Mathematical analysis of cell–target encounter rates in three dimensions

Effect of chemotaxis

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ABSTRACT Efficient and rapid immune response upon challenge by an infectious agent is vital to host defense. The encounter of leukocytes (white blood cells of the immune system) with their targets is the first step in this response. Analysis of the kinetics of this process is essential not only to understanding dynamic behavior of the immune response, but also to elucidating the consequences of many leukocyte functional abnormalities.

The motion of leukocytes in the presence of targets typically involves a directed, or chemotactic component. These immune cells orient the direction of their motion in the presence of gradients in chemical attractants generated by pathogens. Fisher and Lauffenburger (1987. *Biophys. J.* 51:705–716) developed a model for macrophage/bacterium encounter in two dimensions which includes chemotaxis, and applied it to the particular system of alveolar macrophages (phagocytic leukocytes on the lung surface). Their model showed that macrophage/target encounter is likely the rate-limiting step in clearance of bacteria from the lung surface (Fisher, E. S., D. A. Lauffenburger, and R. P. Daniele. 1988. *Am. Rev. Resp. Dis.* 137:1129–1134). We have extended this model to analyze the effects of cell motility properties and geometric parameters on cell-target encounter in three dimensions. The differential equation governing encounter time in three dimensions is essentially the same as that in two dimensions, except for changed probability values.

Our results show that more highly directed motion is necessary in three dimensions to achieve substantially decreased encounter times than in two dimensions, because of the increased search dimensionality. These general results were applied to the particular system of neutrophils operating in three dimensions in response to a bacterial challenge in connective tissue. Our results provide a plausible rationalization for both the chemotactic and chemokinetic behavior observed in neutrophils. That is, these cells exhibit in vitro a greater chemotactic bias and a more dramatic variation of speed with attractant concentration than alveolar macrophages, and our results indicate that these behaviors can have a greater influence in three-dimensional connective tissue infection situations than in two-dimensional lung surface infection cases. In addition, we show that encounter apparently is not generally the rate-limiting step in this neutrophil response. These findings have important implications for correlating in vitro measured defects in cell motility and chemotaxis properties with in vivo functions of host defense against infection.

INTRODUCTION

The immune system is comprised of several interacting classes of cells. One such class of immune cell are the phagocytes, the cells responsible for the elimination of invading pathogens through intracellular killing. The two main types of phagocytic cells are macrophages and neutrophils. Along with their phagocytic role, macrophages are responsible for the amplification of the immune response. They accomplish this amplification through the secretion of various growth factors (lymphokines) and through presentation of phagocytosed antigen to T lymphocytes. Both phagocytosis and antigen presentation require cell–target and cell–cell contact. In contrast to the macrophages, which are located in specific tissues and have a wide range of functions, neutrophils are constantly circulating in the bloodstream and are primarily responsible for the elimination of bacterial challenges. They must be able to be rapidly mobilized from the vessels and into the tissue in order to respond to localized infections (see Fig. 1) (for further background, see, for example, Paul [20]).

Both macrophages and neutrophils have been observed to trace a "biased random walk" in space in response to gradients of chemical attractants released by various pathogens (1, 29) (see Fig. 2). The binding of these chemoattractants to cell surface receptors allows the cell to detect a gradient in the attractant and alter its direction of motion accordingly, in a phenomenon known as chemotaxis (for an excellent recent review, see Devreotes and Zigmond [6]). Defects in macrophage and neutrophil motility and chemotaxis have been shown to be correlated with increased incidence and severity of a number of infectious diseases (4, 21, 26), including AIDS (7, 23).

Fisher and Lauffenburger (8) developed a two-dimensional model for the time necessary for alveolar macro-

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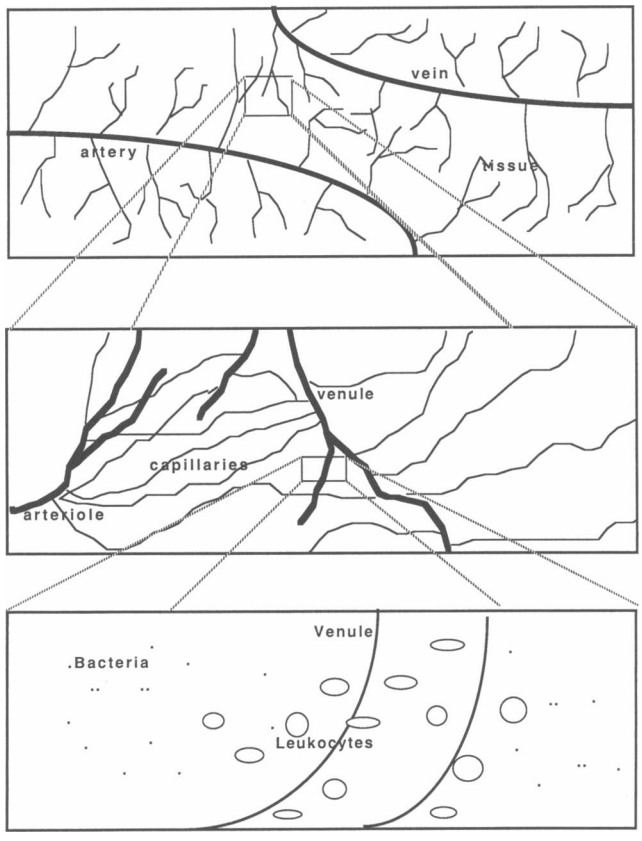

FIGURE 1 Expanded view of the tissue, with emphasis on vascular network. Leukocytes circulating in the vessels must normally migrate into the tissues to combat bacterial infection.

phages to encounter their targets on the lung surface. Their model was able to show that macrophage-bacterium encounter is likely to be the rate-limiting step in bacterial clearance from the lungs. Application of this model to bacterial clearance kinetic data showed that the rate of clearance could be explained very well by in vitro measurements of alveolar macrophage motility and chemotaxis properties (9). However, most immune system cells, including other tissue macrophages, neutrophils, lymphocytes, and killer cells, operate in three dimensions. An analysis of the effects of chemotaxis and cell motility parameters on cell–target and cell–cell encounter time in three dimensions is therefore likely to be helpful in understanding the dynamics of many immune response phenomena.

This paper extends the Fisher-Lauffenburger two-dimensional cell–target encounter time model into three dimensions. Expressions analogous to those developed for the two-dimensional case are derived and applied to the system of neutrophils responding to a bacterial infection in the tissue. The results are compared to those from the two-dimensional system to ascertain the effect of increased dimension on encounter time as well as to examine the relative importance of chemotaxis and cell motility parameters in three dimensions.

MATHEMATICAL MODEL

The development of the three-dimensional model parallels that for the two-dimensional model of Fisher and Lauffenburger (8). We are modeling the encounter process to obtain an equation for the mean time for a cell moving with chemotaxis in three dimensions to reach a target. This encounter time is an average over all possible paths that a cell can take to reach a target from a particular starting position. The targets are assumed to be fixed, and are also assumed to be eliminated upon the first contact with the motile cells. The assumption of fixed targets is warranted when one considers that (*a*) not all bacteria are freely motile, and (*b*) those bacteria that do exhibit motility are severely slowed in the tissue matrix relative to the cells seeking them out. A target is eliminated when the centers of the cell and the target are a distance A apart (see Fig. 3). This distance A is defined as a contact radius, or the sum of the radii of cell and target. For relatively small bacterial targets, A can be approximated by the cell radius, or the effective radius of the moving cell as it extends a pseudopod in the direction of motion.

The region of infection will be modeled as a series of unit spheres of radius R (see Fig. 3), each with a target

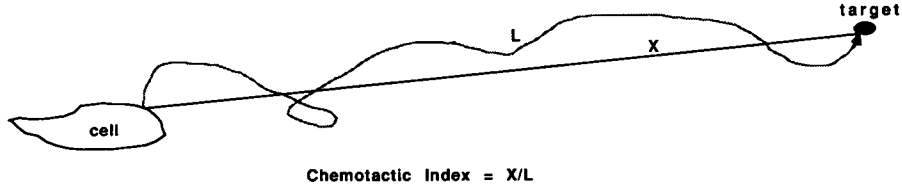

FIGURE 2. Typical cell path. Dotted line represents the cell path; solid line represents straight line distance between cell and target. The chemotactic index is equal to the length of X over the length of L. The time of a step is defined as τ (directional persistence time); the size of a step, δ, is the product of τ and s, the cell speed.

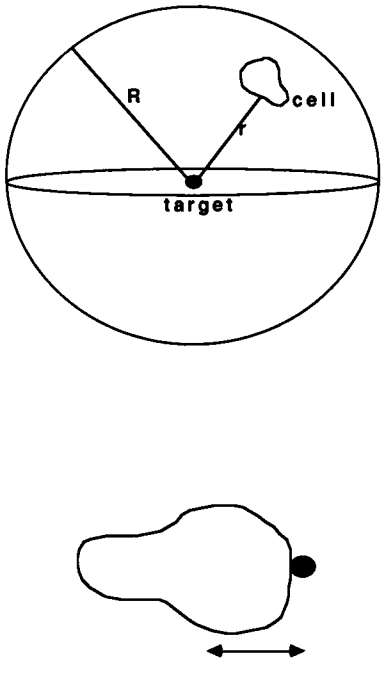

FIGURE 3 Diagram of the unit sphere. A cell's position relative to a target is described in spherical coordinates by r, the radial distance between the cell and target, and θ and ϕ, the angular components of the cell's position relative to some arbitrary plane containing the target. The target is eliminated when the distance between its center and the center of the cell is equal to A, the contact radius.

located at its center. We assume a uniform target distribution, and thus can calculate the radius of each unit sphere by the following: If there are T targets per unit volume, then the number in a unit sphere, 1, is equal to the product of T and the volume of a unit space,

$$1 = T\left(\frac{4}{3}\pi R^3\right) \text{ or } R = \left(\frac{3}{4\pi T}\right)^{1/3}, \qquad (1)$$

where T can be obtained from experimental data. The assumption of uniform target distribution is warranted when one considers an analogous situation described by Berg and Purcell (2), that of a current of molecular ligands to cell surface receptors. They found that this current, when assuming a uniform target distribution, is only minimally different from the current obtained when assuming a random distribution. We will further restrict our attention to cases in which the immune cell density is considerably less than the target density, so that cell competition for targets can be neglected.

For simplicity, we allow a cell to step in any one of the six perpendicular directions available to it in three dimensions, with a probability assigned to each direction (see Fig. 4). As Fig. 3 shows, the position of the cell relative to the target is described in spherical coordinates by r, θ, and ϕ. Motion directly towards or away from the target changes r by the step size, δ, which is defined as the product of the characteristic persistence time, τ, and the cell speed, s. As the persistence time does not depend on the presence of a gradient (18) or the direction of the cell relative to a gradient if present (19), τ is assumed to be constant. We will neglect any chemokinetic effects which would change the cell speed, s, so we may assume constant cell speed. Therefore step size is assumed to be constant.

While the perpendicular steps mainly represent changes in θ and ϕ, they also slightly change r. Fisher and Lauffenburger (8) showed that this small change, dr, is equal to $\delta^2/2r$ for a perpendicular step. By spherical symmetry, this equation holds for steps in any of the four perpendicular directions available to the cell in three dimensions.

Although the probabilities of motion in the six directions can be estimated using experimental data, they depend on the particular type of attractant or gradient used. We will therefore focus on the effect of varying the probabilities on encounter time, rather than on a particular set of probabilities for a given system. The probabilities can be related to other measurable quantities that describe the directedness of the cell's motion; among them, the chemotactic index, CI. The chemotactic index is defined as the net path length traversed by a cell towards a source divided by the total distance travelled (17) (see Fig. 2). A CI of zero (averaged over many cells) would correspond to purely random motion, whereas a CI of one would correspond to perfectly directed motion. A cell taking N steps will take pN steps toward the target, qN steps away from the target, and $4mN$ steps perpendic-

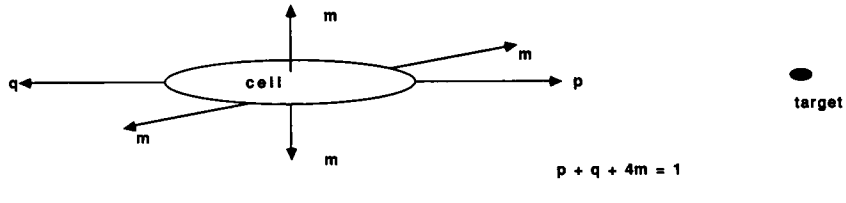

FIGURE 4 Probabilities of motion in three dimensions. Each of the six perpendicular directions has a probability assigned to it.

ular to the target. If we assume that the perpendicular steps cancel over large N, we calculate the net number of steps toward the targets as

$$(p - q)N, \tag{2}$$

and the chemotactic index can therefore be related to the probabilities as:

$$\mathrm{CI} = p - q. \tag{3}$$

It should be emphasized that the chemotactic index does not uniquely describe the cell path, as it is independent of m.

A difference equation for the encounter time of a cell (with a chemotactic component to its motion) reaching a target as a function of the initial distance between them can be derived using a modification of a method used by Berg and Purcell (2). The encounter time, W, for a cell at a given position is equal to the time elapsed as the cell moves to a new position (the persistence time) plus the weighted average of the encounter times associated with the possible new positions. The weighting factors in our case are the probabilities, p, q, and m, of the cell moving to the new positions.

In the limit as step size, δ, becomes much smaller than R, the radius of the unit sphere, and for step times, τ, much smaller than the observation time of the system, the difference equation yields the following differential equation for encounter time as a function of initial position.

$$\frac{d^2W}{dr^2} + \left[\frac{2(q-p)}{\delta(p+q)} + \frac{(1-p-q)}{r(p+q)}\right]\frac{dW}{dr} = \frac{-2}{s^2\tau(p+q)}, \tag{4}$$

with the boundary conditions,

$$W(A) = 0$$

$$dW/dr\,(R) = 0,$$

where s is cell speed, τ is persistence time, δ is step size (equal to $s\tau$), R is the radius of the unit sphere, and A is the contact radius. The encounter time does not depend on the initial angular components of the distance between cell and target relative to some plane. This is a result of the radial symmetry of the concentration profile of the attractants being produced by the target. If we assume low cell and target densities, then this profile will be unaffected by the presence of other cells or targets, and the condition that W is independent of θ and ϕ will hold. For this reason, the angular dependences were neglected in the above derivation.

This equation is analogous to that developed by Fisher and Lauffenburger (8) for the encounter time in two dimensions. The only difference between the two equations lies in the fact that the probabilities change for a given degree of directed motion in going from two dimensions to three dimensions. For example, if CI = 0, in two dimensions, $p = q = m = 0.25$, whereas in three dimensions $p = q = m = 0.17$. As a result, the solution of the differential equation for encounter time in three dimensions is identical to that of the Fisher and Lauffenburger equation for encounter time in two dimensions (except for the differences in probabilities).

The boundary condition at $r = A$ follows from the assumption that a target is eliminated immediately when the distance between its center and that of the phagocytic cell is equal to the contact radius, A. The second boundary condition follows from symmetry, and is most easily understood by noting that the volume of infection is comprised of many adjacent, identical unit spheres. We thus require that the encounter time be continuous from one unit sphere to the next.

Simplification of the analyses to follow is accomplished through dedimensionalization of the encounter time equation. Encounter time is scaled to the cell speed rather than the persistence time, as we expect that τ will be much smaller than W in the limiting case for which the above differential equation was derived. Although there are other choices of scaling factor for W (8), we will use cell speed as it is a measured characteristic of individual cells. The step size, contact radius, and starting distance will all be scaled to the radius of the unit sphere.

The resulting dedimensionalized equation for the encounter time is

$$\epsilon\frac{d^2\omega}{d\rho^2} + \left[2\frac{(q-p)}{(p+q)} + \epsilon\frac{(1-p-q)}{\rho(p+q)}\right]\frac{d\omega}{d\rho} = \frac{-2}{(p+q)}, \tag{5}$$

with the boundary conditions

$$\omega(A/R) = 0$$

$$d\omega/d\rho(1) = 0,$$

where ω is dimensionless encounter time (equal to Ws/R), ρ is r/R, and ϵ is δ/R. Note that the effects of cell speed and persistence time are now incorporated into the single parameter ϵ.

This inhomogenous, second-order linear equation can be solved using a variation of parameters method (3). The result is a closed form solution made up of three integrals that must be evaluated numerically for most values of the parameters (ϵ, p, and q) involved. The final equation for dimensionless encounter time as a function of starting position is

$$\omega = \frac{2}{\epsilon(p+q)}\left[\int_{A/R}^{\rho} e^{-k\zeta}\zeta^{-b}\int_{A/R}^{\zeta} e^{ks}s^{-b}\,ds\,d\zeta + \left(\int_{A/R}^{\rho} e^{ks}s^{-b}\,ds\right)\left(\int_{\rho}^{1} e^{-k\zeta}\zeta^{-b}\,d\zeta\right)\right], \tag{6}$$

where

$$k = \frac{2(p - q)}{\epsilon(p + q)}$$

$$b = \frac{1}{(p + q)} - 1.$$

These integrals can be evaluated using the IMSL quadrature routines DBLIN or DMLIN, depending on the form of the integrand.

Certain sets of the probabilities, p, q, and m, permit an exact solution of this differential equation to be obtained. The case of one-dimensional motion ($m = 0$) was described by Fisher and Lauffenburger (8). Clearly, as the model equation of Fisher and Lauffenburger for two-dimensional encounter differs from our model equation only in the values for the parameters p, q, and m, our model reduces to the same result in one dimension. A second simplifying case, in which $p = q$, is slightly different in three dimensions.

The solution of Eq. 5 when $p = q = m = 1/6$ corresponds to purely random motion of a cell. The result obtained is exactly the same as that obtained by Berg and Purcell (2) for their description of random particle motion,

$$W = \frac{1}{s^2\tau}\left(\frac{2R^3}{A} - \frac{2R^3}{r} + A^2 - r^2\right). \tag{7}$$

However, if p and q are equal but not equal to $1/6$, we obtain the following equation for encounter time:

$$\omega = \frac{2}{\epsilon}\left\{\frac{1}{C}[r^C - (A/R)^C] + \frac{1}{2}[(A/R)^2 - \rho^2]\right\}, \tag{8}$$

where

$$C = 2 - \frac{1}{2p}.$$

As expected, this is the same result obtained by Fisher and Lauffenburger (8) for the case where the chemotactic index is zero ($p = q$), but p and q are not equal to $1/4$.

To facilitate the analyses to follow, we can eliminate the dependence of encounter time on position by taking its average over all possible distances between the cell and target:

$$\bar{\omega} = \frac{3\int_{A/R}^{1} \omega(\rho)\rho^2 \, d\rho}{1 - (A/R)^3}. \tag{9}$$

RESULTS

We now have an expression for the cell–target encounter time as a function of cell speed and persistence time (cell motility parameters), the probabilities of motion towards and away from the target (directional bias parameters), and the dimensions of the volume over which the cell searches for its target (unit sphere parameters).

To evaluate the effects that each of these parameters have on encounter time, we must obtain reasonable estimates for each parameter, so as to hold constant all of the parameter values save that which we are studying. Table 1 presents the parameter values used by Fisher and Lauffenburger (8) to model the system of alveolar macrophages, as well as the values used in this analysis to model the system of neutrophils operating in the tissues. We performed two sets of studies: (*a*) the effect of *dimension* alone on encounter time, and (*b*) the combined effect of *dimension* and *parameter changes* on encounter time.

The first study was performed by applying the three-dimensional model with the parameters used by Fisher and Lauffenburger to model the alveolar macrophages. This is equivalent to the hypothetical case of placing these macrophages in a three-dimensional setting where they would perceive bacterial volume densities that yield a unit

TABLE 1 Parameter estimates

Parameter	Alveolar macrophage/lung surface	Neutrophil/connective tissue	References
R	500 μm	50 μm	8, 14*
δ	10 μm	10 μm	8‡
ϵ	0.02	0.2	§
s (wide range)	1–10 μm/min	2–10 μm/min	8, 11–13, 17
s (small range)	1–3 μm/min	2.5–7.5 μm/min	8, 11–13, 17
τ	1–10 min (5 min)	1–5 min (2 min)	8, 11–13, 28
A	10 μm	10 μm	8‖

*Fisher and Lauffenburger calculated R from bacterial densities on the lung surface; we calculate R from bacterial densities in the tissue (see Eq. 1).

‡Fisher and Lauffenburger calculate $\delta = s\tau$ = (2 μm/min)(5 min) = 10 μm, for ease of comparison, we choose (5.0 μm/min)(2 min) = 10 μm.

§Recall that $\epsilon = \delta/R$.

‖As the contact radius varies, for ease of comparison we choose its value to be that utilized by Fisher and Lauffenburger. Note in the text that the exact value of A does not noticeably affect the results, so the value here is not an unreasonable choice for neutrophils.

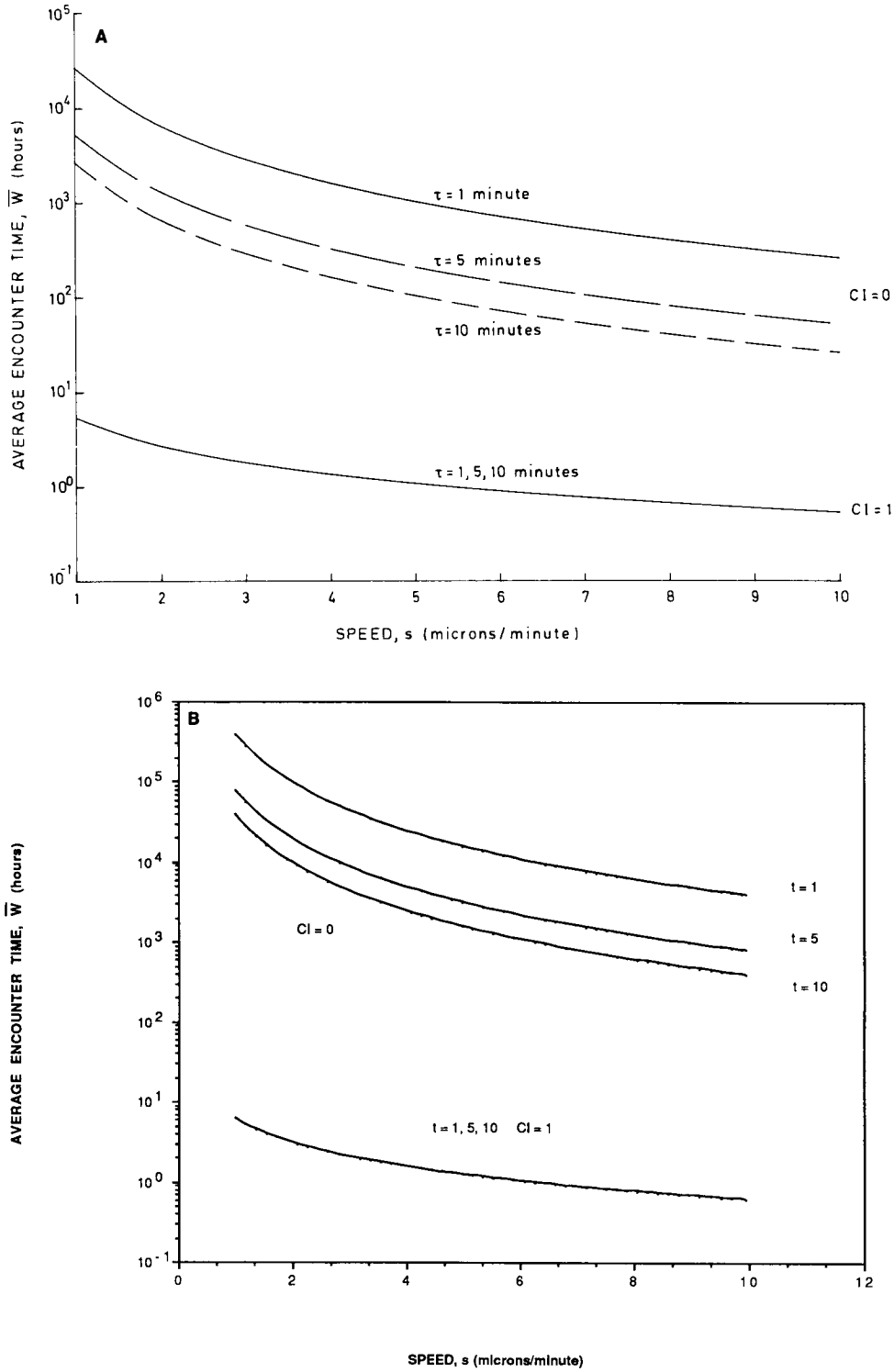

FIGURE 5 Effect of cell speed on average encounter time. Curves are parameterized in persistence time, τ, and CI. (*A*) 2-D model (8), $R = 500\ \mu m$, $A/R = 0.02$. (*B*) 3-D model, $R = 500\ \mu m$, $A/R = 0.02$. (*C*) 3-D model, $R = 50\ \mu m$, $A/R = 0.2$.

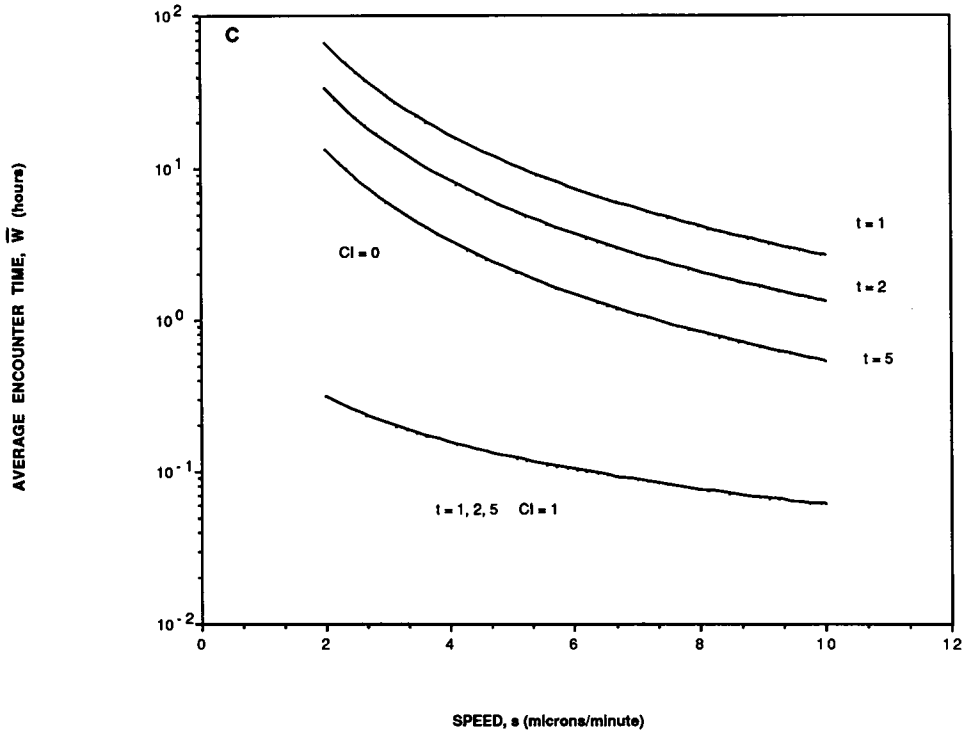

FIGURE 5 (continued)

sphere radius equal to the unit circle radius obtained from the bacterial surface densities on the lung surface. The second study involved applying the three-dimensional model to the system of neutrophils operating in the tissues, and thus all three parameter types correspond to those of neutrophils operating in the tissues. For comparison, in each parametric analysis, the figures from Fisher and Lauffenburger (8) are shown, followed by figures reflecting the two studies described above.

We first examined the effects of cell speed and persistence time on encounter time (Fig. 5). The speeds and persistence times shown in the figure are characteristic for neutrophils (11–13, 18, 29). In all three systems an increase in either speed or persistence time decreases the encounter time. Encounter time for purely random motion is more sensitive to changes in cell speed and persistence time than encounter time for perfectly directed motion. In going from two to three dimensions, the encounter time increases by an order of magnitude. This effect is also more pronounced in the limit of purely random motion, for, as the chemotactic index approaches unity (perfectly directed motion), the cell's path toward the target approaches a straight line. As this is the case regardless of dimension, the effect of an increase from two to three dimensions is not seen to effect the encounter times for perfectly directed motion, whereas the effect on purely random motion is noticeable.

Note also that when the intrinsic neutrophil/tissue parameters are applied, the model predicts encounter times orders of magnitude *lower* than for the macrophage/surface parameters. This is primarily due to the reduction in the size of the unit sphere radius, R. Observe that at specified values of both speed and persistence time, the neutrophil/tissue encounter time is much lower than that for the alveolar macrophages (at CI = 0). Thus, only a unit space parameter could have produced this effect.

Another important observation is that whereas for the macrophage/surface case increases in cell speed (at CI = 0) do not bring the encounter times close to that for purely directed motion, in the neutrophil/tissue case increases in cell speeds can produce much of the same beneficial effect as purely directed motion. Neutrophils can exhibit cell speeds even higher than those in the range of Fig. 5. Clearly, high cell speeds for CI = 0 (purely random motion) can decrease the encounter time to that predicted at lower cell speeds for CI = 1 (perfectly directed motion). Fig. 9, which will be discussed in greater detail later, also presents the effect of cell speed nicely. Even moderate cell speeds (on the neutrophil speed scale) at CI = 0 can reduce the encounter time to that predicted at lower cell speeds for CI = 1. For great initial target densities, increases in cell speed may even decrease the encounter time for purely random motion to

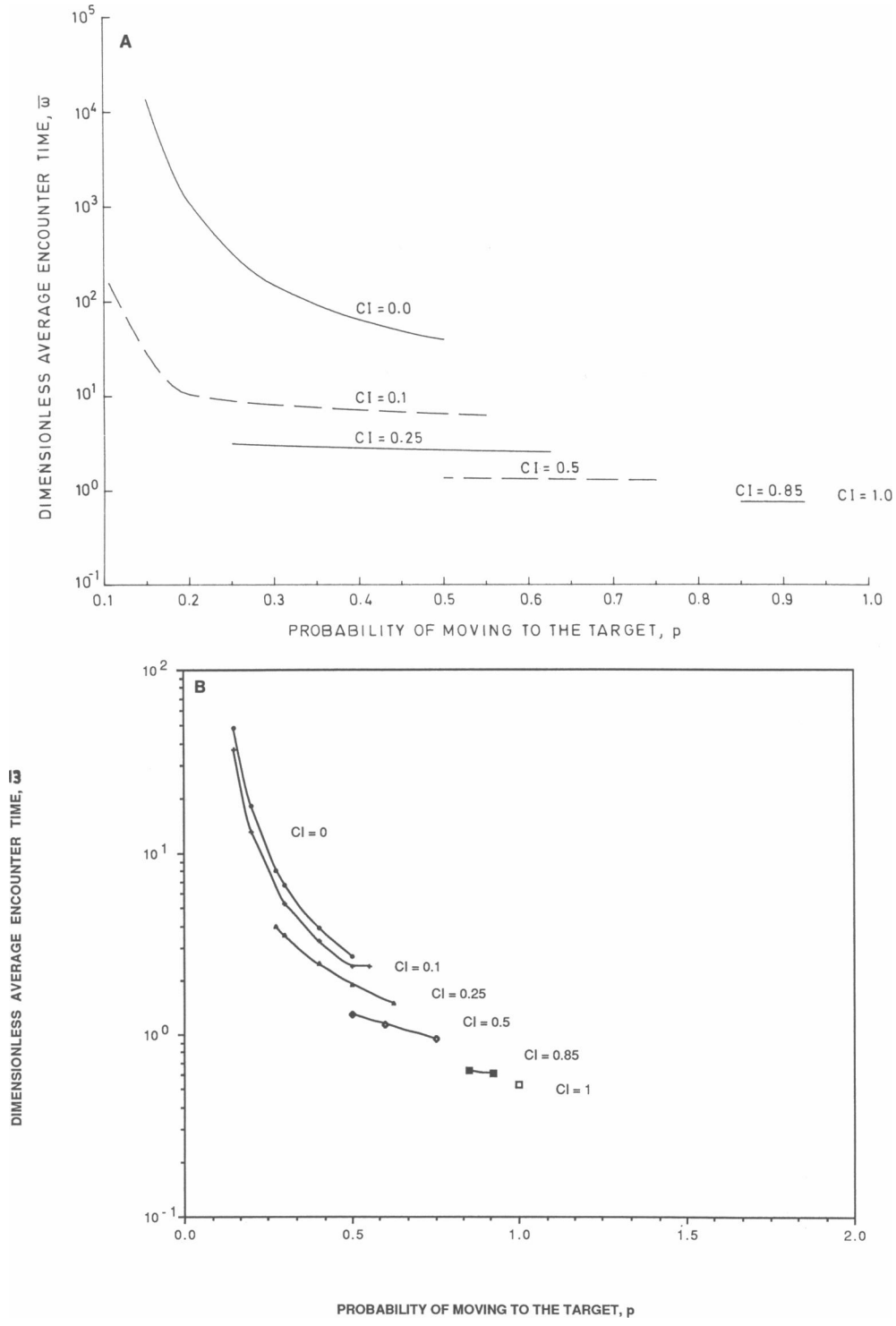

FIGURE 6 Effect of the probability of a cell moving directly towards a target on dimensionless average encounter time. Curves are parameterized in CI. CI = $p - q$. (*A*) 2-D model (8), $A/R = 0.02$, $\epsilon = 0.02$ and 3-D model, $A/R = 0.02$, $\epsilon = 0.02$. (*B*) 3-D model, $A/R = 0.2$, $\epsilon = 0.2$.

a value *below* that predicted at lower cell speeds for CI = 1. Clearly, increases in cell speed in response to pathogenic challenges are of extreme benefit in the neutrophil/tissue situation. This was not the case for the macrophage/surface situation.

The cell motility parameters clearly have a pronounced effect on the encounter time. The directional bias parameters show a substantial effect on encounter time as well. Consider Fig. 6, in which the encounter time is plotted versus the probability of motion directly toward the target, p. The curves are parameterized in values of chemotactic index ranging from zero to one. The encounter time is seen to be more sensitive to changes in p at lower values of CI than at values of CI corresponding to more directed motion. Fig. 6 *A* represents both the 2-D and 3-D case with alveolar macrophage/lung surface parameters, as a given value of CI (equal to $p - q$) and p, as well as constant values for the cell motility parameters, completely determines the encounter time. Fig. 6 *B* differs solely as a result of changes in the unit space radius. Fisher and Lauffenburger (8) noted that there were dramatic decreases in encounter time with increased CI up to a value of ~0.2. Fig. 6 *B* shows that this beneficial effect on encounter time for the neutrophil/tissue case is not realized until the value of CI approaches ~0.6–0.8.

By selecting the value of m, one can reduce the problem of describing the cell's motion from having two degrees of freedom to one. Following the arguments of Fisher and Lauffenburger (8), we choose the value of m to be equal to that of q. They base this decision on the fact that choosing m to be equal to q reduces the number of degrees of freedom in describing cell motion from two to one.

Having done this, we can now analyze the effect of chemotactic index on encounter time. In Fig. 7, we plot the ratio of encounter time to that encounter time corresponding to purely random motion. For alveolar macrophages operating in 2-D, an increase in chemotactic index from 0 to 0.2 brings about a two-orders-of-magnitude decrease in encounter time. Note that to obtain the same two orders of magnitude decrease in encounter time obtained by alveolar macrophages at CI = 0.2, the neutrophils must exhibit CI values of 0.6–0.8, which is the range of values obtained experimentally (12, 18, 28). Once again, this appears to be primarily due to the reduction of the unit space radius, R. In Fig. 8, which will be further described shortly, we note that at the higher

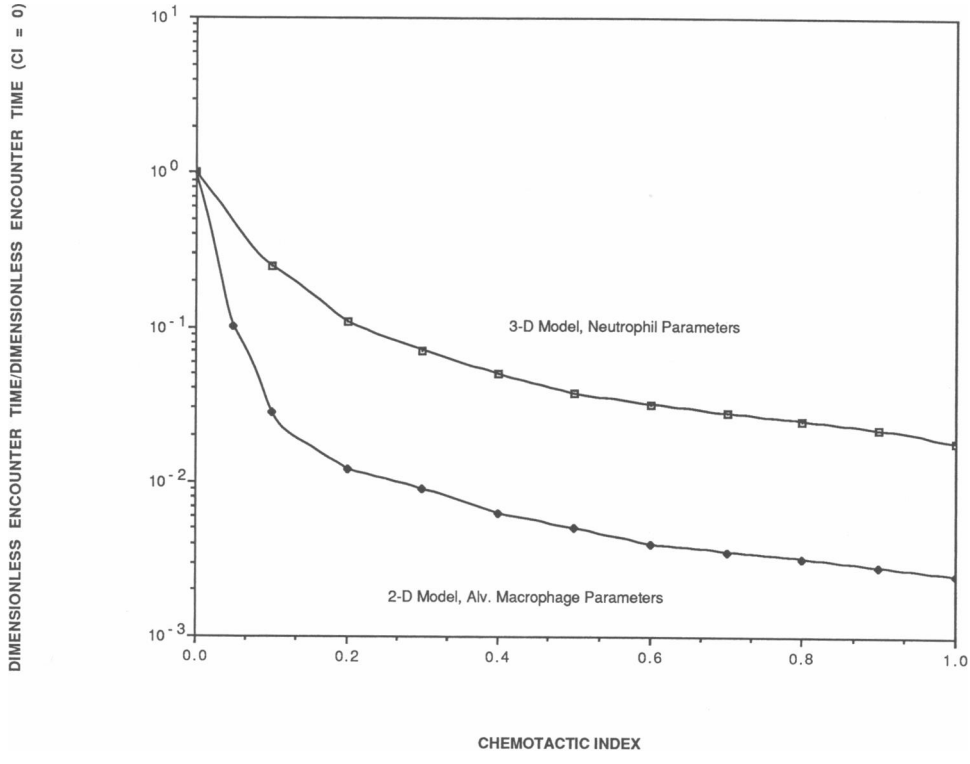

FIGURE 7 Effect of chemotactic index on relative dimensionless average encounter time. Curves representing both alveolar macrophage/lung surface parameters and neutrophil/connective tissue parameters are plotted as the ratio of dimensionless average encounter time to that dimensionless average encounter time corresponding to purely random motion versus chemotactic index.

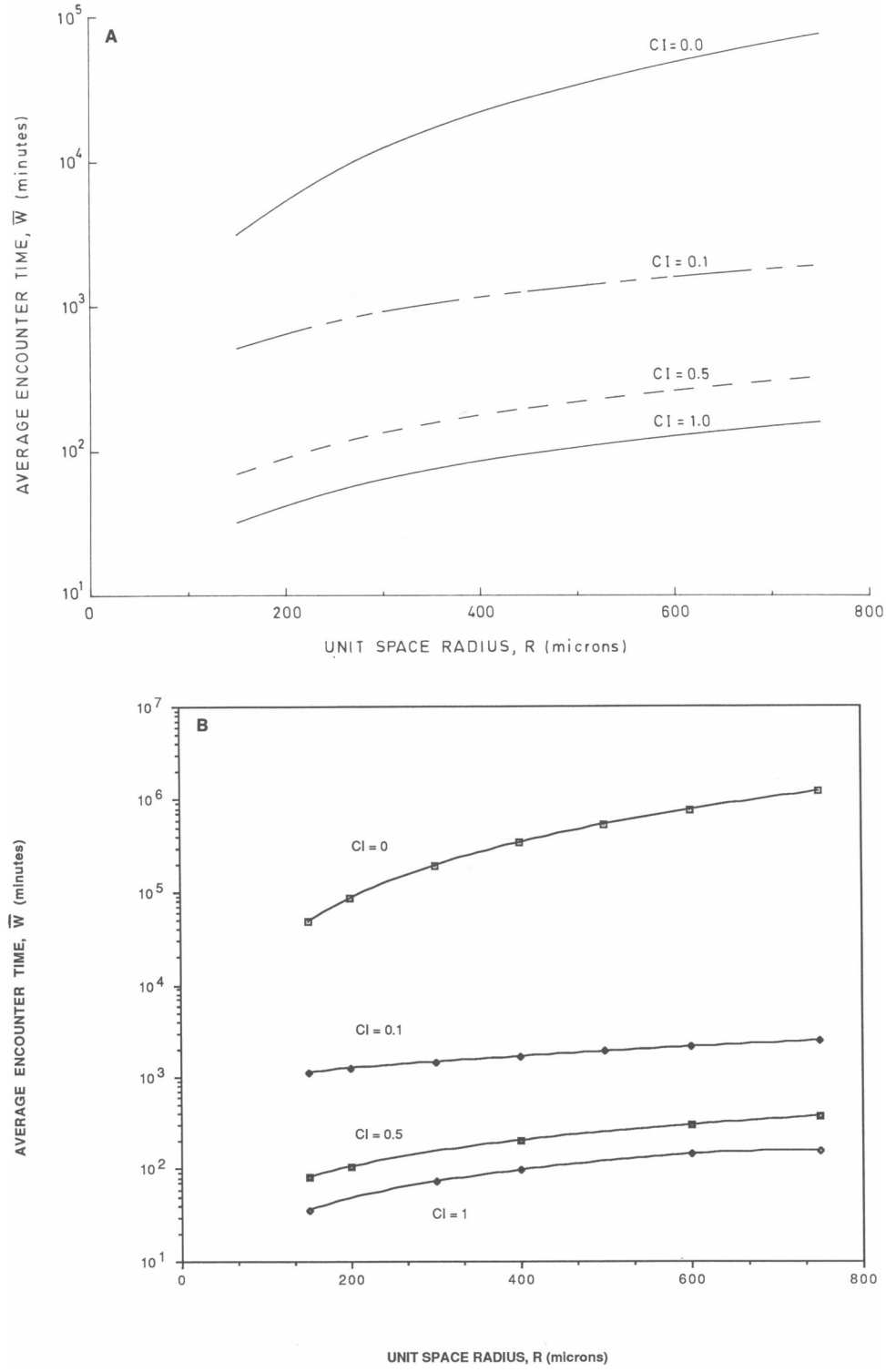

FIGURE 8 Effect of unit space radius on average encounter time. Curves are parameterized in CI. (*A*) 2-D model (8). (*B* and *C*) 3-D model. For all plots: $q = m$, $s = 3\ \mu m/min$, $\tau = 5$ min, $A/R = 0.2$.

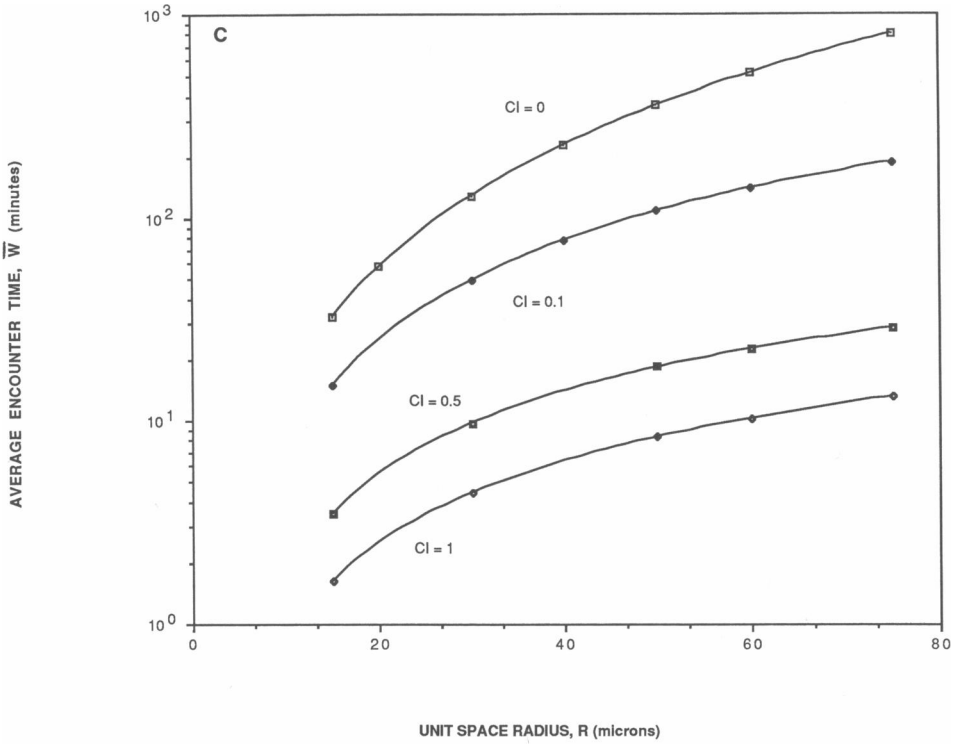

FIGURE 8 (continued)

values of R dealt with by the lung macrophages, a two-orders-of-magnitude decrease in encounter time can be achieved with an increase in CI from 0 to 0.2 (which is in their experimental range [24]). For values of R characteristic of target densities encountered by neutrophils, this decrease is not achieved until values of CI = 0.6–0.8. Because all other parameters have been held constant for these figures, this effect can only be a result of the reduction in unit space radius, R.

We have now seen that both cell motility parameters as well as directional bias parameters can have substantial effects on the encounter time. We now turn our attention to the unit space parameters. We intuitively expect that the encounter time will increase with increasing starting distance from the target. Although encounter time is fairly sensitive to changes in starting distance, this is not the case for contact radius. Motile cells ruffle their membranes and extend pseudopods in the direction of their motion (1); this, as a result, slightly changes the effective contact radius. Although the encounter time decreases with increased contact radius, this effect is small compared with that of the other parameters discussed. Thus the assumption of constant contact radius should apply even though this parameter varies for moving cells. Variations in contact radius do not dramatically affect our results (computations not shown).

Fig. 8 shows the effect of unit sphere radius on encounter time. Encounter time increases with unit sphere radius (decreased target density), and decreased radius actually can provide much of the same beneficial effect on encounter time in three dimensions as cell speed, i.e., a decrease in R for purely random motion can bring the encounter time down to a value comparable with that for directed motion.

We have thus examined the effect of each parameter type on encounter time. Most of the parameters have substantial effect on the encounter time, with the exception of contact radius, A, which has a relatively minor effect. Clearly, increased speed or persistence, increased directional bias, and decreased unit sphere radius have the greatest beneficial effect on encounter time.

DISCUSSION

Fisher and Lauffenburger (8) derived a model for cell–target encounter times in two dimensions. Their model showed that encounter could be considered the rate-limiting step in the immune response of these macrophages, and that the rate of clearance of bacteria by the macrophages could be explained by measured motility and chemotaxis properties (9). We have extended the Fisher-Lauffenburger model into three dimensions and applied it to the system of neutrophils operating in the

tissue, a three-dimensional system. The equation which describes three-dimensional encounter time as a function of starting distance from the target has exactly the same form as the equation for two-dimensional encounter time. The difference between the two equations lies in the values of the probabilities of motion towards and away from the target, p and q.

The encounter time was found to increase by an order of magnitude with the increase in dimensionality, all other parameters being held constant. This effect was

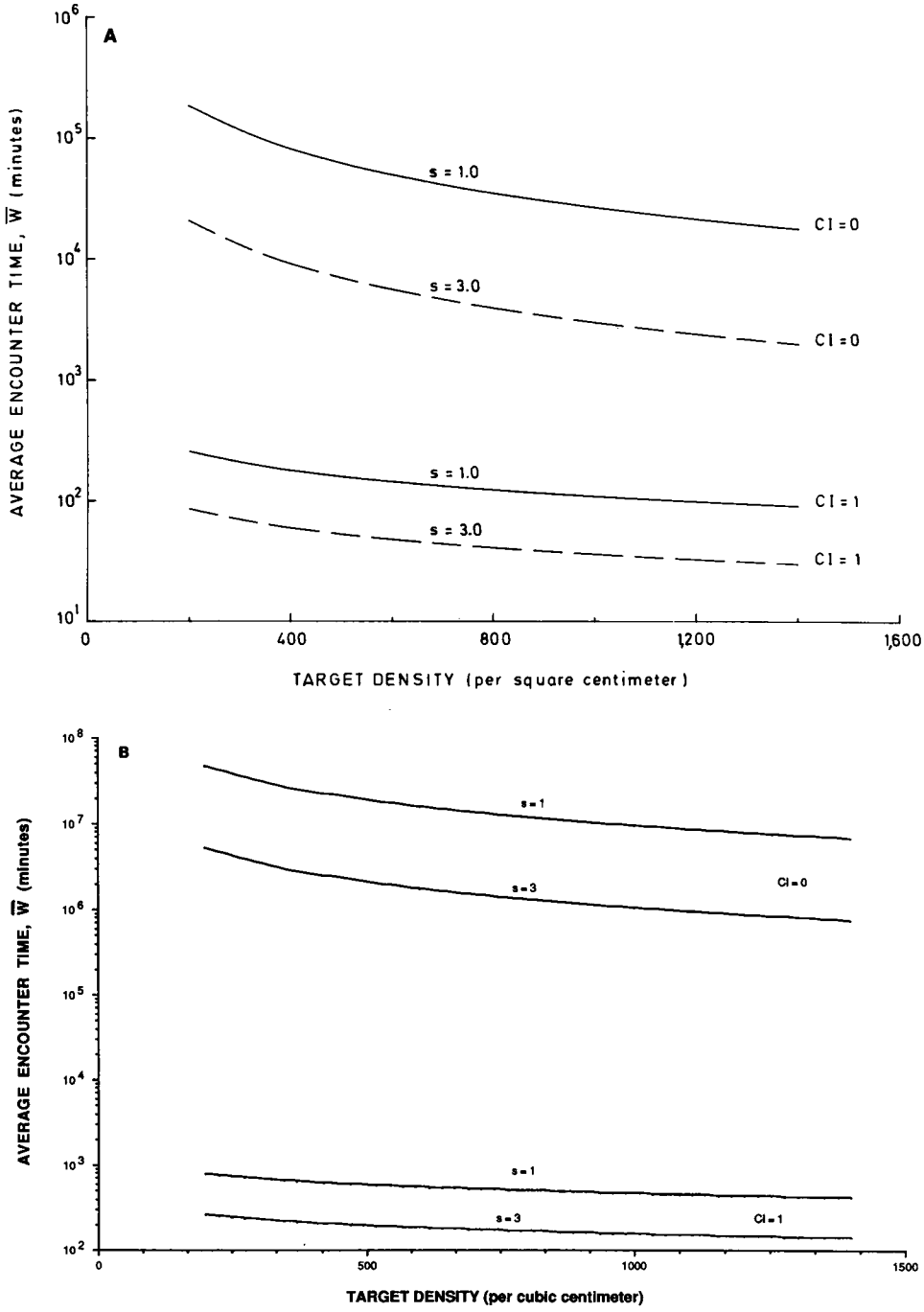

FIGURE 9 Effect of target density on average encounter time. Curves are parameterized in cell speed and in CI. (A) 2-D model (8), $A = 10\ \mu m$, $\tau = 5$ min. (B) 3-D model, $A = 10\ \mu m$, $\tau = 5$ min. (C) $A = 10\ \mu m$, $\tau = 2$ min.

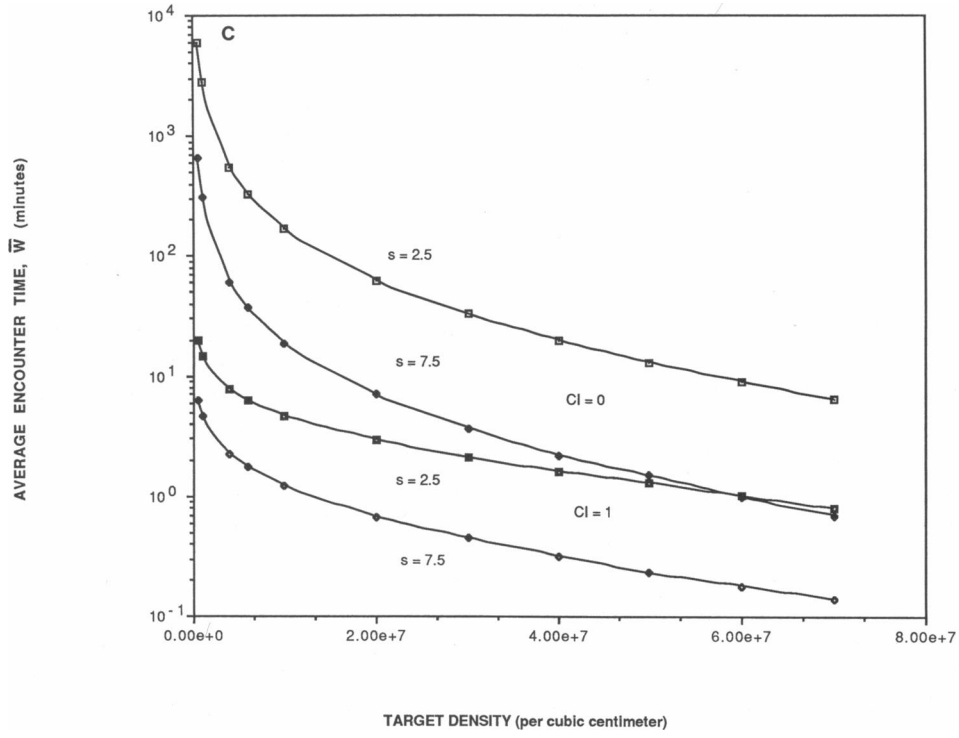

FIGURE 9 (continued)

most pronounced near the limit of purely random motion, as near the limit of perfectly directed motion, the cell path toward the target becomes more one-dimensional, and the effect of increased dimensionality is thus lessened.

The results shown in Fig. 9 help provide a plausible rationalization for the strong chemokinetic behavior (i.e., dependence of speed on attractant concentration) observed for neutrophils (30). Note that the highest target density, $8 \times 10^7/cm^3$, corresponds to a volume fraction of 5.24×10^{-3} (assuming an average bacterial radius of 5 μm), so the assumption that target density is small is valid even at the highest target density in Fig. 9. Fig. 9 *C* shows that, in three dimensions, with target densities in the range that neutrophils might encounter in the tissues, increased cell speed can provide much of the same beneficial effect as nearly perfect directed motion. That is, the curve for speed equal to 7.5 μm/min and CI = 0 almost superimposes with the curve for speed equal to 2.5 μm/min at CI = 1, except for low target densities. Fig. 9 *A*, in contrast, shows that this is not the case for alveolar macrophages, which do not show as strong a chemokinetic response (10).

The model also provides a basis for the physiological utility of the relatively high chemotactic indices exhibited by neutrophils of ~0.6–0.8 (12, 18, 28). This increase in CI is necessary to achieve the same beneficial effect (two orders of magnitude decrease in encounter time) in three dimensions that alveolar macrophages achieve in two dimensions with CI = 0.2 (which, interestingly, is roughly that experimentally observed for these lung cells [24]). Neutrophils undergo a three-dimensional search for their targets in the tissue. The path toward a particular target may involve passage through the tissue matrix as well as crawling along vessel walls or between tightly packed regions of tissue. Although the mechanism of movement in each of these cases may differ considerably, the model predicts that the chemotactic index in either case should fall between 0.6 and 0.8, as this is the CI necessary to obtain a two-orders-of-magnitude decrease in encounter time for a neutrophil searching for its target in three dimensions regardless of the mechanism used to find this target. This is indeed the case. Buettner et al. (5), using the millipore filter assay in which the neutrophils migrate in three dimensions through a nitrocellulose filter, obtained a value for the chemotactic sensitivity, χ_0, of 6×10^{-6} cm. Tranquillo et al. (25) used the linear under agarose migration assay, in which the neutrophils migrate in two dimensions on a glass slide under an agarose slab, to determine a value for $\chi_0 = 4 \times 10^{-6}$ cm. As the chemotactic sensitivity can be related to CI (22), these results together show that the neutrophils do exhibit similar values of CI regardless of the mechanism of movement used to find their targets in the three-dimensional tissue matrix. As alveolar macrophages are

specialized for elimination of targets in a two-dimensional space, they beneficially exhibit the lower value of CI predicted by the model. One might then expect macrophages specialized for target elimination within tissue spaces to exhibit values for CI which would provide the two-orders-of-magnitude decrease in encounter time given values for their particular cell speed and persistence time. Unfortunately, rigorous quantitative data on speed, persistence time, and chemotactic orientation bias are not, to our knowledge, available for phagocytic cells other than the alveolar macrophages and neutrophils we have discussed here.

Our model predicts shorter encounter times for the neutrophil/tissue case than for the macrophage/surface case, dur primarily to the greater bacterial densities (and thus lower unit sphere radii) encountered in the tissue by neutrophils. This leads to an important observation: that encounter might not be a rate-limiting step in neutrophil response as it is for alveolar macrophage response (8). Fig. 9 shows the effect of target density on encounter time. The curves are parameterized in cell speeds characteristic for the respective cell types in question (8, 11–13, 18). Fisher and Lauffenburger (8) concluded that even at maximum cell speed and with perfectly directed motion, alveolar macrophage encounter time was still much greater than the phagocytosis times for these cells, roughly a few minutes (15). This is confirmed in Fig. 9 *A*. The characteristic phagocytosis time for neutrophils is somewhat lower, on the order of 1 min (15), but the encounter times are also much lower, except at low target densities. As some neutrophils can exhibit speeds roughly two times the maximum speed shown in Fig. 9 *C* (11–13, 18), we see that encounter times for neutrophils are actually of about the same order of magnitude as phagocytosis times. Encounter apparently can therefore *not* be considered the rate-limiting step in the neutrophil response. Our finding here that encounter may not be rate-limiting for neutrophil phagocytosis of bacteria in typical infectious challenges has possibly important implications for correlating in vitro measured defects in motility and chemotaxis properties with in vivo functions of host defense against infection. That is, a quite severe cell motility defect, which increases the encounter time by at least an order of magnitude, may be necessary for the inflammatory response to be noticeably impaired. In addition, it would be reasonable to expect defects in the mechanism of chemotaxis to be more detrimental to function of alveolar macrophages, for which encounter is rate-limting, than to tissue macrophages or neutrophils, for which encounter is apparently not as likely to be rate-limiting. It must be emphasized, though, that the target (bacterial) density can greatly affect these conclusions. The lower the target density, the more likely encounter will be rate-limiting (see Fig. 9).

As a result of the fact that encounter may not be the rate-limiting step in neutrophil response, a model including both the encounter time component presented here as well as a component based on data available for the phagocytosis rate of neutrophils in response to various infections (16, 27) would have to be developed to study the overall dynamics of neutrophil response in a way similar to that used by Fisher et al. (9) to study lung clearance by alveolar macrophages. The encounter time calculated here would be inverted to obtain a rate constant for the encounter process, which would then give the encounter rate as the product of this rate constant and the cell volume density at any time, t. By then including rate expressions for the phagocytic component of this dynamic model, one could predict the target density as a function of time during the initial stages of infection. As mentioned earlier, modifications would have to be made to the encounter time model if one wished to model the later stages of infection. At that point, the cell density may approach the target density (14), or surpass it, requiring the unit spheres to be based on the cells rather than on the targets. In addition, during the later stages of infection, competition between cells for targets may not be negligible.

Finally, it must be pointed out that for the smaller unit spaces analyzed here in some of the computations, the ratio of unit space radius to cell step size is not large. In this regime, the limit taken to obtain the differential form of the model (Eq. 4) from the difference form may break down. In such a regime, direct solution of the difference form, or alternatively, computer simulations of the moving cells, may yield more exact numerical results. Given the level of approximation involved in the overall model, however, we believe that the results presented here are a very useful first step.

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REFERENCES

1. Allan, R. B., and P. C. Wilkinson. 1978. A visual analysis of chemotactic and chemokinetic locomotion of human neutrophil leukocytes. *Exp. Cell Res.* 111:191–203.
2. Berg, H. C., and E. M. Purcell. 1977. Physics of chemoreception. *Biophys. J.* 20:193–219.
3. Boyce, W. E., and R. C. DiPrima. 1977. Elementary Differential Equations and Boundary Value Problems. John Wiley & Sons, Inc., New York.

4. Brown, C. C., and J. I. Gallin. 1988. Chemotactic Disorders. *Hematol./Oncol.* 2:61–79.

5. Buettner, H. M., D. A. Lauffenburger, and S. H. Zigmond. 1989. Measurement of leukocyte motility and chemotaxis parameters with the millipore filter assay. *J. Immunol. Methods.* 123:25–37.

6. Devreotes, P. N., and S. H. Zigmond. 1988. Chemotaxis in eukaryotic cells: a focus on leukocytes and dictyostelium. *Annu. Rev. Cell Biol.* 4:649–686.

7. Ellis, M., S. Gupta, S. Galant, S. Hakim, C. Vande Ven, C. Toy, and M. S. Cairo. 1988. Impaired neutrophil function in patients with AIDS or AIDS-related complex: a comprehensive evaluation. *J. Infect. Dis.* 158:1268–1276.

8. Fisher, E. S., and D. A. Lauffenburger. 1987. Mathematical analysis of cell-target encounter rates in two dimensions: the effect of chemotaxis. *Biophys. J.* 51:705–716.

9. Fisher, E. S., D. A. Lauffenburger, and R. P. Daniele. 1988. The effect of alveolar macrophage chemotaxis on bacterial clearance from the lung surface. *Am. Rev. Resp. Dis.* 137:1129–1134.

10. Glasgow, J. E., B. E. Farrell, E. S. Fisher, D. A. Lauffenburger, and R. P. Daniele. 1989. The motile response of alveolar macrophages. *Am. Rev. Respir. Dis.* 139:320–329.

11. Gruler, H. 1989. Biophysics of leukocytes: neutrophil chemotaxis, characteristics, and mechanisms. *In* The Neutrophil: Cellular Biochemistry and Physiology. CRC Press, Inc., Boca Raton, FL. 63–95.

12. Gruler, H., and B. D. Bultmann. 1984. Analysis of cell movement. *Blood Cells.* 10:61–77.

13. Lackie, J. M., and P. C. Wilkinson. 1984. Adhesion and locomotion of neutrophil leukocytes on 2-D substrata and in 3-D matrices. *In* White Cell Mechanics: Basic Science and Clinical Aspects. 237–254.

14. Lauffenburger, D. A., and C. R. Kennedy. 1981. Analysis of a lumped model for tissue inflammation dynamics. *Math. Biosci.* 53:189–221.

15. Leijh, P. C. J., M. T. Van den Barselaar, and R. Van Furth. 1981. Kinetics of phagocytosis and intracellular killing of *Staphylococcus aureus* and *Escherichia coli* by human monocytes. *Scand. J. Immunol.* 13:159–174.

16. Leijh, P. C. J., M. T. Van den Barselaar, T. L. Van Zwet, I. Dubbeldeman-Rempt, and R. Van Furth. 1979. Kinetics of phagocytosis of *Staphylococcus aureus* and *Escherichia coli* by human granulocytes. *Immunology.* 37:453–465.

17. McCutcheon, M. 1946. Chemotaxis in leukocytes. *Physiol. Rev.* 26:319–336.

18. Maher, J., J. V. Martell, B. A. Brantley, E. B. Cox, J. E. Niedel, and W. F. Rosse. 1984. The response of human neutrophils to a chemotactic tripeptide (*N*-formyl-methionyl-leucyl-phenylalanine) studied by microcinematography. *Blood.* 64:221–228.

19. Nossal, R., and S. H. Zigmond. 1976. Chemotropism indices for polymorphonuclear leukocytes. *Biophys. J.* 16:1171–1182.

20. Paul, W. E. 1984. Fundamental Immunology. Raven Press, New York.

21. Ricevuti, G., and A. Mazzone. 1987. Clinical aspects of neutrophil locomotion disorders. *Biomed. & Pharmacother.* 41:355–367.

22. Rivero, M. A., R. T. Tranquillo, H. M. Buettner, and D. A. Lauffenburger. 1990. Transport models for chemotactic cell populations based on individual cell behavior. *Chem. Eng. Sci.* In press.

23. Torre, D., S. Rossi, C. Sampietro, M. Broggini, and C. Quadrelli. 1988. Defective lymphocyte chemotaxis in patients with AIDS. *J. Infect. Dis.* 158:1398–1399.

24. Tranquillo, R. T., E. S. Fisher, B. E. Farrell, and D. A. Lauffenburger. 1988. A stochastic model for chemosensory cell movement: application to neutrophil and macrophage persistence and orientation. *Math. Biosci.* 90:287–303.

25. Tranquillo, R. T., S. H. Zigmond, and D. A. Lauffenburger. 1988. Measurement of the chemotaxis coefficient for human neutrophils in the under-agarose migration assay. *Cell Motil. Cytoskeleton.* 11:1–15.

26. Van Dyke, T. E. 1985. Role of the neutrophil in oral disease: receptor deficiency in leukocytes from pateints with juvenile periodontitis. *Rev. Infect. Dis.* 7:419–425.

27. Verbrugh, H. A., R. Peters, P. K. Peterson, and J. Verhoef. 1978. Phagocytosis of staphylococci by human polymorphonuclear and mononuclear leukoctyes. *J. Clin. Pathol. (Lond.).* 31:539–545.

28. Zigmond, S. H. 1974. Mechanisms of sensing chemical gradients by polymorphonuclear leukocytes. *Nature (Lond.).* 249:450–452.

29. Zigmond, S. H., R. D. Klausner, R. Tranquillo, and D. A. Lauffenburger. 1985. Analysis of the requirements for time-averaging of the receptor occupancy for gradient detection by polymorphonuclear leukocytes. *In* Membrane Receptors and Cellular Regulation. Alan R. Liss, Inc., New York. 347–356.

30. Zigmond, S. H., H. I. Levitsky, and B. J. Kreel. 1981. Cell polarity: an examination of its behavioral expression and its consequences for polymorphonuclear leukocyte chemotaxis. *J. Cell Biol.* 89:585–592.