

These concerns aside, the study by Martin and Goodnow will undoubtedly stimulate future investigations into the functional differences between IgM and IgD BCRs and those expressed by B cells after CSR, as well as the molecular mechanisms responsible for such differences. Published data concerning this area are limited, despite the fact that a major fraction of memory B cells in mice and humans expresses class-switched BCRs. Because all forms of BCRs are found associated with the signal-transducing Ig α and Ig β coreceptors¹¹, differences in activity among the classes of BCR are likely accounted for by unique interactions between their membrane and cytoplasmic domains and yet-to-be-identified integral membrane and cytoplasmic factors. Such specific interactions could lead to differences in plasma membrane distribution and density, transmembrane signaling and endocytic potential. The latter idea is supported by previous *in vitro* studies in the case of mouse IgG1 and IgG2a isotypes¹².

Taken together with previous studies of the “maturation” of IgV region structure and function during the development of the memory B cell compartment, the study by Martin and Goodnow inspires a new working model for BCR function at different stages of B cell development (Fig. 1). Primary B cells

express IgM and IgD BCRs, whose V regions have not been altered by somatic hypermutation. Such “affector” BCRs efficiently promote foreign antigen-independent development in central lymphoid organs and mediate tolerance induction to the self-antigens to which they bind with high avidity. Once B cells enter the periphery, the low-avidity self-antigen reactivity of subpopulations of “affector” BCRs may further direct primary development towards particular mature B cell subsets that are phenotypically and functionally distinct^{8,9,13}.

At the onset of an immune response, “affector” BCRs also mediate clonal selection into the response. However, the development of protective immunity to most pathogens requires the transformation of “affector” into “effector” BCRs. This takes place during memory B cell development, most probably in the GC. The V regions of “affector” BCRs are subjected to iterative hypermutation and both positive selection by the driving foreign antigen and negative selection by self-antigens¹⁴. The resulting “effector” V regions possess increased affinity and specificity for the driving foreign antigen, properties that confer exquisite sensitivity for this antigen to memory B cells. Many memory B cell precursors also undergo CSR, which endows their progeny with the second

component of an “effector” BCR, an IgG, IgA or IgE C region. This results in the acquisition of unique functions that promote efficient secondary differentiation, such as rapid and sustained proliferation and generation of AFCs in the case of IgG1. As alluded to by Martin and Goodnow³, this model predicts that humoral immunity is accounted for not so much by quantitative differences in the frequency of pathogen-specific B cells, but by qualitative differences in the function of the BCRs expressed by primary *versus* memory B cells.

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The last molecular fortress in leukocyte trans-endothelial migration

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To efficiently protect the body from infectious organisms, leukocytes circulate as nonadherent cells in the blood and lymph where, upon encountering an inflammatory stimulus, they arrest and migrate into the affected tissues^{1–3}. Once arrested from the bloodstream, the leukocyte faces a formidable barrier made of endothelial cells linked to each other by interendothelial junctions (Fig. 1)⁴. To initiate the transmigration process through this vascular barrier—which is also called diapedesis or extravasation—activated leukocytes must sense the interendothelial junction and engage

in molecular interactions in order to crawl through the cleft between adjacent endothelial cells and gain access to the underlying basement membrane. In contrast to leukocyte adhesion and arrest, the molecular details of leukocyte transmigration are not well understood. In this issue of *Nature Immunology*, Muller⁵ and Weber⁶ and colleagues explore the molecular interactions that are crucial for leukocyte transmigration into the tissues. In addition, they identify candidate molecules that help propel leukocytes through the vascular barrier and into sites of infection.

Leukocyte arrest on inflamed endothelium constitutes only the first phase of their recruitment into the tissues. New data points to the roles played by JAM-1 and CD99 in leukocyte passage through the barrier posed by the vascular endothelium during inflammatory responses.

Leukocyte homing begins with a multi-step adhesion process that captures the leukocyte from circulating blood and adheres it to the vascular wall^{1–3}. Adhesive selectin molecules initiate the rolling of leukocytes along inflamed endothelium. Chemokines induce the activation of integrins on rolling leukocytes that then bind to vascular ligands; this process leads to the tight adhesion of leukocytes to the endothelium⁷. One of these integrins is the heterodimeric lymphocyte function-associated antigen 1 (LFA-1), which interacts with intercellular adhesion

molecule 1 (ICAM-1) expressed by vascular endothelium upon inflammation⁷. However, considerably less is known about the mechanisms used by leukocytes to cross the endothelium. Until now, platelet-endothelial cell adhesion molecule 1 (PECAM-1, also known as CD31) was the only known molecule involved in diapedesis and expressed on leukocytes and endothelial cells at the intercellular contacts^{8,9}.

Muller and colleagues now identify a second molecule, CD99, that is essential for monocyte transmigration and is concentrated at interendothelial contacts⁵. They show that diapedesis occurs as a multistep cascade involving sequential molecular interactions between transmigrating leukocytes and endothelial cells. Using a panel of antibodies generated against endothelial cells, they show that transmigrating monocytes first use homophilic PECAM-1 interactions to link leukocytes to the luminal surface of endothelial cells and initiate diapedesis. Homophilic CD99 interactions then allow the invading leukocytes to transmigrate through clefts in the endothelial wall. This mechanism probably occurs under inflammatory and noninflammatory conditions because the distribution of PECAM-1 and CD99 at endothelial cell-cell contact regions is not affected by inflammatory stimuli. These two molecules may thus form the minimal basic housekeeping elements for leukocyte transmigration.

A role for inflammatory stimulation of endothelial cells in the control of leukocyte transmigration is provided by the study by Weber and colleagues⁶. They identify the molecule junctional adhesion molecule 1 (JAM-1) as a ligand for LFA-1. In contrast to PECAM-1 or CD99, JAM-1 is exclusively localized at junctional complexes between quiescent endothelial cells. Previously, JAM-1 was shown to relocalize to the apical surface of endothelial cells after inflammatory stimulation¹⁰. Weber and colleagues show that JAM-1-

LFA-1 interaction is involved in tight adhesion or transmigration of leukocytes, depending on the apical or junctional localization of JAM-1 on endothelial cells. These results support the hypothesis that endothelial cells actively control the efficiency of leukocyte transmigration by regulating the structure of intercellular junctions.

and are formed by a network of transmembrane proteins that are specific to each type of junction. Gap junctions are clusters of transmembrane, hydrophilic channels that allow the direct exchange of ions and small molecules between adjacent cells. However, no evidence exists for the involvement of gap junctions in transmigration of leukocytes. Adherens junctions are formed by transmembrane proteins of the cadherin family, which exhibit homophilic interactions and are associated with intracellular catenins and the actin cytoskeleton. Numerous studies have shown that the binding of cadherins to the catenin-actin cytoskeleton is essential for morphogenesis and maintenance of adherens junctions at cell-cell contacts. In endothelial cells, adherens junctions are formed by vascular-endothelial (VE)-cadherin, which plays a central role in vasculogenesis and in the regulation of macromolecular permeability. It has been proposed that VE-cadherin acts as a gatekeeper for the passage of leukocytes, which, themselves, do not express VE-cadherin. The migrating leukocyte induces delocalization of VE-cadherin away from adherens junctions, resulting in a gap through which the transmigrating leukocytes can pass¹². Although the mechanism by which delocalization of VE-cadherin occurs is still unknown, it is clear that the opening of the endothelial adherens junction is restricted to a limited region.

The third type of intercellular adhesive complexes are the tight junctions that form close contacts between endothelial cells and are located at the most apical part of the junction between adjacent cells. Electron microscopy studies have shown that the number of tight junctions in endothelial cells varies with the requirement for permeability control¹¹. For example, tight junctions are well developed in the brain vascular bed that forms the blood-brain barrier, whereas tight junctions are barely detectable in the high endothelial venules of lymphoid organs where constitutive extravasation of

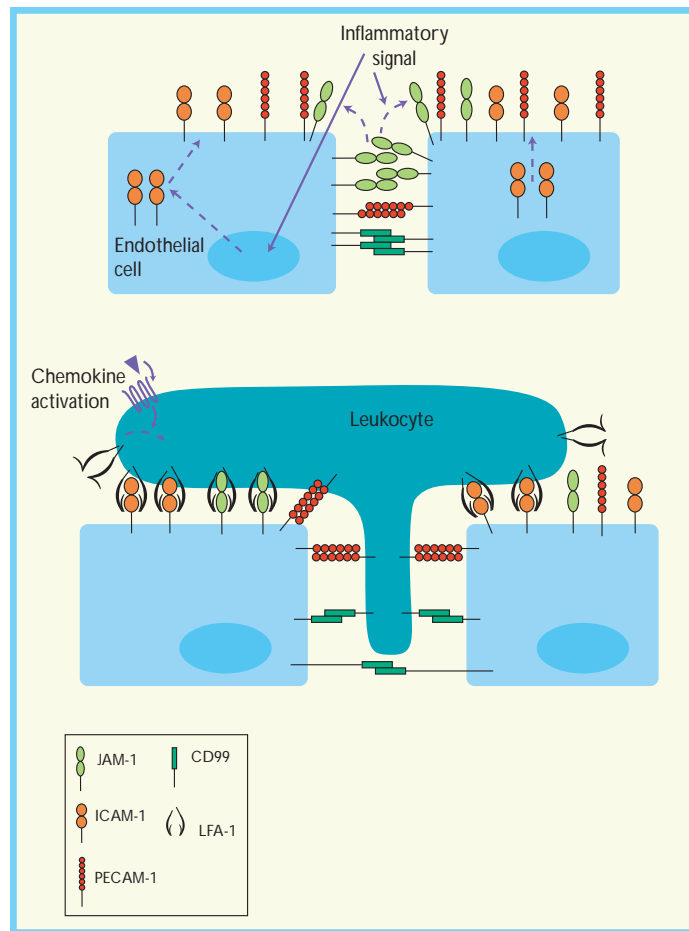


Figure 1. New molecular mechanisms in leukocyte transendothelial migration. (a) An inflammatory stimulus leads to expression of ICAM-1 on the luminal face of the vascular endothelium. The same signal affects the junctional localization of JAM-1 at interendothelial contacts while the lateral distribution of CD99 and PECAM-1 is not affected. (b) After tethering, rolling, triggering and tight adhesion, leukocytes transmigrate through the vascular endothelium using JAM-1, PECAM-1 and CD99. Chemokines activate integrins on leukocytes (triggering), which leads to LFA-1 engagement with vascular ICAM-1 and JAM-1. Subsequently, sequential trans-homophilic interactions of PECAM-1 and CD99 contribute to trans-endothelial migration of leukocytes.

Vascular junctions are molecular complexes that contribute to the barrier function of the blood vessel, hindering leukocyte diapedesis¹¹. At least three types of intercellular adhesive complexes have been described: gap junctions, adherens junctions and tight junctions⁵. These were defined morphologically



lymphocytes occurs. Three types of transmembrane proteins are found in tight junctions: occludin, claudins and the JAMs⁴. Tight junctions consist of strands of the apparently fused plasma membranes of adjacent cells in which the tetraspan proteins occludin and claudins are incorporated. JAM-1 is an immunoglobulin (Ig) superfamily molecule that is peripherally associated with tight junctions *via* cytoplasmic adaptor proteins bridging JAM-1 and claudins. Thus, inflammatory signals may induce the relocalization of JAM-1 on endothelial cells by regulating its association with adaptor proteins.

Monoclonal anti-JAM-1 blocked monocyte transmigration in a murine model¹³. However, because murine monocytes do not express JAM-1, it was postulated that the antibody interfered either with the remodeling of interendothelial junctions during transmigration or that additional ligands for JAM-1 exist on transmigrating cells. Weber and colleagues bring a valuable missing piece to this puzzle by showing that LFA-1 on leukocytes binds to JAM-1 expressed by endothelial cells⁶. They show that the membrane-proximal Ig domain of JAM-1 supports its interaction with LFA-1, whereas the membrane-distal Ig domain of JAM-1 is responsible for its homophilic dimerization at interendothelial junctions¹⁴ (Fig. 1). This structural duality opens the possibility that JAM-1–JAM-1 interactions at interendothelial contacts occur simultaneously to LFA-1–JAM-1 interactions between the transmigrating leukocyte and endothelial cells. In addition, when JAM-1 is localized on the luminal side of the blood vessel, it induces tight adhesion of leukocytes. Until now this function was assigned to ICAM-1 interaction with LFA-1³. However, the exclusive expression of ICAM-1 on the luminal surface of inflammatory endothelium could not explain the involvement of LFA-1 in transmigration. The dual involvement of LFA-1 in tight adhesion and transmigration of leukocytes has now been identified⁶. The scenario outlined above, however, does not provide a role for the expression of JAM-1 on human circulating cells nor does it provide an explanation for the signals that lead to relocalization

of JAM-1 in endothelial cells. Answering this question may be more complex than previously thought because it seems that further molecules are involved in leukocyte diapedesis.

At present it is not clear whether transmigration starts with PECAM-1–PECAM-1 or LFA-1–JAM-1 interactions, whether these mechanisms operate at the same time or whether there are qualitative differences between these adhesion pairs. PECAM-1 can influence the cellular actin cytoskeleton, the machinery for cell migration¹⁵. However, the direct involvement of PECAM-1 in leukocyte migration has not been identified. This contrasts with the integrin LFA-1, which directly participates in leukocyte migration by linking the extracellular environment to the intracellular cytoskeleton. The current model suggests that PECAM-1 ligation transduces signals into cells through its association with the phosphatases SHP-1 and SHP-2. This process occurs either in transmigrating leukocytes or endothelial cells in which it may contribute to open the junctional complexes. Both mechanisms contribute to facilitating leukocyte diapedesis through the vascular wall.

The final step in leukocyte transmigration involves CD99. This molecule was identified in the early 1990s and is expressed by all leukocytes and red blood cells. Muller and colleagues show that vascular endothelial cells constitutively express CD99 at lateral contacts⁵. In contrast to anti-PECAM-1, anti-CD99 arrests leukocytes only after penetrating deep into the interendothelial junctions, just before transmigration is completed. The late arrest of the leukocyte may be explained by blockade of the uropod, the tail of the migrating cell and a membrane region that concentrates high numbers of adhesion molecules, such as ICAM-1 and possibly others. Unfortunately we do not know yet whether CD99 also becomes enriched in this region. By analogy to PECAM-1, the question arises of whether CD99 is directly or indirectly involved in leukocyte migration. CD99 regulates LFA-1 integrin expression and affinity *via* an unknown signal-transduction mechanism¹⁶. Thus, it is likely that CD99 interferes

indirectly with leukocyte transmigration, possibly by regulating the function of integrins in the uropod.

The reports by Muller⁵ and Weber⁶ and colleagues provide the first solid evidence for the existence of a multi-step molecular mechanism involved in leukocyte transmigration. The role of JAM-1 and CD99 in guiding leukocytes through the junctional complexes as well as the refined dissection of this multi-step cascade will be research activities for the immediate future; real-time intravital microscopy will be instrumental for this¹². The results should tell us why a blood vessel does not become leaky while leukocytes proceed through the gap in the endothelium, and confirmation of the two mechanisms *in vivo* will also be of compelling interest. It took ten years for us to understand the three steps of leukocyte adhesion and many pharmaceutical companies have now targeted these steps to identify compounds that halt chronic inflammation. Thus, identification of the molecules involved in leukocyte transmigration may provide important future targets for therapeutic intervention. The current state of affairs suggests that this may be achieved rapidly.

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