)

$$\mu = \tfrac{1}{2}\tau v^2, \tag{11}$$

$$\chi = \frac{\phi v}{\left(\frac{\partial a}{\partial x}\right)}, \tag{12}$$

where τ is the persistence time, defined as the average length of time between changes in the direction of cell movement, v is the cell speed and ϕ is the orientation bias. A measure of the fraction of cells in a population oriented in the direction of the stimulus, ϕ is related to the gradient in bound receptors across its dimension through the expression

$$\phi = \chi_o \frac{\partial R_b}{\partial a}\frac{\partial a}{\partial x}, \tag{13}$$

in which R_b is the number of bound receptors at any point and χ_o is a sensitivity parameter representing the ability of the cell to translate a gradient in bound receptors into an orientation response. Although the observation of individual cell movement necessary for determining τ, v, and ϕ is not possible in the opaque Millipore filter, τ is known for other systems, and as a first approximation the same value is reasonably assumed for the filter assay. As indicated in Eq. 7, the gradient in bound receptors across the cell surface is estimated from binding experiments to be of the form

$$\frac{\partial R_b}{\partial a} = \frac{R_T(a)K_d}{(K_d + a)^2}, \tag{14}$$

where $R_T(a)$ is the total number of receptors on the cell surface at a given attractant concentration and K_d is the dissociation constant for binding of attractant to receptor. Substituting Eqs. 13 and 14 into Eq. 12 yields an expression for χ in terms of experimentally measurable variables, as given by Eq. 4. Both R_T and K_d are known for leukocyte surface receptors binding FNLLP, and v can be obtained from μ. Substituting these quantities into Eq. 4 leaves just one unknown, χ_o, to be fit to the data. Theoretical values of χ were estimated in this manner for each of the midpoint concentrations at which a value of χ was measured. Fitting Eq. 4 to the data yielded a best fit value of $\chi_o = 6 \times 10^{-6}$ cm. The resulting theoretical chemotaxis coefficient and the experimentally measured values of χ are compared in Figure 9, where both quantities have been multiplied by the midpoint concentration, $\bar{a}$, to yield the chemotactic response, expressed in more intuitive units of cm^2/s. The theoretical response differs from the experimental response at each point by less than an order of magnitude. Further verification of these relationships in the filter assay will rely upon measurements made in transparent materials such as collagen or fibrin gels that have recently been introduced for such purposes (Islam et al., 1985). However, our preliminary results agree with those obtained by Lauffenburger et al. (1983) and Tranquillo et al. (1988) for the under-agarose assay.

In the under-agarose assay, cells move in the narrow gap between a glass microscope slide and a layer of agarose poured over the slide to maintain a chemoattractive gradient. Values of μ and χ in the under-agarose assay are 1–2 orders of magnitude higher than in the filter assay, but the difference seems to be accounted for primarily in the speed (which can be measured independently of μ in the under-agarose assay). Substituting the larger v from the under-agarose assay into Eq. 11 along with the same receptor binding data used above yields a similar estimate of χ_o when compared with the under-agarose data for χ. This is a reasonable result considering that the cells must traverse a more tortuous path in the filter, which would not necessarily change their orientation bias but might easily be expected to slow down their effective speed. The advantage of relationships like Eqs. 11 and 12 is that they can provide significant insight into the mechanism of movement and help to pinpoint more precisely the cause of abnormal cell transport when it occurs. They may also provide more specific clues as to appropriate treatment to be taken in clinical situations and aid in the development of pharmaceuticals.

Overall Inflammatory Response

As a model of the inflammatory response, the filter assay represents the simplest approximation, i.e., both the blood vessel

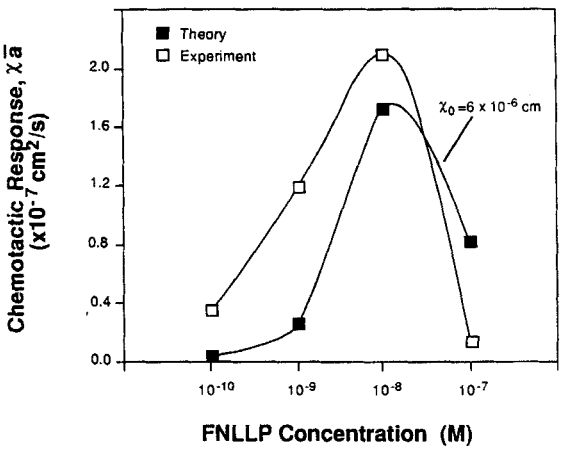

Figure 9. Comparision of the theoretical and experimental chemotactic response, $\chi\bar{a}$.

The experimental response was obtained by multiplying the experimentally measured values of $\chi(\bar{a})$ by the midpoint concentration, $\bar{a}$. The theoretical response was obtained by multiplying Eq. 12 by $\bar{a}$, with the orientation bias, ϕ, determined from Eqs. 13 and 14. The best fit of the theory to the data was obtained for a value of $\chi_o = 6 \times 10^{-6}$ cm.

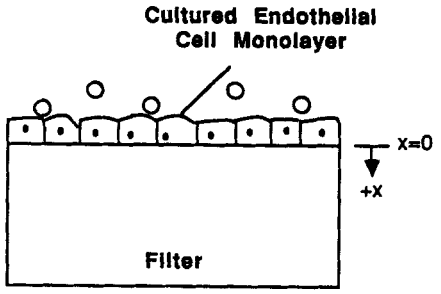

Figure 10. A composite assay for migration through the blood vessel wall and the tissue.

wall and the tissue are modeled by a single layer, the filter. An obvious extension of this model is the culture of endothelial analogs of the blood vessel wall on the top surface of the filter (Darby, 1983), forming the composite system illustrated in Figure 10. The effect of the endothelial layer might then be modeled as a condition at the boundary of the filter in the following way

$$V\frac{dc_s}{dt} = Ah_c[c(0) - c_s] \qquad \text{in the cell layer,}$$

$$V\frac{\partial c}{\partial t} = A\left[\mu\frac{\partial c}{\partial x} - \chi c\frac{\partial a}{\partial x} + h_c(c - c_s)\right] \qquad x = 0,$$

where c_s is the density of cells resting on the endothelial layer and h_c is a transfer coefficient for the migration of leukocytes across the endothelial layer. Since solution of Eq. 4 subject to these conditions would require knowledge of the cell density between the two layers, $c(0)$, as a function of time, the best approach might be to construct the filter layer from a transparent gel, as mentioned above.

Conclusions

We have shown that the cell migration in the *in vitro* Millipore filter assay can be accurately modeled by a general equation for chemotaxis, in which the key parameters are the random motility coefficient, μ, and the chemotaxis coefficient, χ. Values for μ and χ determined from the assay corresponded reasonably with previous measurements in a different system, suggesting that μ and χ are, in fact, fundamental parameters describing cell movement independently of the particular system in which they are moving. The success of this approach suggests that it may be a useful tool in analyzing further aspects of the inflammatory response, such as transport of cells across the vessel wall, as well as the migration of other cell types.

Acknowledgment

The support of NIH Grants AI21538 and DE00143 to D.A.L. is gratefully acknowledged.

Notation

a = attractant concentration
$\bar{a}$ = attractant concentration at the midpoint of the filter
a_1 = initial attractant concentration in the upper well of the filter chamber
a_2 = initial attractant concentration in the lower well of the filter chamber
A = cross-sectional area of the filter perpendicular to the x-axis
c = cell density
c_0 = initial cell density on the surface of the filter
c_s = density of leukocytes in the endothelial cell layer
h = height of each fluid compartment in the filter chamber
h_c = coefficient of transfer of leukocytes across endothelial cell layer
J_c = cell flux
K_d = dissociation constant of binding of attractant to cell surface receptors
R_b = number of bound receptors on the cell surface
L = height of one cell monolayer
R_T = total number of receptors on the cell surface
x = distance into the filter
v = cell speed
V = volume of suspension in which cells are resting on top surface of filter
μ = random motility coefficient
τ = persistence time of cell movement
ϕ = cell orientation bias
χ = chemotaxis coefficient
χ_o = cell orientation sensitivity

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Manuscript received Feb. 11, 1988, and revision received Nov. 9, 1988.