The American Journal of Pathology, Vol. 184, No. 4, April 2014

ELSEVIER

The American Journal of PATHOLOGY

ajp.amjpathol.org

ROUS-WHIPPLE AWARD LECTURE

How Endothelial Cells Regulate Transmigration of Leukocytes in the Inflammatory Response

William A. Muller

From the Department of Pathology, Northwestern University Feinberg School of Medicine, Chicago, Illinois

CME Accreditation Statement: This activity ("ASIP 2014 AJP CME Program in Pathogenesis") has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of the American Society for Clinical Pathology (ASCP) and the American Society for Investigative Pathology (ASIP). ASCP is accredited by the ACCME to provide continuing medical education for physicians.

The ASCP designates this journal-based CME activity ("ASIP 2014 AJP CME Program in Pathogenesis") for a maximum of 48 *AMA PRA Category 1 Credit(s)*™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

CME Disclosures: The authors of this article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.

Accepted for publication December 9, 2013.

Address correspondence to William A. Muller, M.D., Ph.D., Magerstadt Professor and Chairman, Department of Pathology, Northwestern University Feinberg School of Medicine, 303 E. Chicago Ave., Chicago, IL 60611. E-mail: wamuller@northwestern.edu.

Leukocytes attach to vascular endothelial cells at the site of inflammation via a series of intercellular adhesive interactions. In a separate step in leukocyte extravasation, transendothelial migration is regulated by molecules that play no role in the preceding steps of tethering, rolling, adhesion, and locomotion. Transendothelial migration itself can be dissected into a series of distinct interactions regulated sequentially by molecules concentrated at the endothelial cell border; these include platelet/endothelial cell adhesion molecule, poliovirus receptor (CD155), and CD99. These molecules are components of the lateral border recycling compartment (LBRC), a perijunctional network of interconnected tubulovesicular membrane that traffics to surround the leukocyte as it passes across the endothelial cell. This targeted recycling of LBRC requires kinesin to move the membrane along microtubules, and interfering with LBRC trafficking blocks transmigration of neutrophils, monocytes, and lymphocytes. The LBRC is also recruited to mediate transcellular migration when that occurs. Movement of the LBRC is coordinated with events on the luminal surface, such as clustering of intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 under the migrating leukocyte, as well as movement of vascular endothelial cadherin and its associated catenins out of the junction at the site of transendothelial migration. How these events are coordinated is not known, but their regulation shares common signaling pathways that may serve to connect these steps. *(Am J Pathol 2014, 184: 886–896; http://dx.doi.org/10.1016/j.ajpath.2013.12.033)*

Transendothelial Migration

The Point of No Return in the Inflammatory Response

The inflammatory response, the body's rather stereotyped response to tissue damage of any kind, evolved to heal wounds and fight infections. However, recent improvements in human health mean that we are living long enough to experience the other side of the double-edged sword of inflammation. There has not been time enough on an evolutionary scale to select against the baggage that comes with our ability to heal wounds and kill foreign microorganisms. Inflammation is at the root of virtually all pathology. Thus, there has been great interest in understanding how inflammation is regulated and so to design pharmaceutical and other interventions to control it.

Supported by NIH grants R01-HL046849 and R37-HL064774.

The Rous–Whipple Award is given by the American Society for Investigative Pathology to a senior pathologist with a distinguished career in experimental pathology research and continued productivity at the time of the award. William A. Muller, recipient of the 2013 ASIP Rous–Whipple Award, delivered a lecture entitled "How Endothelial Cells Regulate Transmigration of Leukocytes in the Inflammatory Response," on April 21, 2013, at the annual meeting of the American Society for Investigative Pathology in Boston, MA.

Disclosures: None declared.

http://dx.doi.org/10.1016/j.ajpath.2013.12.033

Acute inflammation involves soluble and cellular mediators that act over the first minutes to hours of the inflammatory response. At the site of inflammation, generally in postcapillary venules, inflammatory mediators such as histamine secreted by mast cells cause local endothelial permeability, allowing preformed agents such as antibody and complement to cross endothelial junctions into the tissue. It is a common misconception that the increase in vascular permeability is causally related to increased leukocyte extravasation. Although these phenomena are both part of the acute inflammatory response, peak vascular leakage occurs well before leukocyte extravasation begins.[1] Furthermore, the gaps between endothelial cells created by these agents measure only a few hundred angstroms (tens of nanometers), not nearly large enough to allow white blood cells to pass through. Other mechanisms are therefore required to allow leukocytes into the area. Recent *in vivo* studies demonstrate conclusively that vascular permeability and transendothelial migration (TEM) are separate phenomena. Mice made genetically deficient in the actin-bundling protein cortactin have vessels that are constitutively leaky and have an exaggerated vascular leak response to inflammation. However, leukocyte emigration in these mice is actually reduced, compared with wild-type littermates, because of reduced intercellular adhesion molecule 1 (ICAM-1) clustering and therefore reduced leukocyte adhesion and transmigration.[2]

The Multistep Extravasation Paradigm

The process of leukocyte extravasation has been broken down into a series of adhesive and signaling interactions between leukocytes and endothelial cells. Over the past two decades, we have learned much about the cellular and molecular interactions that take place during this process.[3–5] Histamine, thrombin, and other mediators of acute inflammation stimulate exocytosis of Weibel–Palade bodies from endothelial cells, bringing P-selectin to the luminal surface. The same increased vascular permeability that allows plasma to leave the bloodstream causes local hemoconcentration, which decreases the rate of blood flow, allowing selectin ligands expressed on the surface of the slowly moving leukocytes to contact P-selectin and thus causing the leukocytes to roll along the luminal surface. Rolling brings the leukocytes close enough for their chemokine receptors to be activated by chemokines attached to the endothelial surface via glycosaminoglycans. Under certain conditions, other cell-surface receptors (eg, platelet-activating factor receptor) bind to and are activated by nonchemokine ligands expressed on the endothelial surface. The activated receptors, through an inside-out signaling cascade, activate leukocyte integrins for tighter adhesion to endothelial surface. This leads first to slow rolling on the endothelium and then to firm arrest. Attached leukocytes then locomote over the endothelial surface, using primarily the β_2 integrin CD11b/CD18 bound to ICAM-1[6–8] to reach the junction. When viewed *in vivo* by intravital microscopy, leukocytes are sometimes seen to migrate over several endothelial cells (occasionally migrating against the direction of blood flow) before they cross the endothelial cells, usually passing through the endothelial junctions.

Rolling, activation, adhesion, and locomotion (intraluminal crawling) are all critical for leukocyte extravasation, but these are reversible. In fact, most leukocytes that enter a venule at the site of inflammation do not roll, most leukocytes that roll do not adhere, and most that adhere do not extravasate.[9] However, once leukocytes make the commitment to cross the endothelium into the tissue, with notable exceptions,[10] they never go back—at least not as the same type of cell. And all of the good, the bad, and the ugly of inflammation takes place once leukocytes cross blood vessels. Thus, diapedesis or TEM is a logical process to study if one hopes to control an ongoing inflammatory response.[5,11]

Diapedesis Is a Distinct Step in TEM

Electron microscopy studies of leukocytes in the process of TEM show that the leukocytes squeeze in amoeboid fashion between tightly apposed endothelial cells.[12,13] Neutrophils have the pharmacological armamentarium to blow a hole in the vessel wall, but instead of barging in like a SWAT team, they sneak in like spies, minimizing leakage of vascular contents into the interstitial tissues. It was this tight apposition of leukocyte and endothelial cell membranes that fascinated me. Considering that it takes only 1 to 2 minutes for the leukocyte to completely traverse the endothelial border *in vitro*[6] or *in vivo*,[10] this suggests that very specialized membrane–membrane interactions are taking place. In the 1980s, when I started my training, leukocytes were assumed to direct the inflammatory response. They were known to have receptors for specific chemoattractants as well as adhesion molecules (later defined as integrins) promoting their migration. Endothelial cells were assumed to serve only as a relatively inert Teflon-like lining to blood vessels, preventing blood from clotting. However, the ability to culture endothelial cells *in vitro*, which was pioneered at the Gimbrone and Jaffe laboratories in the early 1970s,[14,15] catapulted vascular biology into the molecular era. It soon became apparent that endothelial cells play a major role in the regulation of most metabolic and physiological processes, including blood pressure, glucose metabolism, thrombosis, hemostasis, and inflammation. I wondered whether there were specific endothelial molecules at the junctions important for intercellular adhesion that regulate diapedesis, in the same way that the selectins and vascular cell adhesion molecule 1 (VCAM-1) and ICAM-1 on the apical surface are important for leukocyte rolling and adhesion, respectively.[16–19]

Adhesion Molecules at the Junction with Unique Roles in TEM

Monoclonal antibodies (mAbs) were made against endothelial cells and selected for those that bound to antigens concentrated