B) Experimental and Clinical-Thrombosis

Factors Influencing Leukocyte Adherence in Microvessels

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Summary

In vivo studies of the microcirculation of an untraumatized and unanesthetized animal preparation has shown that leukocyte adherence to vascular endothelium is an extremely rare occurrence. Induction of leukocyte adherence can be produced in a variety of ways including direct trauma to the vessels, remote tissue injury via laser irradiation, and denuding the epithelium overlying the observed vessels. The role of blood flow and local hemodynamics on the leukocyte adherence process is quite complex and still not fully understood. From the results reported it may be concluded that blood flow stasis will not produce leukocyte adherence but will augment pre-existing adherence. Studies using 2 quantitative measures of adherence, leukocyte flux and leukocyte velocity have shown these parameters to be affected differently by local hemodynamics. Initial adherence appears to be critically dependent on the magnitude of the blood shear stress at the vessel wall as evidenced by the lack of observable leukocyte flux above some threshold value. Subsequent behavior of the leukocytes as characterized by their average rolling velocity shows no apparent relationship to shear stress but, for low velocities, may be related to the linear blood velocity.

Introduction

Leukocyte adherence to the walls of blood vessels is commonly seen during *in vivo* observation of the microcirculation. Since first reported by Dutrochet (1824) many details of the leukocyte sticking process and in some instances their emigration through the vessel wall to adjacent tissue has been lucidly described in the now classic works of Conheim (1867), Metchnikoff (1893), and the Clarks (1935). The microscopic character of the leukocyte-wall interaction process is in many ways similar in a variety of tissues including the rabbit ear, rat and mouse mesentery, hamster cheek pouch, frog web and bat wing. Induction of the process itself has been linked to certain forms of tissue injury (Zweifach 1973) and is now an accepted hallmark of the inflammatory process (Grant 1973). Recent observations have raised the possibility of a link between leukocyte emigration and cancer metastasis. In addition evidence is beginning to accumulate suggesting the possibility of leukocyte involvement in the genesis of vessel wall pathology (Stewart et al. 1974). In each of these clinically important areas involving to varying

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degrees, initial leukocyte adherence, maintained "sticking", diapadesis, and extravascular migration, it is clear that without the initial adherence phase the subsequent phases and their consequences (good or bad) can not occur. However, outside of describing that adherence occurs, little progress in unraveling the mechanisms causing the initial leukocyte adherence has been reported. Several difficulties have contributed to this, but probably foremost is the complexity of the problem itself. The following formidable goal may be formulated. Describe in specific terms (1) the nature of the mutual interaction between leukocytes and either vascular endothelium or leukocytes and the endothelial-blood interface, which results in leukocyte adherence and (2) the factors which promote and influence this interaction. The formulation of the first part of this goal suggests that at present it is unknown whether the initial leukocyte adherence is produced by alterations in the endothelial cell membrane, by changes in the adjacent minute boundary layer between flowing blood and cell or possibly both. Further, by having specified the necessity of a mutual interaction, the leukocyte, its membrane, and local environment may be implicated. The appropriate experimental approach to study the interfacial and membrane aspects as they relate to the present *in vivo* problem have yet to be studied. The second part of the fundamental goal is more manageable and, in fact, represents the goal toward which most experimental work has been directed and is also the main focus of the present paper. Underlying such research there is usually the hope (whether stated or not) that the more that is known about the factors that influence the adherence process then the greater will be the likelihood of making inroads into the basic mechanisms causing it. The authors have attempted in the following discussion to examine some of the basic issues related to leukocyte adherence and to present some recent findings and speculations which relate to these issues.

Leukocyte Adherence in Undisturbed Vessels

From their studies on amphibians and in the rabbit ear chamber, the Clarks (1935) concluded that leukocyte sticking to vessel walls was not in itself a pathological process. It appears that this conclusion was based in part on the frequency with which this phenomena was observed and on the relatively slight stimuli required to produce it. Grant (1973) has suggested that perhaps all preparations employed in the study of leukocyte sticking have a built-in artifact which renders leukocyte sticking omnipresent in "normal" circumstances.

The nature of such an artifact probably is that in most *in vivo* preparations in which the microvessels are visible with sufficient resolution to study leukocyte phenomena, the associated surgery and trauma are sufficient stimuli to induce leukocyte adherence. Atherton and Born (1972) have shown large peaks in the number of observable leukocytes following preparation of the hamster cheek pouch, and a maintained level of rolling leukocytes for the duration of their experimental protocol. The presence of this background level of leukocyte activity makes it difficult to study the initial character of the adherence phenomena. To eliminate the problem of surgery, the authors have used the bat wing vasculature to study the character of leukocyte-vessel wall interaction in microvessels. The techniques for preparing the bat wing for microscopic study is well documented (Wiedeman 1973). Briefly, the procedure requires the placing of the unanesthetized animal in a tubular chamber, extending its wing over a large optical flat and securing the wing in position using small spring loaded clips. To enhance visibility a small amount of mineral oil is placed on the undersurface of wing and in some instances an additional thin layer spread on the top of the wing. Using this preparation it is then possible to study intravascular events in both arterial and venous vessels in an animal which is not subjected to either anesthesia or other traumatic influences. Systematic study of the arterial vasculature prepared for observation in this fashion, using magnifications up to 1,200 X and observation times up to 3 hr, have failed to show leukocyte sticking to the walls of these vessels.

had returned to control levels. The results of these studies show that although flow stasis is not sufficient to induce leukocyte adherence, it does augment pre-existing leukocyte-vessel wall interactions. Secondly it shows that the nature of this augmentation is transient and reversible. The mechanisms responsible for this augmentation remain speculative although 2 possibilities can be considered.

The blood flow in the arterial vessel under observation increased in a fashion typically seen as a hyperemic response. It could be argued that if normally there are random collisions of flowing leukocytes with the vessel wall, then an increase in the total number of leukocytes being delivered to the observed region due to greater blood flow would result in a larger observed leukocyte flux. To test this possibility continuous measurements of vessel diameter and red blood velocity were made prior to vessel occlusion and after resumption of blood flow. Red blood cell velocity measurements were performed in a manner similar to that reported previously (Mayrovitz et al. 1977b). The image of the vessel under observation is projected onto a viewing screen by means of a right-angle prism inserted into the trinocular barrel. Two matched phototransitors are inserted into small slits in the viewing screen so that they are positioned in the center of the projected image of the vessel in which velocity is to be measured. The phototransitors are aligned parallel to the vessel axis. The electrical signals obtained from the sensors represents the instantaneous optical density at two axially separated vessel sites. After preamplification of these signals, on-line velocity determinations are obtained by cross correlating the up- and downstream optical signals using a velocitometer. This device computes the time delay between up- and downstream optical signals and, together with a calibration factor for the effective axial separation of the sensors, produces an output voltage representing the red blood cell velocity.

Calibration and reliability of the velocity tracking system used in these experiments have been verified by comparing its output with that obtained (1) from a Hewlett-Packard model 3721A correlator when small tubing of known diameter was perfused with blood at a known flow rate, (2) by electronic simulation signals of known time delay, and (3) by moving a calibrated microscope stage micrometer at a known velocity.

In a total of 10 experiments blood velocity and blood flow computed as the average velocity times the vessel cross sectional area was found to increase during the post occlusion phase in 3 experiments, to decrease in 3 experiments, and to remain unchanged in 4 of the experiments. The mean and standard deviation of the control and post occlusion velocities were found to be 2.54 ± 1.25 and 2.47 ± 1.62 mmsec^{-1}. However in spite of the variability in post occlusion velocity changes, all post occlusion leukocyte fluxes were elevated. This finding that leukocyte flux increases independent of the blood flow change argues for the possibility that, though hemodynamics play a role in the leukocyte-wall interaction process, the increase produced by stasis is mediated by factors which enhance the affinity of the endothelial cell region for leukocytes.

A second possibility to account for enhanced leukocyte adherence after stasis takes root in the temporal character of the post occlusion response, which substantially increased during the first minute after return of blood flow with a rapid return toward pre-occlusion levels. This kind of temporal response would be expected if endothelial cells experienced transient alterations of their membrane properties due to changes in the local chemical environment during the stasis period. Return of blood flow might subsequently reverse the chemical derangement and permit leukocyte flux to return to pre-occlusion levels. However the fact that a background level of leukocyte flux is observed after removal of the epithelium suggests the presence of a maintained and continuous level of leukotactic signal or leukocyte-vessel wall interaction force.

erage leukocyte velocity could be found. This lack of correlation suggests that once adherence does occur there is a shift in the nature of the leukocyte-vessel wall interaction process. Atherton and Born (1973) have reported that in their studies on rolling leukocytes in venules, the leukocyte velocity was directly related to red blood cell velocity providing that the blood velocity was not larger than 1 mm/sec. The factors influencing leukocyte rolling velocity are probably only in part related to blood velocity. Preliminary experiments in the arterial vessels of the bat wing during continuous blood velocity measurement show considerable differences in the average velocity of individual leukocytes in the same vessel, at precisely the same time, and at the same blood velocity. Further differences in factors influencing leukocyte flux and the velocity with which they travel along the wall are suggested by other recent experiments (Mayrovitz et al. 1975). A background level of rolling leukocytes was established and control values of leukocyte flux and velocity determined. Following a suitable period the tissue adjacent to the vessel under observation was irradiated with a standard dose from a single pulse ruby laser. Although there was no significant change in the post irradiation leukocyte flux, there was, after a brief latency time, a 2-fold decrease in the velocity of the rolling leukocytes. These results suggest that it is important at this stage to conceptually maintain a separation between questions related to the initial adherence of leukocytes and the mechanisms which play a role once a critical level of adherence has been established.

In addition to the hemodynamic interrelationships already dealt with there are several others that need to be considered. Schmid-Schoenbein et al. (1975) have raised the issue of the role of the red blood cell as factor influencing the local shear stress. They have used a hydrodynamic model to evaluate both the drag coefficient associated with adherent leukocytes and attempted to calculate the magnitude of the shear force required for leukocyte dislodgement. Another factor which may be related to leukocyte dislodgement is the presence of a significant pulsatile component (Mayrovitz et al. 1976 a, b, Intaglietta et al. 1971) in the arterial microvessels. Though the study of the specific role of this pulsatility as it relates to the leukocyte-vessel wall interaction is just beginning, leukocyte movement in synchrony with the heart beat has been frequently observed. This observation may simply be related to a waxing and waning of the local shear forces around a threshold level to produce movement of rather firmly adherent leukocytes or may be due to an oscillatory alteration in the nature of the blood-vessel wall interface.

Résumé

Les études de microcirculation in vivo sur une préparation d'animal non traumatisé et non anesthésié ont montré que l'adhérence des leucocytes à l'endothèle vasculaire est extrêmement rare. L'adhérence des leucocytes peut être produite de diverses manières parmi lesquelles une lésion directe de la paroi vasculaire, une lésion de tissus a distance par le laser et une dénudation de l'epithélium des vaisseaux étudiés. Le rôle de l'écoulement du sang et de l'hémodynamique sur l'adhérence des leucocytes est très complexe et pas encore entièrement compris. Des résultats on peut conclure que la stase sanguine ne va pas produire d'adhérence mais augmenter une adhérence préexistante. Les études avec deux méthodes de mesure de l'adhérence, le flux des leucocytes et la vélocité des leucocytes ont montré que ces paramètres sont affectés différemment par les conditions hémodynamiques locales. L'adhérence initiale dépend de la force de cisaillement au niveau de la paroi vasculaire comme le met en évidence l'absence de flux des leucocytes au-dessus d'une certaine valeur limite. Le comportement ultérieur des leucocytes caractérisé par leur vitesse moyenne de roulement n'est apparemment pas en relation avec la force de cisaillement, mais aux faibles vitesses pourrait être en rapport avec la vitesse linéaire du sang.

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