Adhesion molecule knockouts: one step forward and one step backward.

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Editorial

Mice with genetic deletions ("knockouts") of either ICAM-1 or P-selectin, or both, are phenotypically normal, but induced neutrophil accumulation in the peritoneal cavity (after instillation of *Streptococci pneumoniae*) is greatly reduced. Yet, in the lung, neutrophil recruitment is not affected under the same circumstances (1). This pattern is similar to findings in rabbit lung in which neutrophil responses to the same microorganisms are independent of a requirement for CD18, yet in subcutaneously implanted sponges containing *S. pneumoniae* neutrophil recruitment is CD18-dependent (2). Accordingly, adhesion molecule requirements for neutrophil recruitment may, to some extent, be organ specific. Since in rabbit lung and in subcutaneously implanted sponges neutrophil accumulation in the presence of *Escherichia coli* is CD18-dependent, it can also be concluded that under certain conditions adhesion molecule requirements for neutrophil recruitment are stimulus-specific. Using knockout mice of the type described above, it will be important to investigate neutrophil accumulation in lung and in the peritoneal cavity in response to *E. coli*. It seems likely that under these conditions requirements for both ICAM-1 and P-selectin will be found.

Induction of neutrophil-enriched peritoneal exudates by instillation of thioglycollate has demonstrated the requirement for P-selectin (3), although there is other evidence that P- and E-selectins function in concert under similar conditions. This conclusion has been based on selectin-suppression achieved through the use of blocking antibody or knockout technology, or both (4). However, the degree of impairment in neutrophil accumulation when either P-selectin or E-selectin is unavailable is much less than that reported by Bullard et al. (1). Once again, the differences may be due to the nature of the challenge. As compared with thioglycollate, a replicative agent such as *S. pneumoniae* may lead to a much more complex array of engaged mediation pathways (e.g., activation of complement; cytokine elaboration; stimulation of neutrophils, macrophages, and platelets; etc.). Accordingly, interruption of a critical adhesion molecule by knockout technology may produce more dramatic results, a hypothesis that is supported by data (1).

Even though blockade of E-selectin by monoclonal antibodies yields rather dramatic reductions in neutrophil accumulation (for review see reference 5), in mice with E-selectin knockout neutrophil accumulation is little affected and seems to have a co-requirement for P-selectin (4). Why is genetically induced deletion of an adhesion molecule in an embryo less effective than when a blocking antibody is employed in an adult mouse? One possibility is that blocking antibodies reacting with endothelial or leukocytic molecules may trigger secondary events that perturb the ability of the cell to respond subsequently to agonists. A more likely explanation is the possibility that knockout mice have adapted to the genetic deletion. It is possible, for example, that in P- or E-selectin knockout mice compensating mechanisms result in over-expression of either L-selectin on leukocytes and/or over-expression of endothelial ligands for selectins. This possibility can be tested with current technology. If it becomes feasible to induce knockouts in adult mice, it seems likely that, in contrast to embryos, adults will not be able to adapt to genetic deletions. This possibility remains to be determined.

While the dogma developed over the past several years suggests that rolling of blood leukocytes is the earliest step (in cats, rats, and mice) in a sequence of in vivo adhesive interactions, virtually all of this work has been done with mesenteric spreads, in which surgical manipulation alters the vasculature and leads to the rolling of neutrophils along surfaces of post capillary venules, followed by firm attachment of neutrophils to the endothelium via $\beta 2$ integrin and ICAM-1–dependent pathways. Whether the rolling phenomenon occurs in vessels of other tissues is unknown. While in IgG immune complex induced dermal vasculitis in guinea pigs and rats, the efflux of neutrophils occurs in postcapillary venules (6), in rabbit lung neutrophil efflux occurs in capillaries, not in postcapillary venules (7). The same is probably true in most other organs such as the central nervous system, heart, liver, kidney, etc. In these organs neutrophil accumulation appears to develop around capillaries, not larger vessels. The geometric dimensions in lung capillaries do not allow neutrophils to "bounce" around on capillary surfaces. Indeed, the caliber of rabbit lung capillaries and the "stiffness" of activated neutrophils makes their rolling through pulmonary capillaries a remote possibility (7). If rolling is not a necessary precursor to subsequent adhesive events between neutrophils and endothelial cells, this could suggest that adhesion molecules do not act sequentially but, rather, simultaneously. If this is the case, then blocking or deletion of a single adhesion molecule would result in less than complete interference of adhesive interactions. Data in the literature would support such a concept. Resolution of this issue will have important implications for therapeutic blockade of adhesion molecules in human inflammatory diseases. Like all good and stimulating studies, the data of Bullard et al (1) raise many more questions than answers and provide the basis for additional investigations.

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