A model for the initial phase of cell/surface interactions based on ligand binding phenomena

John HUBBLE,* Robert EISENTHAL† and William J. D. WHISH†

Schools of *Chemical Engineering and †Biochemistry, University of Bath, Bath BA2 7AY, U.K.

The interaction between mammalian cells and solid surfaces plays an important role in a number of biological phenomena. Of particular clinical importance is the migration of cells suspended in blood to the wall of a blood vessel in the event of tissue damage. While the resultant inflammation often represents a desirable response to an external challenge, responses of this type can also lead to adverse consequences. Although the cell mi-

gration phenomenon is well known, a plausible mechanism for controlling the critical 'rolling' stages of adhesion has yet to be proposed. In this report we suggest how a simple consideration of ligand/receptor binding interactions can be used to explain a switch between a situation where a cell population is almost entirely in free suspension, to one where a significant fraction is attached to the solid surface.

INTRODUCTION

The recruitment of neutrophils and other leucocytes to sites of inflammation is preceded by 'rolling' where the cells show a characteristic motion along the wall of the blood vessel [1]. It has been suggested that a progressive decrease in rolling velocity leads to an increased contact time between cell and wall such that adhesion can occur [2]. The importance of these interactions in a number of clinical conditions is well established and a recent review considers the potential of binding antagonists as potential therapeutic agents in such cases [3]. However, although the molecular components that mediate rolling have been identified, the mechanism by which this occurs has yet to be adequately explained [4]. Models published to date have concentrated on force balance calculations, assuming that ligand–receptor interactions can be treated as springs capable of extension prior to breakage [5]. While this approach is attractive for use with covalent adhesive interactions there are two problems with its application to affinity-type interactions. The first is that the models do not take account of the co-operative effect of multiple binding interactions on the extent of cell binding; the second is that deformation and breakage of bonds implies damage, while Lawrence and Springer [4] have found that there is no change in cells or substrate after prolonged rolling.

THEORY

The model proposed here is based on two-site populations where one population represents ligands on the cell surface and the other receptors, e.g. on the endothelial wall, although the terms ligand and receptor are interchangeable. It is assumed that the number of sites per cell that can interact with the surface may change according to changes in cell-surface properties and fluid dynamic conditions in the suspending medium. The receptor population is assumed to be uniformly distributed over the surface area available for cell attachment.

Consider a cell with multiple ligands (L) capable of interacting with receptors (R) on a surface. The material balance for the cell ligands, where concentration is defined with respect to area rather than volume, is

$$[L_t] = [L] + [LR] + 2[LR_2] + 3[LR_3] + \dots + n[LR_n] \quad (1)$$

where $[L_t]$ is the total cell ligand concentration, $[L]$ is the free cell ligand concentration, $[R]$ is the surface receptor concentration, n

is the number of interactions per cell and $[LR_n]$ is the population of cells attached to the surface by n ligand–receptor interactions (Figure 1).

Under equilibrium conditions the initial interaction of the cell with a surface can be described by the dissociation constant

$$K = [L][R]/[LR] \quad (2)$$

Once the cell has undergone the initial binding step, further interactions may take place. However, once spatially constrained by a previous attachment, further attachments will be determined by the presence or absence of a surface receptor within the binding distance of a cell ligand (Figure 1). While the concentration of ligand and receptor will determine the probability of a possible interaction [6], the equilibrium position can be quantified in terms of the dimensionless association constant

$$K' = [LR_n]/[LR_{n-1}] \quad (3)$$

At equilibrium, using these definitions and assuming that the interactions are independent and that the dimensionless constant K' does not vary with the number of cell-surface ligands interacting with receptor sites, the concentration of the species identified in eqn. (1) can be expressed in terms of $[L]$ and $[R]$:

$$[L_t] = [L] + [L][R]/K + 2K'([L][R]/K) + 3K'^2([L][R]/K) + \dots + nK'^{(n-1)}([L][R]/K) \quad (4)$$

Solving for $[L]$ gives:

$$[L] = \frac{[L_t]K}{K + [R](\sum_{i=1}^n iK'^{(i-1)})} \quad (5)$$

Hence the fraction of ligand bound can be expressed as

$$Y = \frac{([L_t] - [L])}{[L_t]} = 1 - \frac{K}{K + [R](\sum_{i=1}^n iK'^{(i-1)})} \quad (6)$$

Figure 1 An illustration of the spatial constraints on the formation of an additional interaction between a ligand on an adsorbed cell and an adjacent surface receptor

Figure 2 Influence of the number of cell-surface interactions on the fraction of total cell ligand bound to surface receptor

Curves are generated for a range of $[R]/K$ ratios from 1 to 1×10^{-7} in order of magnitude increments. An arbitrary value of 1.5 was used for K' . The stepwise appearance of the curves results from the discrete nature of n .

Given that the cell population is assumed to be homogeneous, the fraction of the cell population adsorbed to the surface can be related to the fraction of cell-surface ligand bound. However, when cell binding is considered we are describing a population of cells rather than a homogeneously distributed ligand concentration. Therefore when a cell is bound, its total ligand population is removed from the bulk irrespective of the number of interactions it forms with the surface ($n \geq 1$). In this case eqn. (4) becomes

$$C_t = C + C[R]/K + K'(C[R]/K) + K'^2(C[R]/K) + \dots + K'^{(n-1)}(C[R]/K) \quad (7)$$

and eqn. (6) becomes

$$Y = \frac{(C_t - C)}{C_t} = 1 - \frac{K}{K + [R](\sum_{i=1}^n K'^{(i-1)})} \quad (8)$$

where C_t is the total cell population and C is the unbound cell concentration at equilibrium.

The relationship between $[L]$ and C_t is given by the number of ligands per cell. In the case of neutrophils this has been estimated to be in the region of 20000 [7]. Geometric considerations have been used to calculate the effective contact area over which the distance between cell and surface is sufficiently small to allow ligand-receptor interactions to occur. In the case of a 10 μm diameter sphere the area will be circular with approximate radius of 10% of the sphere radius [8], suggesting that there will be approximately 50 sites within the contact area of a neutrophil. Given that a spherical cell can approach the surface in any orientation, any subset of 50 ligands mediate the binding-interaction such that $[L]$ will be determined by the total number of ligands, not a single binding subset.

As K and K' will be determined by the specific ligand-receptor interaction, this means that the fraction of the cell population that is bound to the surface will be a function of n and the surface site concentration at equilibrium. The variation of fractional-adsorption of cells as a function of n , generated using eqn. (8), is shown in Figure 2. In critical regions a change in as few as five interactions can lead to a switch between 10 and 90% site dissociation. As can be seen from this Figure, the point at which

Figure 3 Influence of the value of K' on the fractional binding of the cell population

Curves were generated for $n = 25$ and the $[R]/K$ ratios used for Figure 2.

the critical range of n is reached is a function of the ratio $[R]/K$. The other factor that will influence the critical region is the value of K' . Figure 3 shows the form of this relationship and suggests that as the influence of the initial single site interaction is reduced by lowering the $[R]/K$ ratio, a higher value of K' is required to give effective cell binding to the surface.

Although we have assumed that K' is independent of the number of participating interactions, this represents a worst-case scenario. In practice it is likely that surface cooperativity, as described by Yon [6] for the affinity adsorption of multimeric proteins, would lead to an increase in the apparent value for K' as the number of participating groups increases.

Given that the number of interactions, n , depends on the spatial relationship of the cell to the surface, any local variations caused by deviations from a perfect sphere geometry are likely to mean that the effective value of n will vary as the cells change position in the fluid. For a vascular/endothelium system, this, combined with blood flow variations resulting from the pulsating action of the heart, will lead to an inherently chaotic system where n fluctuates about the critical value, leading to rapid attachment and detachment. The balance between time attached and detached will dictate the rolling velocity.

As early as 1978 [9], Bell observed that for cells attached via antibody-antigen interactions 'The bonds are reversible so that no force is needed to separate the molecules; a little patience will suffice. However, when many bonds are linking two cells together, patience will not suffice because the probability for all the receptors to be simultaneously unbound is very small.' The results presented here suggest that the second part of this statement is not necessarily true, and that cells held together via multiple weak interactions can be induced to dissociate by a very small change in their environment. Support for this hypothesis is provided by recent binding studies carried out with E-selectin/carbohydrate sialyl Lewis X which showed that it has a low association constant (10^4 M^{-1}) which results from an unusually rapid 'off' constant [10].

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