Leukocyte Recruitment in the Microcirculation: the Rolling Paradigm Revisited

Paul Kubes and Steven M. Kerfoot

Intravital microscopy has done much to elucidate the cascade of events involved in the recruitment of leukocytes to sites of inflammation. Here we review the physiological relevance of leukocyte rolling and some of the important subtleties of this process, highlighting limitations in our knowledge and directions for future investigation.

Fighter planes landing on an aircraft carrier are brought from a high speed to a stop very quickly by catching onto cables strung across the flight deck. Similarly, a leukocyte moving at very high speeds in the mainstream of blood must also tether to and roll along the vessel wall before adhering at a site of injury and/or inflammation. Although the phenomenon of tethering/rolling was certainly described more than 100 years ago by Cohnheim (3), the molecular mechanisms have only been studied extensively in the last decade. Two key observations sparked extensive interest in the study of leukocyte rolling. First, inhibiting leukocyte adhesion did not reduce leukocyte rolling, raising the possibility that rolling and adhesion were two distinct molecular events (1). Second, inhibiting rolling reduced adhesion, suggesting that rolling was a prerequisite of leukocyte adhesion/recruitment and ultimately the inflammatory response (20).

The ability to visualize the microcirculation has played an essential role in our study of the importance of rolling in the recruitment process. Nevertheless, important issues remain unanswered for physiologists studying the microcirculation, mainly because of technical limitations. In this brief overview, we first summarize our current understanding of the physiological importance of leukocyte rolling and how this event may impact on inflammatory cell recruitment. We also highlight technical limitations and areas that clearly require further investigation.

Leukocyte recruitment cascade

Figure 1 is a representative illustration of the type found in most immunology textbooks depicting the universal or unifying theme of leukocyte recruitment from the vasculature into tissues. The basic premise is that leukocyte recruitment occurs within postcapillary venules and is dependent on a cascade of events involving the selectins as the primary molecules that induce and support rolling. Chemokines, lipid mediators, and other proinflammatory molecules presented on the surface of the endothelium then activate a second family of adhesion molecules, the integrins, and cause cells to firmly adhere. It is now well appreciated that α4-integrin is an exception to this rule in that this molecule can mediate both rolling and adhesion. Once adherent, leukocytes can then migrate out of the vasculature. The fact that this is an interdependent series of events has been nicely illustrated in vitro by Lawrence and Springer (13). Under flow conditions in the absence of...
selectins, cells will not roll and hence cannot adhere to integrin ligands [e.g., intracellular adhesion molecule (ICAM)-1]. Immobilizing selectins (P-selectin) and ICAM-1 to coverslips and adding proinflammatory molecules (e.g., N-formylmethionyl-leucyl-phenylalanine) allowed the cells to roll and subsequently adhere via ICAM-1 in the flow chamber system.

Similarly, the use of intravital microscopy on exteriorized translucent tissues, such as the mesentery or cremaster muscle, permitted visualization of leukocytes within blood vessels and demonstrated that an antiselectin antibody inhibited rolling and thereby prevented firm adhesion of leukocytes (11, 20). The latter experiments were performed in postcapillary venules with shear rates that ranged from 200 to 1,500 per second and with diameters three times or more that of leukocytes. These vessel characteristics make the initial selectin-dependent capture or tether entirely necessary. Similar observations have been made in skin, Peyer’s patches, lymph nodes, and some other organs (19a).

Intravital microscopy to study leukocyte rolling

Baseline rolling. A very real limitation of intravital microscopy is the potential complication of enhanced baseline rolling induced by surgical manipulation. It is reasonable to assume that exteriorization of tissues from the body cavity is associated with some inflammation and therefore significant changes in cell rolling. Indeed, Fiebig et al. (4) reported that immediately upon exteriorization of the mesentery leukocyte rolling flux increased, but with time the number of rolling cells declined to a new steady state level. A partial role for mast cells has been suggested as a mechanism for preparation-induced leukocyte rolling. Mast cells are exquisitely sensitive to environmental changes, and they are strategically located in very close proximity to microvessels, where they may release proinflammatory molecules during tissue manipulation and induce cell recruitment. Indeed, reducing the release of mediators from mast cells dramatically reduces baseline rolling in some tissues (11). However, some tissues such as the brain and the liver do not appear to have significant baseline rolling following surgical manipulation, whereas the mouse ear microvasculature has baseline rolling without any surgical manipulation. Perhaps in tissues more closely apposed to the external environment basal rolling is a physiological necessity for surveillance, whereas in other tissues (mesentery) high baseline rolling is simply an artefact of the preparation.

Mediator-induced rolling. Artifactual baseline rolling has made it more difficult to study mediator-induced leukocyte rolling. Three approaches have been used to circumvent the complications of increased baseline rolling to demonstrate that certain mediators can indeed induce an increase in leukocyte rolling. Asako and colleagues (2) reported that very careful surgical preparation resulted in very low levels of rolling and that this permitted observations of responses to mediators like histamine. Second, as already mentioned, stabilization of mast cells also permitted the subsequent study of histamine responses (11). A third approach was to administer a mediator before rapidly exteriorizing and visualizing the mesentery (14). In all cases the early increase in leukocyte rolling was dependent on P-selectin. Although histamine only increased the number of rolling cells, addition of leukotriene C4 (LTC4) (8) caused cells to roll very slowly (discussed below) as well as increased the number of interacting leukocytes, suggesting different rolling mechanisms.

An inference has been made by numerous investigators that the more one increases rolling, the greater the propensity for cell adhesion. A corollary to this view is that adhesion is reduced proportionately to the reduction in the number of rolling leukocytes. It should be noted that the relationship

![FIGURE 1. The leukocyte recruitment cascade. Fast-moving leukocytes in the bloodstream tether and roll on activated endothelium via interactions between selectins and their ligands, or in some cases integrin (α4)-immunoglobulin superfamily [vascular cell adhesion molecule-1] interactions. Chemokines or other proinflammatory mediators released by various sources within the tissue (mast cells or tissue macrophages, for example) are presented on the endothelium to rolling leukocytes, resulting in integrin activation and firm adhesion. Firm adhesion permits leukocyte transmigration across the endothelium and entry into inflamed tissue.](http://physiologyonline.physiology.org/)
between the number of rolling and the number of adhering cells is not proportional, and in fact a reduction in the number of rolling cells with antiselectin therapy does not reduce the number of adhering cells until >90% of rolling cells are inhibited (9, 10).

A further limitation of intravital microscopy is the inability to differentiate the types of rolling leukocytes. Therefore, whether increased rolling represents a proportional increase in all cell types or whether only some populations of cells increase remains unknown. This is by no means trivial, inasmuch as even a 10-fold increase in rolling of a very minor population of leukocytes may not be detected using intravital microscopy and therefore counting total numbers of rolling leukocytes may not be meaningful. For example, interleukin (IL)-4 is a recruiter of eosinophils, which make up <1% of cells in the circulation. IL-4 does not induce a large increase in leukocyte rolling, but it is conceivable that as little as an increase from 0 to 2% rolling eosinophils may be sufficient to recruit these cells to the IL-4-injected site. This contention is based on the fact that IL-4-induced rolling is reduced by 98% in E-selectin/P-selectin double-deficient mice, yet the few cells that continue to roll (perhaps eosinophils via α, integrin) are sufficient to induce identical amounts of eosinophil recruitment in wild-type and E/P-selectin-deficient mice (6). Clearly, it would be optimal if the few rolling eosinophils could be tagged and recognized. The need to know the identity of specific rolling cells will continue to grow as intravital microscopy is applied to more complex models of inflammation and autoimmunity. One solution may be to make transgenic mice wherein a fluorescent marker [e.g., green fluorescent protein] is incorporated into a lineage-specific promoter (e.g., CD4) to generate a mouse in which selected leukocyte populations have endogenous fluorescence.

**Slow rolling**

For a rolling cell to adhere, a reduction in rolling velocity must take place. It is assumed that endothelial-bound chemokines activate integrins that are responsible for the transition from rolling to adhesion. However, some [LTCA, tumor necrosis factor (TNF), ischemia-reperfusion] but not all inflammatory mediators (histamine, H2O2) also cause slow rolling without always inducing firm adhesion. However, the slow rolling may facilitate firm adhesion. The underlying mechanisms for slow rolling may include increased density of selectins. For example, increased expression of P-selectin or E-selectin may cause cells to roll more slowly (8, 15). Increased levels of chemokines and other proinflammatory molecules may also reduce rolling velocity, presumably by low-level integrin activation (8). Although these are likely contributors, different cell types may roll at different velocities and a change in the population of rolling cells over the course of a response (e.g., from neutrophils to mononuclear cells) may also contribute to altered rolling velocities. As already discussed, limitations in the identification of rolling cells with intravital microscopy preempts a test of this hypothesis.

One physiological reason for the reduction in slow rolling may be to permit longer interactions between the leukocyte and the endothelial surface. Kanwar and colleagues (8) demonstrated that neither LTCA nor histamine induced adhesion but only LTCA induced slow rolling. Addition of low concentrations of proadhesive molecules [platelet-activating factor (PAF), IL-8] induced adhesion only in the LTCA system. Only at much higher concentrations of PAF was adhesion observed with histamine. Ley and colleagues (15) made similar observations by inducing slow rolling with TNF and then demonstrating that the slow rolling was dependent on E-selectin. When this molecule was inhibited, cells rolled faster and were less apt to adhere to a local chemokine stimulus. However, it is possible that in both of the above systems the slow rolling is incidental and the engagement of a significant number of selectin ligands (due to increased selectin density) induces signaling and subsequent predisposition for adhesion within rolling leukocytes.

**Is rolling always required for cell recruitment?**

The ease with which one can visualize the muscle, skin, or mesenteric microcirculation has resulted in much information about leukocyte recruitment in these vascular beds. The notion that rolling is an absolute requirement for adhesion was determined in these tissues. However, there may be vascular beds including the liver, lung, and heart that may not require rolling to recruit leukocytes. For example, in the liver the sinusoids are sufficiently narrow so that cells can tether and immediately adhere without apparent rolling. Whether this is simple trapping of activated, more rigid leukocytes or an overt molecular adhesive event remains unclear. Nevertheless, none of the selectins nor α, integrin are necessary for adhesion/trapping of leukocytes within the liver sinusoids (5). Similar results have been reported for the lung (17). The importance of selectins in other tissues such as the heart circulation is unknown. Tissues like the heart and lung cannot be easily exteriorized. Therefore, extensive surgical intervention, which may significantly alter the physiology of cell adhesion, needs to be done to perform intravital microscopy in these tissues. For example, visualizing adhesion within the heart requires a nonbeating preparation. Therefore, blood flow patterns related to a beating heart are absent in such a preparation. Similar limitations apply to intravital microscopy of the lung.

The importance of rolling in the brain microcirculation is not entirely clear. It has been postulated that, following proinflammatory stimulation, early rolling may not occur within the brain microvasculature because of the lack of presynthesized P-selectin. Recent unpublished work from our laboratory suggests that transient rolling leading to rapid adhesion may occur at least in part on platelets (expressing P-selectin) bound to brain microvessels. The leukocytes roll on the platelets rather than directly on brain endothelium, inasmuch as removal of platelets abolishes leukocyte rolling.
Leukocyte interactions in arterial vessels and capillaries

The general view is that leukocytes do not roll or adhere on arterial or capillary endothelium. Higher hydrodynamic forces cannot account for the lack of rolling on the arterioles because a reduction in shear forces in these vessels to approximate the shear forces in venules does not induce rolling (18). Similarly, neither higher $P_0$, nor any particular architectural differences of the arterial blood vessels account for the lack of rolling on the arterial side. This contention is based on the observation that reversing the flow from the venous to the arterial side did not alter leukocyte-endothelial cell interactions. A simple explanation may be that the arterioles lack sufficient adhesion molecule expression to support rolling. Nevertheless, there is a growing body of evidence that certain stimuli considered risk factors for atherosclerosis, including cigarette smoke and oxidized low-density lipoproteins, do induce leukocyte-arterial endothelium interactions (16). Prolonged stimulation of cremaster muscle with TNFα also induces leukocyte-endothelial cell interactions on the arteriole side (12). However, in this system adhesion was not observed, so the physiological importance of this rolling remains unclear.

Intravital microscopy of the lung has revealed arterial rolling and adhesion consistent with extensive histological data, suggesting that leukocytes can adhere to arterial endothelium. In chronic skin window chambers, leukocyte-arterial endothelial cell interactions are also observed; however, these interactions may be a response to the skin window preparation because acute skin flap preparations (even those stimulated with inflammatory stimuli) lack rolling on the arterial side. Only a few attempts have been made to visualize leukocyte-large artery interactions using intravital microscopy (19). This work was performed in situ and revealed no rolling under normal conditions but significant rolling in mice with a propensity for atherosclerosis.

Leukocyte-capsillary interactions are also an often-ignored issue. Certainly, under conditions in which venular rolling is seen, leukocytes appear to pass through mesenteric or cremaster capillaries unrestrained. In contrast, in some tissues, including the liver and lung, a significant amount of adhesion occurs in capillaries. In the inflamed liver, the portal vasculature reveals leukocyte adhesion in pre- and postsinusoidal vessels as well as a very significant amount of adhesion within the sinusoids (capillaries) themselves. Interestingly, cells that adhere within these vessels do so independent of selectins and perhaps even the integrins (5). It is worth mentioning that this profile of leukocyte recruitment in liver may not hold true for all inflammatory conditions, inasmuch as the majority of adherent cells are seen in pre- and postsinusoidal but not sinusoidal vessels following ischemia-reperfusion of the liver (unpublished observations). Under these conditions, selectins contribute more to the overall leukocyte recruitment. Very similar observations have been made in the pulmonary microvasculature—leukocytes adhere in venules and arterioles as well as the capillaries, and the leukocyte recruitment can occur independent of selectins or integrins, but only under some conditions (17).

Conclusion

Rolling has clearly evolved as an important mechanism in the normal operation of the immune system. Continuous surveillance of tissues for potential infections or injury as well as subsequent recruitment responses are important roles for leukocyte rolling. Certainly a free-flowing cell in the circulation does not have the same capacity to survey the endothelium as a rolling cell. The need for rolling to permit adhesion is clearly an essential process in some situations, but perhaps not all. Despite many potential clinical applications, a successful therapeutic inhibitor of leukocyte rolling has not been developed. Part of the problem may be our lack of a complete understanding of the rolling process. Nevertheless, the importance of rolling may be underscored by the fact that parasitic mimicry of the rolling system has evolved. For example, Plasmodium falciparum (malaria) within red blood cells induces the cells to adhere within microvessels to successfully evade the spleen and allow for replication. Interestingly, the parasite has evolved a rolling system that is a prerequisite for firm adhesion and uses some of the same adhesive mechanisms used by leukocytes (7). Clearly, from an evolutionary standpoint rolling is a successful way to achieve firm adhesion in the microvasculature.

References

The renin-angiotensin system is one of the most widely studied endocrine systems. It has an important role in the regulation of normal homeostasis, and disturbances in this system may be important in numerous pathological states. This review will focus on the major insights and important questions raised from gene targeting of this system.

The first gene-targeting studies on the RAS were performed on the mouse renin system (RAS) using the hypothyroid mutant strain 129. Several studies have since been performed using the knockouts of Ren-1a, Ren-1b, and Ren-2. Ren-1a and Ren-1b were found to have only one renin gene in the mouse, whereas Ren-2 has three renin genes.

ACE knockout mice have been generated using a modified Cre-loxP recombination system. These mice lack detectable plasma levels of ACE, and their blood pressure is decreased by 15–20 mmHg from wild-type controls. As expected, ACE knockout mice fail to exhibit a pressor response to infusion of angiotensin I (ANG I) and exhibit an enhanced depressor response to infusions of bradykinin. The same renal phenotype being a decrease in blood pressure in Ren-1c knockout mice has been difficult to obtain because of the chronic alterations in plasma levels of AGT are able to affect leucocyte-endothelial cell interactions in cat mesenteric venous.

Angiotensinogen (AGT) is synthesized in a wide variety of tissues, including blood vessels, brain, and kidney. ANG II is generated by the serial cleavage of AGT first by renin and then by angiotensin-converting enzyme (ACE). The AT1 receptor is the major receptor for ANG II, with two subtypes, AT1A and AT1B, that cannot be distinguished pharmacologically. AT1A is the major receptor in rodents, whereas AT1B is the major receptor in humans. The AT2 receptor is present in the heart, lung, and kidney, but its role is less clear.

The importance of E-selectin and other selectins in leukocyte-endothelial cell interactions in vivo.

The importance of E-selectin and other selectins in leukocyte-endothelial cell interactions in vivo.

Angiotensinogen (AGT) is synthesized in a wide variety of tissues, including blood vessels, brain, and kidney. ANG II is generated by the serial cleavage of AGT first by renin and then by angiotensin-converting enzyme (ACE). The AT1 receptor is the major receptor for ANG II, with two subtypes, AT1A and AT1B, that cannot be distinguished pharmacologically. AT1A is the major receptor in rodents, whereas AT1B is the major receptor in humans. The AT2 receptor is present in the heart, lung, and kidney, but its role is less clear.

The importance of E-selectin and other selectins in leukocyte-endothelial cell interactions in vivo.

References:
18. Perry MA and Granger DN. Role of CD11/CD18 in shear-rate dependent...