Leukocyte Recruitment Into Developing Atherosclerotic Lesions
The Complex Interaction Between Multiple Molecules Keeps Getting More Complex

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A few years ago, I was asked to give a series of lectures to our second-year medical students on basic inflammatory mechanisms. I was taking the place of the late Russell Ross, who had given these lectures for many years. Among the teaching materials Russ had collected was a videotape generated from a 16-mm movie made in the 1940s. The movie showed leukocytes rolling, adhering, and migrating through capillaries in response to an inflammatory stimulus in a rabbit ear chamber. I used this videotape to illustrate the basic steps in a classical inflammatory response but also to introduce to the students the remarkable complexity of the molecular interactions underlying each step in leukocyte recruitment into inflamed tissues. We have come a long way in delineating the nature of these molecular interactions since the 1940s. We now use in vitro adhesion assays and microscopic techniques such as intravital microscopy, coupled with antibodies and receptor antagonists designed to specify which candidate adhesion molecules, counter receptors, and chemokines mediate leukocyte recruitment.1–7 These have provided a basic molecular paradigm for the rolling, activation, arrest, adhesion, and transmigration of leukocytes. L and P selectins appear to be primarily responsible for the initial capture of cells from the flowing blood, E and P selectins for mediating rolling, and 

pecam-1 for adhesion and transmigration.8–10 In addition, products of the arachidonate and complement cascades and chemokines, a large family of small peptides that activate leukocytes via binding to G protein–coupled receptors, also seem to play fundamental roles in the recruitment process.11–13

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Most of our current knowledge of the molecular mediators of leukocyte recruitment has been derived from adhesion assays and studies of the microvasculature. Determining whether the same molecules are involved in recruiting leukocytes into atherosclerotic lesions in the muscular arteries has been more problematic. There is extensive evidence that many of these molecules are expressed by cells in human and experimental atherosclerotic lesions14–23 and that expression of adhesion molecules such as VCAM-1 is temporally related to lesion initiation and progression in animal models.22,24 Furthermore, studies of transgenic mice or mice with targeted mutations have provided some insights into which of these molecules play roles in the atherogenic process. In particular, these studies have so far shown that E and P selectins, ICAM-1, MCP-1, and its receptor CCR2, as well as the IL-8 receptor CXCR2, seem to mediate recruitment of leukocytes into atherosclerotic lesions in hyperlipidemic mice.25–34 However, the in vivo studies have also clearly demonstrated the presence of redundant mechanisms, as in no case has there been a complete ablation of lesion development in the absence of any one of these molecules. A look at the increasing list of known chemokines and the lack of specificity with which they bind to the CCR and CXCR receptors12,13 further emphasizes the extent to which redundancy exists in this system and again points to the underlying complexity of the molecular interactions supporting leukocyte recruitment.

In the current issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Aiello et al35 provide direct evidence that leukotrienes also contribute to monocyte infiltration into atherosclerotic lesions. They convincingly demonstrate that a specific LTb4 receptor antagonist significantly inhibits atherosclerotic lesion development and reduces the macrophage component of the lesions in apoE−/− and LDLR−/− mice. Thus, these investigators have added a new level of complexity to the equation. Although it has been known for many years that LTb4 plays a significant role in the recruitment of neutrophils and that macrophages express LTb4 receptors,36 demonstrating a role for LTb4 in atherosclerosis has awaited the development of highly specific LTb4 receptor antagonists and the availability of convenient models in which to test their effects.

Perhaps it is not surprising that LTb4 plays a role in the atherogenic process being a product of the 5-lipoxygenase pathway. We have speculated for many years about the potential roles of arachidonic acid metabolites in atherosclerosis, in particular those generated by 12/15 lipoxygenase. These range from the oxidation of LDL37,38 to stimulating intracellular signal transduction.39 There is also in vitro evidence that products of 12/15 lipoxygenase mediate leukocyte adhesion.40–42 Furthermore, recent data in mice with disruption of the 12/15 lipoxygenase gene or overexpressing 12/15 lipoxygenase provide direct evidence that products of these enzymes contribute to the atherogenic process.43–46

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Despite evidence of the formation of LTB4 in atherosclerotic lesions, to date there have been very few previous studies suggesting that products of 5-lipoxygenase play any role in atherogenesis.\(^{47}\) Aiello et al\(^{15}\) provide two additional and intriguing observations that suggest that LTB4 mediates leukocyte recruitment into atherosclerotic lesions by indirect mechanisms. First, they demonstrate that the LTB4 receptor antagonist inhibits the LTB4-induced upregulation of CD11b expression in circulating monocytes from the treated mice. CD11b is the \(\beta2\) integrin component of the CD11/CD18 complex on monocytes that mediates binding to ICAM-1 as well as to fibrinogen and heparin.\(^{49}\) It is currently unclear to what extent ICAM-1 contributes to lesion formation as targeted knockout of ICAM-1 in mice only has a modest effect on inhibiting lesion formation.\(^{27}\) Furthermore, Kubo et al\(^{40}\) have shown that bone marrow transplantation with CD11b-deficient cells does not inhibit lesion formation in LDLR\(^{-}\) mice. Thus, it seems unlikely that this effect of LTB4 plays a significant role in recruiting monocytes into lesions in mice. The second observation is much more compelling. Aiello et al\(^{15}\) have also shown that in mice simultaneously deficient in MCP-1 and apoE, there is no inhibitory effect of the LTB4 receptor antagonist. There are two possible interpretations for this observation. The first is rather trivial and suggests that, in the MCP-1 deficient mice, the absence of MCP-1 already reduces monocyte recruitment to the point where any other inhibitory mechanism has no additional effect. This is somewhat unlikely given the degree of redundancy in the chemo- kine system and the fact that CCR2 deficient mice exhibit a reduced rate of lesion initiation with catch-up at later time points.\(^{32}\) The more interesting possibility is that there is cross-talk between MCP-1 and LTB4 signaling. For example, LTB4 may induce expression of MCP-1 and thus functions to recruit monocytes not via a direct chemotactic response, but through MCP-1. Unfortunately, Aiello et al\(^{15}\) don’t provide any evidence that the LTB4 receptor antagonist inhibits MCP-1 expression or conversely that LTB4 induces MCP-1 in the apoE\(^{-}\) or LDLR\(^{-}\) mice. However, as noted by the Aiello et al,\(^{15}\) it has been shown that the same LTB4 receptor antagonist inhibits neutrophil and macrophage recruitment into the peritoneum of mice with cecal ligation and puncture and reduces the levels of MCP-1 in the peritoneal cavity of these mice.\(^{51}\) Furthermore, administration of MCP-1 elevates the levels of LTB4 in the peritoneum and stimulates the production of LTB4 in peritoneal macrophages in vitro. It is highly likely, therefore, that a similar pattern of cross-talk occurs in the artery wall. The observations of Aiello et al\(^{15}\) suggest a new direction for studies of monocyte recruitment as it appears that there is now another set of molecules to add to our list of those mediating leukocyte recruitment into atherosclerotic lesions, the leukotrienes. Regardless of whether they exert a direct chemotactic response or function through the induction of adhesion molecules and chemokines such as MCP-1, it is clear that what was already a complex system has just gotten a bit more complex.

References

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