Vascular cell adhesion molecule-1: more than an endothelial–leukocyte adhesion molecule

Initial studies of vascular cell adhesion molecule-1 (VCAM-1) focused on its role in endothelial–leukocyte interactions (1, 2). A member of the immunoglobulin gene superfamily, this transmembrane protein on the surface of endothelial cells interacts with the β_1 integrin very late antigen-4 expressed by mononuclear leukocytes (3). In the context of atherosclerosis, this molecule warrants particular interest because of its early and focal expression on endothelial cells just where monocytes first accumulate at the sites of fatty streak formation (4, 5).

The case of VCAM-1 illustrates a general principal that the restriction of expression of a molecule decreases as a function of time that the molecule is studied. Early surveys disclosed VCAM-1 expression not only on endothelium but on follicular dendritic cells in lymph nodes (6). Others reported VCAM-1 expression on skeletal muscle cells and postulated a role for this molecule in myogenesis (7). Elsewhere in this issue, O'Brien et al. report that in human atheroma not only endothelial cells, but certain lesional macrophages and smooth muscle cells also express VCAM-1 (8). In addition, atherogenic diets induce VCAM-1 expression by a subset of intimal smooth muscle cells in experimental atherosclerotic lesions in rabbits (9). Interestingly, although luminal endothelial cells express VCAM-1 within the first week after initiating the atherogenic diet, VCAM-1 expression by intimal smooth muscle cells does not occur until after the sixth week of consumption of the atherogenic diet. A similar sequence may pertain during human atherogenesis as O'Brien et al. find only rare expression of VCAM-1 on luminal endothelial cells at a stage in evolution of the human atherosclerotic lesion when intimal smooth muscle cells often bear this molecule.

O'Brien and colleagues made the additional important observation that the endothelial cells of plaque microvessels also express VCAM-1. Advanced human atheroma contain regions of microvascular plexi that provide a large surface area for macrophage recruitment. Indeed, macrophages colocalize to regions of plaque neovascularization (10). Taken together, these observations support the view that the plaque microvasculature may furnish an important portal for monocyte recruitment during human atherogenesis. Nonetheless, it is premature to conclude that VCAM-1 plays an exclusive or even primary role as a monocyte adhesion molecule during human atherogenesis. VCAM-1 does bind selectively monocytes and lymphocytes, the very types of leukocytes found in atheroma, yet other endothelial leukocyte adhesion molecules (e.g. intercellular adhesion molecule-1, E-selectin) or as yet undiscovered molecules may participate in this interaction.

VCAM-1 on smooth muscle cells, a molecule in search of a function

Although it is easy to envisage a role for endothelial VCAM-1 in monocyte recruitment, the function of leukocyte adhesion molecules on smooth muscle cells within the atherosclerotic plaque seems obscure. Phagocytes and T cells tend to wander, exhibiting more mobility than most parenchymal cells. Perhaps smooth muscle cell VCAM-1 retards the migration of monocytes and T cells, encouraging their more permanent residence within the atherosclerotic plaque. However, VCAM-1 may have functions during atherogenesis unrelated to its adhesion function per se. Increasing evidence points to ongoing immune stimulation during human atherogenesis. While the nature of the antigenic stimulus remains unclear, atheroma contain T lymphocytes in a chronic state of activation (11). While vascular smooth muscle cells stimulate allogeneic immune responses relatively poorly, they present foreign antigens splendidly (12). VCAM-1 in other contexts can provide costimulatory signals for T cell activation (13, 14). Thus, VCAM-1 expression by lesional smooth muscle cells may enhance their ability to stimulate T cells.

Activation of intimal smooth muscle cells during atherogenesis

Regardless of its functional consequences, the finding of VCAM-1 in advanced human atherosclerotic plaques and in the maturing experimental atheroma serve as a marker of "activation" of smooth muscle cells. The concept of "phenotypic modulation" of smooth muscle cells during atherogenesis gained currency some years ago (15). The original concept that intimal smooth muscle cells in the atherosclerotic plaque differ from those in the normal tunica media has proven a valuable paradigm over the years. The nomenclature that describes the intimal smooth muscle cells as "synthetic" and the normal medial smooth muscle cells as "contractile" seems less helpful as few actually measure biosynthetic or contractile function when using these terms.

The increasing availability of specific molecular markers permits a more precise definition of the "activated" smooth muscle cell found in the arterial intima during atherosclerosis. Smooth muscle cells in regions of the atherosclerotic plaque express class II histocompatibility antigens, cell surface molecules required for antigen presentation to helper T cells (16). Many cells within the atherosclerotic intima express intercellular adhesion molecule-1, a member of the immunoglobulin superfamily that may also participate in retention of leukocytes (17, 18). Compared with normal medial smooth muscle cells, those in the atherosclerotic intima display increased levels of the complement regulatory decay accelerating factor (19), and genes for certain growth-promoting molecules, including isoforms of platelet-derived growth factor (20) and tumor necrosis factor (21).

What signals induce smooth muscle activation during atherogenesis?

In vitro studies have established that cytokines regulate most of these markers of activation expressed by intimal smooth muscle cells. Interestingly, we find that the lymphokines gamma interferon and IL-4, products of activated T cells, induce VCAM-1 expression on human smooth muscle cells, while the monokines IL-1 and tumor necrosis factor, products of activated mononuclear phagocytes and intrinsic vascular cells, do

J. Clin. Invest.

0021-9738/93/08/0538/02 $2.00
Volume 92, August 1993, 538–539

not (9). These observations raise the intriguing possibility that the signals for VCAM induction in smooth muscle cells during atherogenesis derive from activated T lymphocytes. However, mediators other than cytokines may induce VCAM-1 expression. For example, constituents of modified lipoproteins such as lysophophatidylcholine can induce VCAM-1 expression by human endothelial cells in culture (22). Other potential stimuli such as hypoxia or glycated proteins have yet to be investigated in this regard.

Despite the advances considered here, we have far to go before achieving a complete understanding of atherogenesis. Fortunately, emerging experimental approaches should permit us to test critically many of the notions proposed above and determine whether VCAM-1 and the other activation markers expressed by smooth muscle cells actually serve the functions that we currently postulate, play no functional role at all, or contribute to lesion evolution in ways not yet imagined.

Peter Libby and Hongmei Li
Brigham and Women's Hospital
Boston, Massachusetts

Acknowledgments

We thank our colleagues Myron I. Cybulsky and Michael A. Gimbrone, Jr., for their helpful discussions.

References

1. Osborn, L., C. Hession, R. Tizard, C. Vassallo, S. Luhowskyj, G. Chi-Rosso, and R. Lobb. 1989. Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. *Cell.* 59:1203–1211.

2. Rice, G. E., and M. P. Bevilacqua. 1989. An inducible endothelial cell surface glycoprotein mediates melanoma adhesion. *Science (Wash. DC).* 246:1303–1306.

3. Elices, M. J., L. Osborn, Y. Takada, C. Crouse, S. Luhowskyj, M. E. Hemler, and R. R. Lobb. 1990. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. *Cell.* 60:577–584.

4. Cybulsky, M. I., and M. A. Gimbrone, Jr. 1991. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. *Science (Wash. DC).* 251:788–791.

5. Li, H., M. I. Cybulsky, M. A. Gimbrone, Jr., and P. Libby. 1993. An atherogenic diet rapidly induces VCAM-1, a cytokine regulatable mononuclear leukocyte adhesion molecule, in rabbit endothelium. *Arterioscler. Thromb.* 13:197–204.

6. Rice, G. E., J. M. Munro, C. Corless, and M. P. Bevilacqua. 1991. Vascular and nonvascular expression of INCAM-110. A target for mononuclear leukocyte adhesion in normal and inflamed human tissues. *Am. J. Pathol.* 138:385–393.

7. Rosen, G. D., J. R. Sanes, R. LaChance, J. M. Cunningham, J. Roman, and D. C. Dean. 1992. Roles for the integrin VLA-4 and its counter receptor VCAM-1 in myogenesis. *Cell.* 69:1107–1119.

8. O'Brien, K., M. Allen, T. McDonald, A. Chait, J. Harlan, D. Fishbein, J. McCarty, M. Ferguson, K. Hudkins, C. Benjamin, et al. 1993. Vascular cell adhesion molecule-1 is expressed in human coronary atherosclerotic plaques: implications for the mode of progression of advanced coronary atherosclerosis. *J. Clin. Invest.* 92:945–951.

9. Li, H., M. I. Cybulsky, M. A. Gimbrone, Jr., and P. Libby. 1993. Inducible expression of VCAM-1 in vascular smooth muscle cells in vivo and in vitro. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 7:A490.

10. Brogi, E., J. Winkles, and P. Libby. 1992. Regional expression of acidic and basic fibroblast growth factor in human atheroma and non-atherosclerotic arteries. *Circulation.* 86:I–85.

11. Stemme, S., J. Holm, and G. K. Hansson. 1992. T lymphocytes in human atherosclerotic plaques are memory cells expressing CD45RO and the integrin VLA-1. *Arterioscler. Thromb.* 12:206–211.

12. Fabry, Z., M. M. Waldschmidt, S. A. Moore, and M. N. Hart. 1990. Antigen presentation by brain microvessel smooth muscle and endothelium. *J. Neuroimmunol.* 28:63–71.

13. van-Seventer, G. A., W. Newman, Y. Shimizu, T. B. Nutman, Y. Tanaka, K. J. Horgan, T. V. Gopal, E. Ennis, D. OSullivan, H. Grey, et al. 1991. Analysis of T cell stimulation by superantigen plus major histocompatibility complex class II molecules or by CD3 monoclonal antibody: costimulation by purified adhesion ligands VCAM-1, ICAM-1, but not ELAM-1. *J. Exp. Med.* 174:901–913.

14. Damle, N. K., K. Klussman, P. S. Linsley, and A. Aruffo. 1992. Differential costimulatory effects of adhesion molecules B7, ICAM-1, LFA-3, and VCAM-1 on resting and antigen-primed CD4+ T lymphocytes. *J. Immunol.* 148:1985–1992.

15. Chamley-Campbell, J. H., G. R. Campbell, and R. Ross. 1979. The smooth muscle cell in culture. *Am. Physiol. Soc.* 58:1–61.

16. Hansson, G. K., L. Jonasson, J. Holm, and L. Claesson-Welsh. 1986. Class II MHC antigen expression in the atherosclerotic plaque: smooth muscle cells express HLA-DR, HLA-DQ and the invariant gamma chain. *Clin. Exp. Immunol.* 64:261–268.

17. Poston, R. N., D. O. Haskard, J. R. Coucher, N. P. Gall, and R. R. Johnson-Tidey. 1992. Expression of intercellular adhesion molecule-1 in atherosclerotic plaques. *Am. J. Pathol.* 140:665–673.

18. Printseva, O., M. M. Peclo, and A. M. Gown. 1992. Various cell types in human atherosclerotic lesions express ICAM-1. Further immunocytochemical and immunochemical studies employing monoclonal antibody 10F3. *Am. J. Pathol.* 140:889–896.

19. Seifert, P. S., and G. K. Hansson. 1989. Decay-accelerating factor is expressed on vascular smooth muscle cells in human atherosclerotic lesions. *J. Clin. Invest.* 84:597–604.

20. Wilcox, J. N., K. M. Smith, L. T. Williams, S. M. Schwartz, and D. Gordon. 1988. Platelet-derived growth factor mRNA detection in human atherosclerotic plaques by in situ hybridization. *J. Clin. Invest.* 82:1134–1143.

21. Barath, P., M. C. Fishbein, J. Cao, J. Berenson, R. H. Helfant, and J. S. Forrester. 1990. Detection and localization of tumor necrosis factor in human atheroma. *Am. J. Cardiol.* 65:297–302.

22. Kume, N., M. I. Cybulsky, and M. A. Gimbrone, Jr. 1992. Lysophosphatidylcholine, a component of atherogenic lipoproteins, induces mononuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells. *J. Clin. Invest.* 90:1138–1144.