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Extracellular matrix-mediated chemotaxis can impede cell migration

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Cells use a combination of changes in adhesion, proteolysis and motility (directed and random) during the process of migration. Proteolysis of the extracellular matrix (ECM) results in the creation of haptotactic gradients, which cells use to move in a directed fashion. The proteolytic creation of these gradients also results in the production of digested fragments of ECM. In this study we show that in the human fibrosarcoma cell line HT1080, matrix metalloproteinase-2(MMP-2)-digested fragments of fibronectin exert a chemotactic pull stronger than that of undigested fibronectin. During invasion, this gradient of ECM fragments is established in the wake of an invading cell, running counter to the direction of invasion. The resultant chemotactic pull is anti-invasive, contrary to the traditional view of the role of chemotaxis in invasion. Uncontrolled ECM degradation by high concentrations of MMP can thus result in steep gradients of ECM fragments, which run against the direction of invasion. Consequently, the invasive potential of a cell depends on MMP production in a biphasic manner, implying that MMP inhibitors will upregulate invasion in high-MMP-expressing cells. Hence the therapeutic use of protease inhibitors against tumours expressing high levels of MMP could produce an augmentation of invasion.

Keywords: chemotaxis; invasion; mathematical modelling

1. INTRODUCTION

The migration of cells, a fundamental process in health and disease, is seen in a large number of settings, such as inflammation, metastasis and placentation. A critical feature of cell migration is the ability of the migratory cells to digest extracellular matrix (ECM) as they move through it, by secreting enzymes such as matrix metalloproteases (MMPs). Invasive cells *in vivo* adhere to surrounding ECM molecules via specific receptors such as integrins, and respond to signals transduced by this interaction, together with cytokine and growth-factor signals, to produce and secrete MMPs and other proteases (Seftor *et al*. 1992). The consequent digestion of the ECM allows the cells to move into the spaces thereby created and also sets up a positive gradient, which the cells then exploit to move forwards. Movement up concentration gradients of ECM has been previously reported as a mechanism enabling movement through tissues of a variety of cell types, including neutrophils in inflammation, malignant cells during metastasis, and trophoblasts during placentation (Hynes 1994).

The term chemotaxis is used to describe cell motility in response to a gradient of a soluble attractant. In contrast, haptotaxis denotes motility towards insoluble, substratum-bound attractants such as laminin and fibronectin. The differences between chemotaxis and haptotaxis have been highlighted by the demonstration that separate domains on the NH_2- and COOH-terminal regions of the thrombospondin molecule are responsible for chemotaxis and haptotaxis, respectively, and that the two processes can be differentially inhibited by antibodies to the relevant sites (Taraboletti *et al*. 1987). Further studies have shown that chemotaxis to type-IV collagen was profoundly inhibited by pertussis toxin treatment in A2058 cells, whereas haptotaxis remained largely unaffected (Aznavoorian *et al*. 1990). The insensitivity of haptotaxis to pertussis toxin suggests that chemotaxis and haptotaxis are induced by distinct transduction mechanisms. The significance of haptotactic migration is apparent when considering that, during the process of tumour invasion and metastasis, proteolytic degradation of fixed ECM proteins results in the creation of gradients, which the cells can use to invade in a directed fashion (Liotta 1986). However, this digestion also generates soluble ECM fragments, in the wake of the invading cells, whose role has not previously been considered.

In this paper, we consider the way in which chemotaxis and haptotaxis conspire to regulate invasion. In § 2, we present experimental evidence indicating that both

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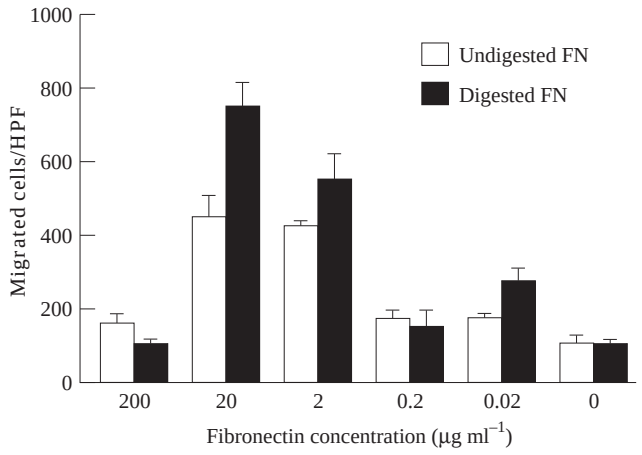

Figure 1. The augmentation of fibronectin-induced chemotaxis through MMP-2 digestion. Chemotaxis was assayed in duplicate by using 24-well chemotaxis dual chambers (Falcon) as previously described (Aznavoorian *et al*. 1990). Briefly, filters were precoated with type-IV collagen (Sigma) (10 μg ml^{-1} in 0.1 M acetic acid) to enhance cell adherence. The chemoattractants (intact and MMP-2-digested fibronectin (Sigma)), dissolved in DMEM with 0.1% BSA, were added to separate lower wells in the indicated concentrations; the upper wells received 10^5 HT1080 cells (ATCC CCL-121) in 200 μl of DMEM with 0.1% BSA(GIBCO-BRL). The MMP-2 was a gift from British Biotech, Oxford, UK. The chambers were incubated at 37 °C with 5% CO_2 for 4 h. Filters were then processed and migration quantitated by averaging migrated cell counts in nine high-power fields (×40). The functions $\chi(c)$ and $\psi(s)$ (discussed in § 3), which represent the modulation of chemotaxis and haptotaxis, were extracted from the data as $\chi(c) = -0.074c^2 + 14.65c + 102.1$ and $\psi(s) = -0.151s^2 + 24.9s + 146.6$ by using a least-squares quadratic fit.

processes are significant for one particular cell–matrix system. Building on these observations, we then (§ 3) develop a mathematical model for the invasive process in one space dimension. In § 4, we return to our experimental system to test the predictions of the mathematical model.

2. CHEMOTACTIC EFFECTS OF DIGESTED FIBRONECTIN

To test the potential for products of ECM degradation to affect cell movement, we used HT1080 cells in a standard dual-chamber chemotaxis assay (Aznavoorian *et al*. 1990). In these assays, two compartments, one containing the cells and the other containing the chemoattractant, are separated by a polycarbonate filter with a uniform pore size, which is smaller than the diameter of the cells. As a chemotactic gradient is established across the filter the cells migrate from the upper to the lower compartment. Several cells (e.g. neutrophils (Springer 1990) and A2058 (Melchiori *et al*. 1995)) have previously been shown to respond to chemotactic stimulation in such dual-chamber chemotaxis assays. Our studies were conducted with HT1080 cells, a rapidly proliferating human fibrosarcoma cell line that has been shown to respond chemotactically to ECM gradients of type-IV collagen and fibronectin (Aznavoorian *et al*. 1990). We used fibronectin as our chemotactic substrate because previous checkerboard analysis has shown that in the presence of fibronectin HT1080 cells predominantly show directed motility with minimal interference from chemokinetic movement (Aznavoorian *et al*. 1990). The HT1080 cells bind to fibronectin through specific receptors, which can be blocked by monoclonal antibodies against the α_5 domain of integrins (Deryugina *et al*. 1996). In our experiments we compared the migratory response of HT1080 cells to intact and MMP-2-digested fibronectin. The digestion of fibronectin by MMP-2 produced fragments of different sizes when seen on SDS–PAGE.

These fibronectin fragments pooled together were strongly chemotactic, with chemotactic potency increased by digestion with moderate levels of MMP-2 (50 μg ml^{-1}) (figure 1). The peak migratory response was seen at a fibronectin concentration of 20 μg ml^{-1}, with a further increase in concentration producing a decreased response, probably because of the saturation of receptors. Other instances of the proteolytic augmentation of ECM-mediated effects have recently been reported for laminin-5 in breast epithelial cell migration (Giannelli *et al*. 1997) and type-IV collagen in angiogenesis (Sage 1997). Our initial finding raised the possibility that gradients of soluble ECM fragments may be acting in concert with gradients of intact ECM during cellular invasion. The latter type of directed movement on intact matrix components is haptotactic, and has been described in a number of previous studies (Aznavoorian *et al*. 1990; Klominek *et al*. 1993); in contrast, the response to the soluble fragments is chemotactic.

To explore the way in which these different motility mechanisms might interact, we constructed a mathematical model to simulate a cellular invasion.

3. MATHEMATICAL MODEL

We denote the concentrations of the HT1080 cells, intact fibronectin, MMP-2, and the MMP-2-digested soluble fibronectin by $u(x,t)$, $c(x,t)$, $p(x,t)$ and $s(x,t)$, respectively; t and x represent time and space in a one-dimensional spatial domain. The equations are of conservation type (Murray 1990):

$$\text{cells: } \frac{\partial u}{\partial t} = \overbrace{k_1 u(k_2 - u)}^{\text{cell division}} - \frac{\partial}{\partial x}\left[\overbrace{k_3\psi(s)u\frac{\partial s}{\partial x}}^{\text{chemotaxis}} + \overbrace{k_4\chi(c)u\frac{\partial c}{\partial x}}^{\text{haptotaxis}}\right]; \tag{1}$$

$$\text{intact fibronectin: } \frac{\partial c}{\partial t} = -\underbrace{k_5 pc}; \tag{2}$$

$$\text{solubilized fibronectin: } \frac{\partial s}{\partial t} = \overbrace{k_5 k_6 pc}^{\text{proteolysis}} + h(p,s) + \overbrace{D_s\frac{\partial^2 s}{\partial x^2}}^{\text{diffusion}}. \tag{3}$$

$$\text{MMP-2: } \frac{\partial p}{\partial t} = \underbrace{k_7 uc}_{\substack{\text{MMP-2}\\ \text{production}}} - \underbrace{k_8 pu - k_9 p}_{\substack{\text{MMP-2}\\ \text{degradation}}} + \underbrace{D_p\frac{\partial^2 p}{\partial x^2}}_{\text{diffusion}} \tag{4}$$

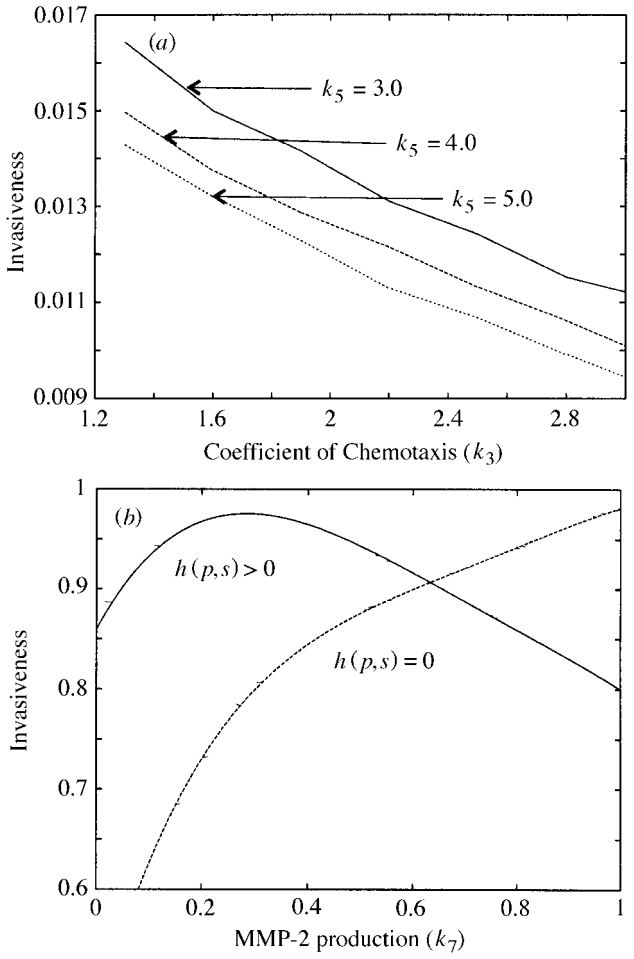

Figure 2. (*a*) Numerical solution of equations 1–4 showing the dependence of the invasion speed on the coefficient of chemotaxis for different levels of MMP-2 production (k_5). The functions $\chi(c)$ and $\psi(s)$ were derived from the data in figure 1. For all the parameter sets we investigated, the invasiveness falls with increasing chemotactic sensitivity. (*b*) The dependence of the invasion speed (normalized) on MMP-2 production with and without $h(p,s)$. When 'dynamic chemotactic drag' is neglected (i.e. $h(p,s) = 0$), invasiveness increases continuously with MMP-2 expression. However, when the dynamic chemotactic drag due to continued fibronectin degradation (shown in figure 3) is considered, invasiveness is parabolically related to MMP-2 expression. The parameter values used in these simulations are $k_1 = 1.0$, $k_2 = 1.0$, $k_3 = 5.0$ (in (*b*)), $k_4 = 0.05$, $k_5 = 3.0$ (in (*b*)), $k_6 = 1.0$, $k_7 = 1.0$ (in (*a*)), $k_8 = 0.1$, $k_9 = 0.1$, $D_s = 0.01$ and $D_p = 0.01$. The initial conditions are $s \equiv p \equiv 0$, with $u = 1$, $c = 0$ for $|x| < 10$, and $u = 0$, $c = 1$ for $|x| > 10$. In (*a*), and for the curve marked $h(p,s) > 0$ in (*b*), we choose the function $h(p,s) = 0.6ps$.

Here the k_is are positive constants. The functions $\psi(s)$ and $\chi(c)$ reflect the extent of chemotaxis and haptotaxis; we determined their functional forms from the data in figure 1, giving the forms listed in the figure legend. The proteolysis of fibronectin is represented by $-pc$; the resultant solubilized fibronectin diffuses in space and the intact fibronectin is anchored in place. The term $h(p,s)$ represents continued action of proteases, whereby fibronectin fragments are themselves broken down to give smaller fragments. This is a key term in the model, which we discuss in detail below. Our representation of the production of MMP-2 by the term uc reflects the fact that protease production *in vivo* is tightly confined to the immediate pericellular environment (Werb 1997; Xie *et al.* 1994) through signals transduced by the interaction of ECM with specific cellular receptors.

We studied numerical simulations of this model for a wide range of parameter values, with initial conditions corresponding to a localized cell population surrounded by intact fibronectin. In all cases the solutions evolve to travelling wave fronts moving in the positive x-direction, corresponding to invading waves of cells and receding waves of ECM, with a pulse wave of MMP-2 localized at the interface between these fronts. The common speed of these waves provides a convenient measure of the invasiveness of the cell population. In all cases, the model predicts that invasiveness decreases as the chemotactic coefficient k_3 is increased (figure 2*a*). This implies that ECM chemotaxis actually inhibits invasion, contrary to its conception as a pro-invasive factor; this is because the gradient in degraded soluble ECM (s) is in the opposite direction to invasion. The predictions of the model depend crucially on the function $h(p,s)$, which represents the possibility of repeated breakdown of ECM fragments by proteases. If this term is excluded from the model, the invasiveness of the cell population increases continuously with increasing protease production (k_3) (figure 2*b*). However, with $h(p,s)$ included, the model predicts a decrease in invasiveness at high rates of protease production (figure 2*b*). To test the biological validity of the term $h(p,s)$ within the mathematical model, we digested fibronectin (1 mg ml^{-1}) with MMP-2 (50 μg ml^{-1}) for various times (0–16 h) and analysed the products on SDS–PAGE. This showed a progressive digestion of fibronectin-yielding fragments with smaller molecular mass with increasing incubation time (figure 3*a*). The progressive nature of this reaction confirms the validity of the term $h(p,s)$.

The prediction of low invasiveness at high rates of MMP production is consistent with the previous observations that cells producing extremely large amounts of serine proteases actually become non-invasive. In these experiments it was shown that the invasive behaviour *in vitro* of cells that produce large amounts of tissue plasminogen activator, or urokinase-type plasminogen activator, is not blocked but enhanced by the addition of plasmin inhibitors or anti-plasmin antibodies (Tsuboi & Rifkin 1990). Conversely, the invasive capacity of cells that produce low levels of these proteases was blocked by the addition of inhibitors. Protease activity in excess of an optimal level producing uncontrolled matrix degradation produces non-invasion. Similar studies with endothelial cells (Montesano *et al.* 1990; Pepper *et al.* 1990) also show that successful angiogenesis requires an optimal level of protease expression. A suggestion has been made (Stetler-Stevenson *et al.* 1993) that this may be a consequence of the uncontrolled breakdown of matrix, marooning the cells without an underlying matrix to crawl on, and thereby abolishing haptotaxis (i.e. $\partial c/\partial x = 0$). However, this explanation assumes that the time-scales of ECM proteolysis and cell movement are widely different and that they occur sequentially, both of which are contrary to the prevailing view (Stetler-Stevenson *et al.* 1993; Lauffenburger 1996).

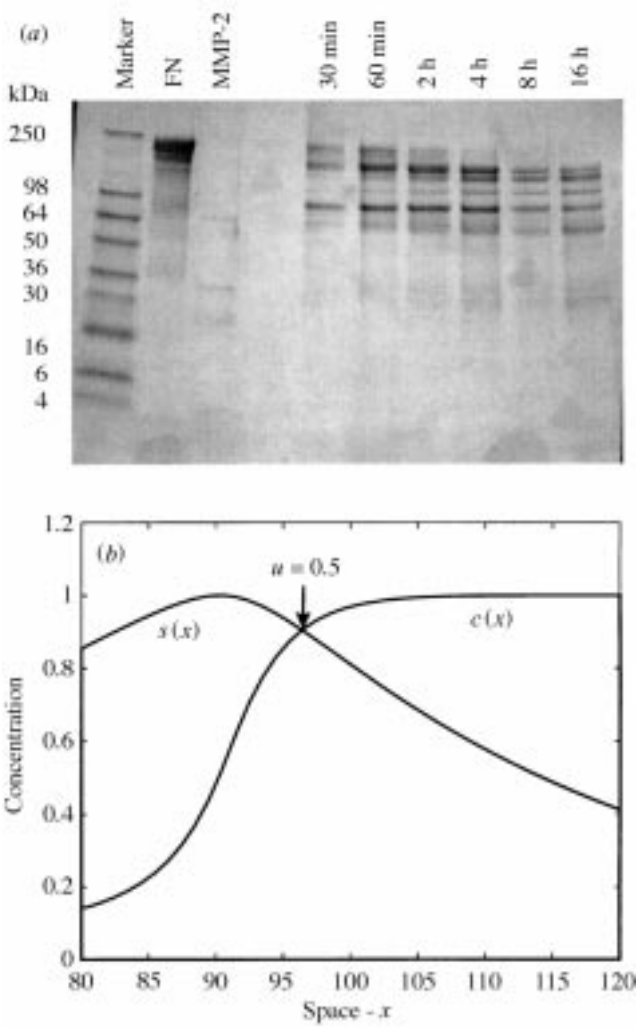

Figure 3. (*a*) 10–20% gradient SDS–PAGE (SeeBlue™ Novex) showing different stages of fibronectin digestion over time producing increasing amounts of smaller-molecular mass fragments. 1 mg ml^{-1} of fibronectin was digested with 50 µg ml^{-1} of MMP-2 for the lengths of time indicated in the figure. (*b*) Numerical solution of equations (1)–(4) showing the interaction of the chemotactic ($s(x)$) and haptotactic ($c(x)$) gradients. The position of the arrow corresponds to the position of the invading front of HT1080 cells measured as $u = 0.5$ in the numerical solution. The parameter values and initial conditions used in this simulation are the same as in figure 2*a*, with $k_5 = 3.0$.

Our model predicts that invasion occurs via a cell being sandwiched between two competing gradients, namely a pro-invasive haptotactic gradient of fixed ECM and an anti-invasive chemotactic gradient of soluble ECM fragments (figure 3*b*). Both of these gradients are produced dynamically as the invasion proceeds; and we refer to this soluble gradient as providing a 'dynamic chemotactic drag', which counteracts the invasive process.

4. COMPETING GRADIENTS OF DIGESTED AND UNDIGESTED FIBRONECTIN

To test these predictions, we investigated the movement of HT1080 cells under competing gradients of intact and digested fibronectin. We again used a standard dual-chamber chemotaxis assay; however, in this case fibronectin was placed in both the upper and lower wells. The addition of putative chemoattractant to both wells of a chemotaxis assay is an established technique, forming the basis for the method of 'checkerboard analysis' used to distinguish between chemotaxis and chemokinesis (Aznavoorian *et al*. 1990). In our experiments, we adapted this approach by using intact fibronectin in the lower well, and digested fibronectin in the upper well; the digested fibronectin is generated by preincubation with MMP-2, as in the experiments described in § 2. This new experimental scenario means that the cell population is situated in opposing gradients of intact and digested ECM, mimicking the situation *in vivo* predicted by our mathematical model. As predicted by the mathematical model, the opposing gradients of intact and digested fibronectin retard cell migration in a dose-dependent fashion (figure 4). In particular, the diagonal elements in the array in figure 4 represent competing gradients of equal concentrations of digested and undigested fibronectin, indicating conservation of fibronectin as would be seen *in vivo*. At low (equal) concentrations, the cells move preferentially towards the undigested fibronectin, but as the concentrations of both fibronectin types are increased, the number of migrating cells decreases dramatically and eventually the level of migration is reduced below that in controls. Thus the digested fibronectin fragments exert the predominant force, overriding the intact fibronectin. The increased chemotactic potency of MMP-2-digested fibronectin may be due to the exposure of cryptic pro-migratory sites or to the cumulative effect of an increased

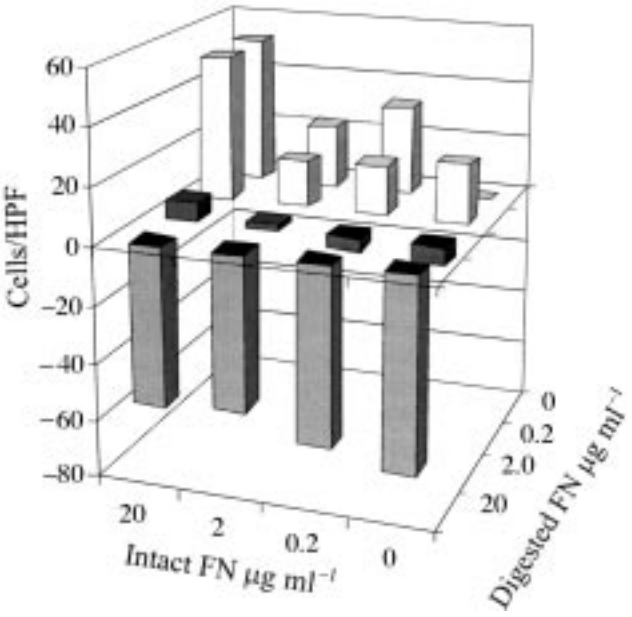

Figure 4. Results of a chemotaxis assay (similar to that used in figure 1) with varying concentrations of undigested fibronectin in the lower well against different concentrations of MMP-2-digested fibronectin in the upper well. The extent of cell migration (cells per high-power field) has been normalized by subtracting migration occurring in the absence of added fibronectin. The diagonal elements, from back left to front right, correspond to gradients with equal amounts of fibronectin, as in a situation *in vivo*; this shows elevated migration (relative to control) for small amounts of fibronectin digestion and reversed migration (normalized) for large fibronectin breakdown.

number of proteolysed fragments that are separately chemotactic. An example of the former mechanism is the MMP-2 cleavage of laminin-5 γ2 subunit at residue 587, which exposes a promigratory site that triggers cell motility (Giannelli *et al*. 1997). The latter mechanism has been observed with *Entamoeba histolytica* trophozoites, where the 70 and 120 kDa fragments of the proteolytic digestion of fibronectin were found to be more chemotactic than intact fibronectin (Franco *et al*. 1997). A similar paradoxical effect of proteolysis is the anti-inflammatory effect of a trisulphated disaccharide ECM fragment derived by heparanase treatment (Lider *et al*. 1995).

5. DISCUSSION

The prediction that ECM-mediated chemotaxis is an anti-invasive force runs counter to traditional views of invasion, which stem from the way in which chemotaxis is usually studied, namely by using dual-chamber chemotaxis assays set up with the chemoattractant concentration gradient running in the same direction as that of overall cell movement. Our results suggest that, in practice, chemoattractant gradients are generated dynamically through ECM degradation during the invasion process, and that these run in the opposite sense, against the direction of invasion. These chemoattractant gradients may eventually become fixed by binding to other ECM proteins (Hynes 1994). This mechanism implies that an optimally invasive phenotype corresponds to a balance between protease production and inhibition, and the relative proportions of digested and intact ECM components. Uncontrolled proteolysis by large amounts of proteases would result in the establishment of steep chemotactic gradients in the wake of invasion, which would impede the outward invasive movement of the cells. Because these chemotactic gradients are soluble they would eventually be dissipated, resulting in continued invasion, albeit at a lower rate. In such cells, the addition of antiproteases such as TIMPs would attenuate the chemotactic drag and cause faster invasion, as has previously been reported for Bowes' melanoma cells (Tsuboi & Rifkin 1990).

The motility-retarding effects of ECM chemotaxis elucidated here have important implications for our understanding of cellular invasion processes in general and of the molecular approaches to anti-cancer therapeutics in particular. The demonstration that chemotaxis and haptotaxis operate through different receptors (Taraboletti *et al*. 1987) and have different signal transduction pathways (Aznavoorian *et al*. 1990) has presented the possibility of two separate targets to retard invasion. However, the results of this study would imply that specifically targeting ECM-mediated chemotaxis could produce the opposite effect, resulting in an augmentation of invasion. Furthermore, the reappraisal of chemotaxis presented here predicts that protease inhibitors can also produce an early increase of invasion in high-protease expressers in appropriate ECM-rich environments. The results of this study show that such unintended augmentation of invasion by antiproteases can be effectively prevented by combining anti-protease therapy with haptotactic blockade. A large number of such potential protease inhibitors are currently in advanced stages of clinical and animal trials (Denis & Verweij 1997). Although similar progress has not been made with haptotactic blockade, studies *in vitro* with specific antibodies (Taraboletti *et al*. 1987) have been shown to be effective. A combination of these two modalities would not only be naturally synergistic, resulting in faster remission, but would also prevent any early increase in invasion.

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