The in and out of monocytes in atherosclerotic plaques: Balancing inflammation through migration

Burkhard Ludewig*[†] and Jon D. Laman*

*Research Department, Kanton Hospital St. Gallen, 9007 St. Gallen, Switzerland; and *Department of Immunology, Erasmus University Medical Center, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands

Recent clinical and experimental evidence indicates that inflammatory processes in the vascular wall are the decisive factor that accounts for the rate of lesion formation and clinical development in patients suffering from atherosclerosis (1). The response-to-injury hypothesis (2) predicts that particular stimuli (= injury) trigger the initiation of an inflammatory cascade in the vessel wall. In addition, alteration of the lipid metabolism is a critical factor intertwined with the complex inflammatory reactions that occur during lesion development. The progressive accumulation of macrophages and other immune cells in the atherosclerotic plaque is one of the hallmarks in atherogenesis (Fig. 1). The ins and outs of macrophage precursors, the blood monocyte, in atherosclerotic lesions have been elegantly dissected in a study by Llodrá *et al.* (3) in this issue of PNAS. The authors suggest that not only recruitment of monocytes to but also their retention within the atherosclerotic lesion contributes to the progression of plaque development.

Immunologists tend to perceive macrophages as simple “antigen crunchers” helping to dispose of damaged cells and to get rid of bacterial pathogens. Lipid-loaded macrophage foam cells in atherosclerotic lesions would thus be responsible for the storage of excess lipids flushed into the lesion via the blood stream or released from dead cells. However, intralésional macrophages are more than a versatile waste-disposal service. Recruitment of monocytes into the lesion is mainly mediated by chemokines (4), with the monocyte chemoattractant protein 1 (MCP-1) being one of the dominant factors (5). Macrophages use different scavenger receptors such as CD36 and scavenger receptor type A to recognize modified forms of low-density lipoprotein (LDL); the consequence of this unregulated receptor-mediated uptake of excess lipids is an enhancement of atherosclerosis (6, 7). As illustrated in Fig. 1, stimulation of macrophages with modified lipids, on the other hand, leads to the production of proinflammatory cytokines such as IL-1, IL-8, and tumor necrosis factor (TNF) and other tissue-damaging compounds such as matrix

Fig. 1. The ins and outs of monocyte-derived antigen-presenting cells in atherosclerotic lesions. Blood monocytes cross the endothelial barrier and develop into either long-lived tissue macrophages or dendritic cells (DC) during their emigration from the tissue via lymphatic vessels or during reverse transmigration through the endothelium into the blood stream. Proatherogenic conditions such as hypercholesterolemia alter this migratory equilibrium. Reduced emigration of blood monocyte-derived antigen-presenting cells together with persisting dyslipidemia results in an accumulation of foam cell macrophages. Increased cellularity in the lesion and the mutual activation of macrophages and T helper cells results in the secretion of inflammatory mediators that promote the progression of the atherosclerotic lesion, as well as eventual thrombotic events and rupture of endstage plaques.

metalloproteinases (MMP) and nitric oxide (8).

The entry of macrophage precursors into the lesion is a matter of attraction, and as mentioned above, is mediated by a certain set of chemokines. However, is the accumulation of foam cell macrophages simply a question of input regulation? The previous work of Randolph

The recruitment of monocytes and their retention within atherosclerotic lesions contribute to plaque development.

and colleagues (9, 10) has taught us that a significant proportion of blood monocytes differentiate into migratory dendritic cells (DC) after they have entered the tissues from the blood stream and migrate subsequently to the local draining lymph node to activate antigen-specific T lymphocytes. This in-out

equilibrium of monocyte-derived cells can be altered by some lipids that are known mediators of atherosclerosis. Llodrá *et al.* (3) could show that both lysophosphatidic acid and an analog of the platelet-activating factor significantly inhibit reverse transmigration of monocyte-derived DC in a cell culture model. These data strongly suggest that the emigration of monocyte-derived cells from the vessel wall may be distorted under conditions that favor the development of atherosclerosis.

Llodrá *et al.* (3) deliver the *in vivo* confirmation of this interpretation by using a transplantation approach requiring remarkable microsurgery skills. Diseased aortic segments from hypercholesterolemic apolipoprotein E (apoE)-deficient mice were transplanted into either apoE-knockout or wild-type control recipient mice. This model is suitable to study both progression and regression of atherosclerotic lesions because transplantation of aortic segments from apoE-deficient mice into normo-

See companion article on page 11779.

[†]To whom correspondence should be addressed. E-mail: burkhard.ludewig@kssg.ch.

© 2004 by The National Academy of Sciences of the USA

cholesterolemic recipients results in lesion regression, whereas lesion development continues progressively after transplantation into hypercholesterolemic mice (11). And indeed, trafficking of monocyte-derived cells out of the atherosclerotic lesion is dramatically reduced under conditions that favor the development of atherosclerosis such as hypercholesterolemia. Emigration is thus a new parameter regulating plaque macrophage numbers. It is noteworthy that, according to Llodrá *et al.* (3), monocyte-derived DC leave the vessel wall not only via draining lymph vessels but also in an abluminal-luminal trafficking process back to the blood stream. It is here where the reader of the companion paper wonders how cells move out of the vessel wall into the arterial lumen with its high hydrostatic pressure. It will be interesting to delineate the mechanisms regulating reverse transmigration of monocyte-derived DC into the arterial blood stream.

The cellular infiltrate found in atherosclerotic lesions not only consists of foam cell macrophages but also contains a considerable number of activated CD4⁺ T helper cells and even activated DC (12). Thus, the question arises whether specific T cell-mediated immune responses contribute to the inflammatory reaction in the lesion or whether the rather high frequency of intralesional T cells is simply a by-product

of the macrophage-driven inflammation. The article by Llodrá *et al.* (3) provides a partial answer to the question. A significantly reduced egress of monocyte-derived DC from the plaque should reduce the presentation of T cell antigens in the draining lymph node. This would,

Blocking mobilization of dendritic cells may be a general mechanism that helps to confine immune responses to distinct lesions.

at least partially, stop the progress of T cell activation and thereby attenuate a generalized inflammatory reaction. This view is supported by a recent report that shows a significant inhibition of monocyte-DC conversion by bacterial lipopolysaccharide or even whole bacteria (13). Blocking the mobilization of monocyte-derived DC under inflammatory conditions may thus be a general mechanism that helps to confine the immune response to a distinct lesion.

The findings of this study may have important implications for another chronic inflammatory disease in which foam cell macrophages are important: in multiple sclerosis these cells abundantly develop in the central nervous system because of ingestion of myelin membranes and apoptotic cells. Cells with macrophage and DC characteristics present myelin protein antigens as well as neutral lipids in the cervical lymph nodes draining the brain (14), with as-yet-unknown consequences for immune reactivity and its regulation. Systematic comparison of these two diseases involving foam cells in chronic lesions may prove fruitful. For instance, Llodrá *et al.* (3) draw attention to the intermediate DC-macrophage characteristics of the cells emigrating from atherosclerotic lesions. Overall, the work of Llodrá *et al.* (3) not only broadens our understanding of the tight regulation of cellular processes that modulate immunopathological reactions in atherosclerosis, it also points out new potential avenues for therapeutic intervention by balancing of the inflammatory response in the atherosclerotic plaque through monocyte migration.

This work was partly supported by Grant 2001B077 from The Netherlands Heart Foundation, a program grant from The Netherlands MS Research Foundation, and Grant 32-63415.00 from the Swiss National Science Foundation.

1. Binder, C. J., Chang, M. K., Shaw, P. X., Miller, Y. I., Hartvigsen, K., Dewan, A. & Witztum, J. L. (2002) *Nat. Med.* **8**, 1218–1226.
2. Ross, R. (1999) *N. Engl. J. Med.* **340**, 115–126.
3. Llodrá, J., Angeli, V., Liu, J., Trogan, E., Fisher, E. A. & Randolph, G. J. (2004) *Proc. Natl. Acad. Sci. USA* **101**, 11779–11784.
4. Bursill, C. A., Channon, K. M. & Greaves, D. R. (2004) *Curr. Opin. Lipidol.* **15**, 145–149.
5. Gu, L., Okada, Y., Clinton, S. K., Gerard, C., Sukhova, G. K., Libby, P. & Rollins, B. J. (1998) *Mol. Cell* **2**, 275–281.
6. Suzuki, H., Kurihara, Y., Takeya, M., Kamada, N., Kataoka, M., Jishage, K., Ueda, O., Sakaguchi, H., Higashi, T., Suzuki, T., *et al.* (1997) *Nature* **386**, 292–296.
7. Podrez, E. A., Febbraio, M., Sheibani, N., Schmitt, D., Silverstein, R. L., Hajjar, D. P., Cohen, P. A., Frazier, W. A., Hoff, H. F. & Hazen, S. L. (2000) *J. Clin. Invest.* **105**, 1095–1108.
8. Greaves, D. R. & Channon, K. M. (2002) *Trends Immunol.* **23**, 535–541.
9. Randolph, G. J., Beaulieu, S., Lebecque, S., Steinman, R. M. & Muller, W. A. (1998) *Science* **282**, 480–483.
10. Randolph, G. J., Inaba, K., Robbiani, D. F., Steinman, R. M. & Muller, W. A. (1999) *Immunity* **11**, 753–761.
11. Reis, E. D., Li, J., Fayad, Z. A., Rong, J. X., Hansoty, D., Aguinaldo, J. G., Fallon, J. T. & Fisher, E. A. (2001) *J. Vasc. Surg.* **34**, 541–547.
12. Millonig, G., Schwentner, C., Mueller, P., Mayerl, C. & Wick, G. (2001) *Curr. Opin. Lipidol.* **12**, 547–553.
13. Rotta, G., Edwards, E. W., Sangaletti, S., Bennett, C., Ronzoni, S., Colombo, M. P., Steinman, R. M., Randolph, G. J. & Rescigno, M. (2003) *J. Exp. Med.* **198**, 1253–1263.
14. de Vos, A. F., van Meurs, M., Brok, H. P., Boven, L. A., Hintzen, R. Q., van der Valk, P., Ravid, R., Rensing, S., Boon, L., 't Hart, B. A. & Laman, J. D. (2002) *J. Immunol.* **169**, 5415–5423.