Leukocyte rolling: a prominent feature of venules in intact skin of anesthetized hairless mice

HARVEY N. MAYROVITZ
Microvascular and Physiological Studies Unit, Wound Care and Vascular Department, Miami Heart Institute, Miami Beach, Florida 33140

Mayrovitz, Harvey N. Leukocyte rolling: a prominent feature of venules in intact skin of anesthetized hairless mice. Am. J. Physiol. 262 (Heart Circ. Physiol. 31): H157–H161, 1992.—Leukocyte (white blood cell; WBC) rolling in postcapillary venules is a frequently reported phenomenon in the microvasculature of experimental preparations. In most reports where this phenomenon has been systematically studied the confounding effects of various procedures associated with tissue preparation have been present. Thus there is sparse information on the extent of WBC rolling under fairly normal conditions. Here observations and features of this phenomenon in venules in the intact skin microvasculature of the homozygous hairless mouse ear are described. One venule in each of 10 mice was observed and continuously video recorded for 90 min. The parameters determined (means ± SD) were diameter, 15.9 ± 3.1 μm; red blood cell velocity, 358 ± 227 μm/s; flux of rolling WBCs, 3.2 ± 2.6/min; velocity of rolling WBCs, 9.6 ± 1.1 μm/s; systemic WBC count (CWBC), 3,220 ± 1,072/μl; and total WBC flux, estimated as the product of CWBC and calculated venule blood flow, 8.5 ± 3.0/min. Overall, 44.8 ± 13.8% of the total WBC flux exhibited rolling with a velocity that was 3.6 ± 2.9% of the red blood cell velocity. During the total 15-h combined observation time, no WBCs were seen to be adherent. These findings establish that in small venules of normal skin, WBC rolling is common, since on the average nearly one of two WBCs delivered to the venule exhibits rolling. Furthermore, because the translational rolling speed is very low, they contribute to the marginated pool, which, according to the present data, might be better termed the “rolling” pool.

LEUKOCYTE ROLLING along venular endothelium is a common observation following tissue exposure (3, 4, 10, 14, 18). The fact that leukocyte rolling usually precedes their firm adherence to endothelium has been taken by some to suggest that the rolling process itself is abnormal, perhaps induced by the surgery or trauma required for microvascular viewing. However, a key question remains open: Does the rolling reflect a generalized change in a “normal” leukocyte-endothelial interaction in which rolling was initially absent, or does it reflect a modulation of rolling already present? In arterioles, data from non-traumatized tissue show that normally leukocyte rolling is absent (24), although it can be readily induced by tissue injury (22) and increased by ischemia (21). In venules, the fact that trauma can further modulate these events is also clear from studies that show that the number of rolling leukocytes diminish from a postpreparation peak toward a relatively constant background level (3, 4) or in some cases increase monotonically (9). Maintained and lengthy tissue exposure and microvascular “deterioration” are often associated with an increase in leukocyte adherence that is presumably preceded by an increase in the number of rolling leukocytes. These types of observations do not rule out the possibility that leukocyte rolling is initially present in the venules and is merely exacerbated by surgical or other trauma to the tissue or microvascular environment to be observed. The objective of the present investigation was to determine if such leukocyte rolling could be detected in venules within a tissue in which surgery and trauma to the microvasculature under study were absent. For this purpose the ear skin microvasculature of the homozygous hairless (H/H) mouse was systematically studied, and, somewhat contrary to expectations, significant leukocyte rolling was indeed detected. The magnitude of this rolling, some of its dynamic features, and its potential physiological implications are the topics of this report.

METHODS

Initial Procedures

This investigation used 10 H/H mice (19) (SKH-1; Charles River Laboratories), 8–10 wk of age, weighing between 30 and 40 g. Following anesthesia induction (pentobarbital sodium, 6 mg/100 g ip) the mouse was placed under a surgical microscope (Zeiss OPMI6 S), and a 27-gauge Minicath (Abbott Laboratories) was inserted into the anterior intraperitoneal cavity for the administration of maintenance anesthesia. The mouse was then wrapped in a 4 × 4 in. cotton gauze pad to minimize convective heat loss and placed in the supine position on an observation board. The board supports the animal’s body and has an attached standard microscope slide on which the ear is placed for microscopic observation. The ear was carefully positioned with the dorsal surface gently positioned against the slide to obtain as flat a field as possible without compressing the tissue. Paraffin oil was placed on both the dorsal and ventral surfaces of the ear before placement of a no. 1 thickenee (0.1 g) micro-cover slip. The cover slip was supported by the underlying oil film. Temperatures were monitored using two thermistor probes (Bailey Instruments), one inserted rectally for monitoring core temperature and another positioned in the external meatus of the ear. ‘Temperatures were maintained using a heat lamp mounted on the microscope, and the animal’s core temperature was maintained between 32 and 35°C and ear temperature between 28 and 31°C. Heart rate was monitored using a Medsonics photopulse adaptor (model PA 13) with the cuff attached to the proximal portion of the tail, and data were recorded on a chart recorder (Grass RPS 7C 8 A).

The observation board with the mouse secured was then placed on the stage of a trinocular microscope (Zeiss, Laborlux) that had a calibrated zoom magnifier in the trinocular port that was coupled to a low light level television camera (MTI 65) and associated video cassette recorder (Panasonic model AG-1560).

Venule Selection

A single postcapillary venule from each animal was randomly selected for a 90-min observation interval. The selection proc-
ess was accomplished by first randomly locating one of the very large number of capillary loops (17, 28) and then by tracing one of these capillaries to its venous confluence. This venule then became the one selected for study. This process eliminated any bias for choosing a venule with either a large or small number of rolling leukocytes because the venule was chosen before it was observed. The elapsed time from positioning the ear until the beginning of venule observation was <5 min.

Data Acquisition

The venule was visualized on the television monitor, and its image was recorded using a x50 dry objective (numerical aperture = 0.75). Effective magnifications to the monitor (range >230×>650) were adjusted for optimum determinations of venule diameter (D), red blood cell velocity (V_RBC), and leukocyte dynamics. The directly measured leukocyte parameters were the number of rollers observed passing a fixed observation site and the velocity of these rollers (white blood cell or WBC velocity, V_WBC). V_RBC was determined by timing the transit of the rolling leukocytes over a calibrated axial distance on playback of the videotape. Venule diameter was measured from the video image using a calibrated video analyzer (CVI model 321). DIameters were measured at three sites within each venule, and the average of these was used to characterize its diameter. To determine V_RBC, the venule was imaged using transillumination with a 420-nm narrow band filter interposed between the light source and the condenser to enhance red blood cell contrast. Use of this filter renders the image inadequate for optimum leukocyte viewing. To circumvent this problem, 2.5-min intervals of V_RBC determination were sequentially alternated with 5-min intervals in which the filter was removed. During the 5 min interval, leukocyte parameters were determined, and this sequence was maintained throughout the 90-min data acquisition time. Sequential leukocyte and V_RBC data are reported, corresponding to the time midpoint for each consecutive leukocyte observation interval. V_RBC was measured from the playback of the video recordings using the dual-window video desitometric method. In this procedure, two video cursors, separated by a known distance, are inserted into the video image and positioned on the capillary, one upstream and one downstream. The video signals within these cursors, which correspond to the optical signature of the flowing blood, were obtained (IPM video photo analyzer 204), and the output signals were transmitted to a microcomputer. With the use of custom software, V_RBC was determined by cross-correlating the upstream and downstream signals to obtain the average transit time of the optical signature of the blood. Data were automatically acquired in sequential sampling segments of 5 s, yielding 38 discrete velocity determinations in each 2.5-min interval. Individual velocity determinations with a normalized covariance <0.70 were rejected, and the remainder were averaged to produce a single velocity value for each 2.5-min segment.

Calculated Quantities

The V_RBC for each 5-min leukocyte observation interval was determined as the average of the values determined immediately before and after this interval. Volumetric blood flow was calculated as the product of mean blood velocity (V_m) and venule cross-sectional area assuming a cylindrical shape. V_m was calculated from V_RBC as V_m/1.6 (6). The total leukocyte flux passing through the venule was calculated as the product of volumetric blood flow and systemic leukocyte concentration. The latter was determined from a blood sample drawn at the end of the 90-min data acquisition interval and subsequent leukocyte counting.

Calculated and Measured Leukocyte Flux

To determine how well calculated leukocyte flux predicts actual flux, an additional group of mice (n = 5) was studied. In this group each animal received an intravenous injection of acridine orange, which renders the circulating leukocytes brilliantly fluorescent (20), thereby permitting an accurate assessment of the total leukocyte flux in the observed venules. Except for the insertion of an intravenous catheter, mice receiving the acridine orange were prepared for microscopic study the same as already described. The total number of leukocytes passing a fixed position in the venule being observed was determined for 5 min from video recordings made under fluorescence video microscopy. The videotape was replayed, and the velocity (V_leuk) of nonrolling freely flowing leukocytes during this same interval was determined by tracking (frame by frame) the cells over a fixed axial distance. The V_RBC was estimated from V_leuk by the equation V_RBC = 1.05V_leuk (16), and V_m was determined as previously described. The total calculated flux was determined as the product of V_m, venule cross-sectional area, and systemic leukocyte count. The leukocyte count was determined as previously described. The calculated leukocyte flux was then compared with the directly measured flux expressed as cells per minute.

RESULTS

Acridine Orange Group (n = 5)

The result of this part of the study is shown in Fig. 1 where the relationship between the average measured and calculated total venular leukocyte flux is depicted. The calculated and measured cell fluxes are highly correlated (r = 0.999), with a slope of 0.9 for the linear regression equation. All values fall slightly below the line of identity (dashed line), which has a slope of 1.0. Thus there is a tendency for the calculated flux to slightly underestimate the measured value. A possible source of this discrepancy is the conversion factor of 1.05 used to estimate V_RBC from V_leuk. Strictly speaking, it is applicable to the hamster cheek pouch from which it was obtained. If a slightly larger value is applicable to the present situation, then the slope would be closer to unity and the small discrepancy would be reduced. A conversion factor of ~1.1 would result in near coincidence of the calculated curve and the line of identity. The result depicted in Fig. 1 indicates that with reasonable accuracy total venular leukocyte flux can be estimated by calculation for the present conditions. Furthermore, because
**LEUKOCYTE ROLLING IN SKIN VENULES**

---

\( V_{RBC} \) is measured for the main study, no conversion factor is required, and the calculated flux values are independent of any conversion factor related error. Thus the method of calculating total flux as the venule flow-systemic concentration product would appear to be adequate for the present purposes.

**Main Study Group (\( n = 10 \))**

**Physiological parameters.** Heart rate, respiratory rate, and core and skin temperature remained relatively constant over the experimental observation interval with no evidence of a trend in any parameter. Means \( \pm SD \) of these parameters (averaged over the total observation interval) were \( 321 \pm 22 \text{ min}^{-1} \), \( 65 \pm 4 \text{ min}^{-1} \), \( 33.8 \pm 0.8 \text{°C} \), and \( 29.1 \pm 2.1 \text{°C} \), respectively. These data suggest reasonably stable systemic parameters during the experimental time.

**Number of rolling leukocytes.** The number of leukocytes that were observed to be rolling (\( F_r \)) and the calculated total leukocyte flux (\( F_t \)) over the 90 min observation interval are shown in Table 1. No trends in either the flux of rollers or in the total number of leukocytes were detected. The means \( \pm SD \) of these quantities were found to be \( 3.2 \pm 2.1 \) and \( 8.5 \pm 3.0 \) cells/min, respectively. Some leukocytes were observed to be rolling in each of the 10 mice studied (\( D_e = 15.9 \pm 3.1 \mu m \)), with individual animal values ranging from a minimum of \( 0.72 \times 10^{-3} \) to a maximum of \( 8.46 \times 10^{-3} \). Overall leukocyte counts for the 10 animals were \( 3.22 \times 10^7 \) cells/\( \mu l \) with a granulocyte count of \( 48.9 \pm 26.5 \% \). As shown in Fig. 2, the average number of rollers in each animal was positively correlated with the total number of leukocytes passing through the venule under observation. Contrastingly, there was an indication of a negative correlation between the percentage of the total leukocyte flux that exhibited rolling and \( D_e \). This is shown in Fig. 3 along with the regression equation and its significance. The percentage of the total venular leukocyte flux that were rollers is shown for each observation time in Table 1. There was no trend in this ratio over the entire 90-min observation interval, and the overall average was 44.8%.

**Velocity of rolling leukocytes.** Table 1 also shows the sequential \( V_{WBC} \) and \( V_{RBC} \) measurements over the 90-min observation interval. No trend in either parameter was detected. Means \( \pm SD \) of \( V_{WBC} \) and \( V_{RBC} \) were \( 9.6 \pm 1.1 \) and \( 359 \pm 227 \mu m/s \), respectively. Also shown in Table 1 is \( V_{WBC} \) expressed as a percentage of \( V_{RBC} \), which over all intervals and animals was \( 3.5 \pm 2.9 \% \) of \( V_{RBC} \). On an individual animal basis, this ratio ranged from a minimum of 1.6% to a high of 10.9%. Although there was a significant correlation between the number of rollers and \( V_{RBC} \) as illustrated in Fig. 4, no statistically significant relationship between \( V_{WBC} \) and either \( V_{RBC} \) or wall shear rate was detected (data not shown).

**DISCUSSION**

Leukocyte rolling, as a component of the inflammatory process, has long been recognized, and many features of the sequence leading to the firm adherence of leukocytes to endothelium and their diapedesis are also generally agreed on (29, 30). Although significant questions regarding mechanisms exist, it is thought that some fraction of the leukocytes flowing in a microvessel are brought to the vicinity of the endothelial margin due to a combination of rheologic, hemodynamic, and random factors (5, 8, 12, 24, 27). The presence of some flowing leukocytes in the vicinity of the vessel wall occurs under all conditions, although the percentage can be increased or decreased depending on a variety of factors (11, 15, 25, 26, 29). In the absence of an appropriate leukocyte-endothelial cell interaction, leukocytes within the peripheral margin would on the average move with speeds determined by the hydrodynamic forces in its vicinity. However, when leukocyte-endothelial interactions develop, either by modifications to the leukocyte or to the endothelium, the leukocyte translational speed is altered in both magnitude and form. This rolling process, which is well described in the literature (3, 4), is seen in most, if not all, microvascular preparations surgically prepared for observation. Although leukocyte rolling is principally viewed as a phenomenon that occurs in the postcapillary vessels, it can be induced to occur in arteriolar vessels (22). Because rolling has been mainly observed under conditions in which surgery, tissue trauma, or tissue exteriorization were required for microvascular observation, it is easy to think of the rolling as an “abnormal” process. The main result of the present study suggests that this is not the case, since significant leukocyte rolling was detected in venules in intact skin in a model that required minimal manipulation for microvascular study. Some rolling was observed in each of the 10 mice.

---

**Table 1. Cellular fluxes and velocities**

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Flux, WBC/min</th>
<th>Velocity, ( \mu m/s )</th>
<th>Ratios, %</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( F_r )</td>
<td>( F_t )</td>
<td>( V_{WBC} )</td>
<td>( V_{RBC} )</td>
</tr>
<tr>
<td>5</td>
<td>2.9±1.0</td>
<td>8.1±1.7</td>
<td>7.2±1.5</td>
<td>363±76</td>
</tr>
<tr>
<td>15</td>
<td>3.2±1.0</td>
<td>7.3±1.3</td>
<td>9.8±1.8</td>
<td>315±56</td>
</tr>
<tr>
<td>25</td>
<td>3.3±0.9</td>
<td>7.5±1.5</td>
<td>9.6±2.1</td>
<td>316±57</td>
</tr>
<tr>
<td>35</td>
<td>3.2±0.9</td>
<td>8.3±1.9</td>
<td>11.0±2.4</td>
<td>351±73</td>
</tr>
<tr>
<td>45</td>
<td>3.1±0.9</td>
<td>7.7±1.8</td>
<td>9.8±1.7</td>
<td>377±70</td>
</tr>
<tr>
<td>55</td>
<td>2.8±0.7</td>
<td>7.8±1.7</td>
<td>8.1±1.7</td>
<td>329±60</td>
</tr>
<tr>
<td>65</td>
<td>3.2±0.8</td>
<td>8.5±1.9</td>
<td>10.5±2.0</td>
<td>357±73</td>
</tr>
<tr>
<td>75</td>
<td>3.6±0.8</td>
<td>9.5±2.0</td>
<td>10.8±1.9</td>
<td>378±74</td>
</tr>
<tr>
<td>85</td>
<td>3.1±0.8</td>
<td>9.7±2.3</td>
<td>12.1±2.4</td>
<td>385±87</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 10 \) mice. \( F_r \), flux of rolling leukocytes (white blood cells; WBC); \( F_t \), total calculated WBC flux; \( V_{WBC} \), velocity of rolling WBC; \( V_{RBC} \), red blood cell velocity.
studied, with 44.8% of the leukocytes passing through the venules being observed as rollers. This percentage is consistent with data from small postcapillary venules in the exposed cremaster muscle microvasculature where ~48% of the total leukocyte flux were rollers (18). The average speed of the rollers determined here (9.6 μm/s) was only a small fraction (3.6%) of the prevailing erythrocyte speed. The low rolling velocity reported here was consistent with values reported in the literature for surgically exposed preparations (3, 4, 10). For V_{RBC} values <1 mm/s, which constituted all of the cases studied here, a mean V_{WBC} of ~40 μm/s in venules with diameters ranging from 20.3 to 60.8 μm was reported (10). From these data one can calculate a corresponding V_{WBC}/V_{RBC} ratio of ~5%, which is close to the value determined here.

The number of rollers is dependent on the total number of leukocytes delivered, as well as on local rheological and hemodynamic conditions. In arterioles (23) and in venules (10) it has been shown that this number decreases with increasing Newtonian wall shear rate in a nonlinear fashion. In the present study, overall shear rates were 109 ± 58 (SD) s^{-1} (range 45–250 s^{-1}), with 80% of the values <125 s^{-1}. Thus for this model significant rolling is present in small venules even at moderate shear rates.

**Potential Physiological Implications**

Although a significant body of indirect evidence would suggest that rollinglike dynamics must be present to account for the “marginated” leukocyte pool (2, 13, 29, 30), the present findings appear to be the first to directly demonstrate this phenomenon in systemic venules in intact tissue in a near normal state. Furthermore, until now there has been controversy as to whether the various procedures necessary to prepare tissue for microvascular observation induce the leukocyte rolling. The present findings do not dispel the concept that such procedures augment rolling and adherence; however, they do show that, at least in skin venules under reasonably normal conditions, significant leukocyte rolling is indeed present. The principle abnormal condition connected to the present study was the fact that the animals were anesthetized. Although a possible direct or indirect effect of the anesthesia cannot be ruled out, the present findings suggest that the systemic venules may play a role in modulating the level of circulating leukocytes in response to a variety of stimuli. It is known that the lungs (1) and pulmonary circulation (7) play an important role in this regulation process. The mechanisms whereby the lungs store and release leukocytes are not known in great detail, although several hypotheses have been put forward, including modulation of the status of a marginated pool within the pulmonary circulation (13). There is also evidence that systemic factors may be involved. Epinephrine administration produces leukocytosis first in arterial blood and then after 30–120 s in venous blood (7). The latter rise in venous leukocyte counts was assumed to be either a delayed reflection of the arterial leukocytosis or due to a release from a different (non-pulmonary) source. Because it was found that the venous counts exceeded the arterial counts, an additional source is indeed likely. The conversion of rolling leukocytes in the systemic venules to circulating cells could be that source. Indeed, measurements of the overall circulating and marginated pools indicate about one-half in each under normal conditions. This is about the ratio of circulating to rolling leukocyte determined here. The results of the present study indicate that, at least under some conditions, the systemic venules have the potential for being both a source and a sink for leukocytes that could be rapidly mobilized or sequestered in accordance with prevailing physiological stimuli. The magnitude of this “rolling pool” could be substantial, since, according
to the present data, almost one-half of the leukocytes are found to be in this category. The overall potential of the skin rolling pool to contribute to changes in systemic leukocyte count depends on the fraction of the cardiac output passing through the skin microvasculature. Because this has a wide range, the potential effects are likely highly variable. If the present observations on skin venules are indicative of events within systemic venules of other organs, then the magnitude would increase proportionately.

In summary, the present data provide evidence in support of the concept that leukocyte rolling may be a normally occurring process within the venules of the skin microvasculature. The potential functional significance of this process is speculative, as is its extension to other organs and tissues. In skin, the very low translational speeds of these rolling cells endows them with the property of being a rolling storage pool having the potential of being a rolling storage pool having the potential to function in a manner akin to the classic "marginated" pool. The importance of the skin storage pool in the acute regulation of the systemic leukocyte count and the impact of the rolling on venular hemodynamics in humans remain to be established.

The technical assistance provided by John Moore and Dr. Elizabeth Sorrentino is gratefully acknowledged. This research was supported in part by the Juvenile Diabetes Foundation International Grant 1801090.

Address for reprint requests: Microvascular and Physiological Studies Unit, Miami Heart Institute, 4701 N. Meridian Ave., Miami Beach, FL 33140.

Received 29 June 1990; accepted in final form 2 September 1991.

REFERENCES