

Circulating monocytes and atherogenesis: From animal experiments to human studies

Eduard Shantsila¹; Andrew Devitt²; Gregory Y. H. Lip^{1,2}

¹University of Birmingham Centre for Cardiovascular Sciences, City Hospital, Birmingham, UK; ²School of Life and Health Sciences, Aston University, Birmingham, UK

Despite significant improvements in the prevention and management of atherosclerosis, its consequences still remain a major cause of disability and mortality. Better understanding of pathophysiological processes implicated in the initial stages of atherogenesis is critical for the discovery of effective approaches to prevent ischaemic stage of the disease and to reduce huge economic and social burden currently associated with atherosclerosis (1).

According to the current paradigm the atherosclerotic process is primarily initiated in the vascular wall itself where monocyte-derived macrophages transform to 'foam' cells by accumulating lipids (2). Consequent apoptosis or death of macrophages is largely responsible for necrotic core generation, progressive accumulation of free cholesterol and expansion of the necrotic core within plaques. Often, unbalanced generation of inflammatory cytokines by plaque monocyte-derived macrophages (e.g. via up-regulation of Toll-like receptors) as well as angiogenic promoting pathological plaque neovascularisation, and enzymes degrading extracellular matrix results in destabilisation of atherosclerotic plaques (3–5).

Whilst a critical role of vascular wall monocyte-derived macrophages in atherosclerotic plaque formation is widely recognised, the impact of circulating monocytes on atherogenesis is less established. For example, accumulation of modified

(mainly oxidised) lipids via scavenger receptors was, until recently, exclusively attributed to vascular wall resident macrophages rather than circulating monocytes (6). Oxidised low-density lipoproteins (LDL) taken up via scavenger receptors (e.g. CD36 and CD204) are delivered to lysosomes, where they undergo esterification by cholesterol esterase and are largely converted into free cholesterol and fatty acids (6). It has been reported that CD36 contributes 60–70% of cholesterol ester accumulation in macrophages exposed to oxidised LDL (7). Deletion of the genes encoding CD36 and CD204 retards the development of atherosclerotic lesions in animal models of atherosclerosis (6). However, circulating monocytes are equipped with these same scavenger receptors and may thus start accumulating lipids even prior to their migration to tissues and differentiation to macrophages (8). Exposure to modified LDL induces rapid 'foam' cell formation from freshly isolated peripheral blood monocytes, accompanied by up-regulation of CD204 and CD36 (8). The clinical relevance of these findings is supported by the observation that an absence of CD36 on circulating monocytes in individuals from a Japanese population results in 40–50% reduction in oxidised LDL binding to monocyte-derived-macrophages compared to normal subjects (9). Certainly, monocyte counts are increased in patients with stenotic coronary artery disease and the Intermountain Heart Collaborative Study Group recently reported a population of 3,227 patients with or without coronary artery disease whereby high monocyte counts were independent predictors of future myocardial infarction and death (10).

However, the impact of monocytes in atherogenesis may go far beyond direct lipid accumulation as they are also actively involved in inflammatory responses attributable to their principal function within the innate immune system. Indeed,

monocytes carry numerous surface antigens acting as pattern recognition receptors: Toll-like receptors, CD14, Fc-receptors as well as scavenger receptors discussed above. Such receptors by definition are not specific to individual bacterial pathogens and may even be activated following ligation with the host's own molecules (e.g. heat shock proteins) thus triggering potent inflammatory responses (11). The role of chronic inflammation in atherogenesis and atherothrombosis is progressively recognised (12–14).

Monocytes and their subsets in atherogenesis: mouse models

Circulating monocytes are not a homogeneous population of cells – whether defined phenotypically and functionally – and include several subpopulations, which play complex roles in atherogenesis, each with their own potential for promotion of plaque formation. A substantial part of our knowledge on the role of monocyte subsets in atherogenesis derives from studies of mouse monocytes which include two major subsets: Ly-6C^{hi} monocytes and Ly-6C^{lo} monocytes.

Counts of Ly-6C^{hi} monocytes are increased dramatically in hypercholesterolaemic apoE-deficient mice consuming a high-fat diet, actively adhere to activated endothelium, infiltrate atherosclerotic lesions, and become lesional macrophages (15). Ly-6C^{lo} monocytes enter atherosclerotic lesions less frequently are prone to developing into plaque cells expressing the dendritic cell-associated marker *CD11c*, and appear to have enhanced atherogenic activity, indicating that phagocyte heterogeneity within plaques is linked to distinct sub-populations of infiltrating monocytes.

Plaque recruitment of Ly-6C^{lo} cells is CCR5 (but not CX3CR1)-dependent whilst Ly-6C^{hi} monocytes unexpectedly

Correspondence to:

Dr. Eduard Shantsila
University of Birmingham
Centre for Cardiovascular Sciences, City Hospital
Birmingham, B18 7QH, UK
Tel: +44 121 554 3801, Fax: +44 121 554 4083
E-mail: e.shantsila@bham.ac.uk

Received: March 12, 2010

Accepted: March 16, 2010

Prepublished online: April 29, 2010

doi:10.1160/TH10-03-0176

Thromb Haemost 2010; 104: 191–193

Thrombosis and Haemostasis 104.2/2010

required CX3CR1 (normally expressed at low levels on these cells) in addition to CCR2 and CCR5 to accumulate within plaques (16). Genetic deletion of CCR2, CX3CR1 or their ligands markedly reduces atherosclerotic lesion size in animal models of atherosclerosis (17). Moreover, double deletion of CX3CL1 and CCR2 expression in mice has an additive effect and dramatically reduces macrophage accumulation in the artery wall and development of atherosclerosis (17). Recently, triple inhibition of CCL2, CX3CR1, and CCR5 in hypercholesterolaemic mice has been shown to lead to a marked and additive 90% reduction in atherosclerosis (18). Of interest, in this study the lesion size highly correlated with the absolute number of circulating monocytes, but particularly so with the Ly-6^{lo} subset (18).

Oxidised LDL promotes conversion of CD11c⁻ monocytes to CD11c⁺ cells and a high-fat diet significantly increases the proportion of LyC6^{lo}/CD11c⁺ monocytes in apoE-deficient mice resulting in an increased proportion of circulating CD11c⁺ monocytes with abundant lipid in the cytoplasm and the phenotype of 'foam' cells (19). This formation of CD11⁺ circulating 'foam' cells was associated with CD204 expression on their surface and was accompanied by abundant accumulation CD11c⁺ cells in the atherosclerotic plaques (19).

Monocyte and their subsets in humans: relevance to atherogenesis

Whilst human monocytes do not have direct equivalent markers used for discrimination of mouse monocyte subsets, it is clear that subpopulations exist. At present, human monocyte subsets are characterised by surface expression of CD14 (lipopolysaccharide and apoptotic cell receptor) and CD16 (Fcγ receptor type III). About 85% of circulating monocytes are CD14⁺CD16⁻ and minor CD16⁺ populations (normally below 15%). CD14⁺CD16⁻ monocytes are considered as counterparts of LyC6^{hi} mice monocytes and CD14^{low}CD16⁺ of LyC6^{lo} mouse subset (20).

The proportion of CD14^{low}/CD16⁺ monocytes has been previously shown to positively correlate with serum cholesterol

and triglyceride levels, and negatively correlate with HDL cholesterol levels in patients with hypercholesterolaemia and is increased in those with developed coronary artery disease (21, 22). Also, CD14⁺CD16⁻ monocytes from patients with familial hypercholesterolaemia preferentially take up native LDL whereas CD14^{low}CD16⁺ monocytes exhibit an increased uptake of oxidised LDL via CD36, an increased expression of CD11c and macrophage markers and a higher adherence to activated endothelial cells in response to oxidised or native LDL stimulation (23). Although CD16⁺ monocytes express fewer CD36 binding sites at their surface than CD16⁻ monocytes, they take up significantly more oxidised LDL consistent with our observations of high expression of CD204 on these monocyte subsets (unpublished data) (24). In light of recent data that mouse Ly6Clo monocytes (analogous of CD16⁺ human monocytes), in contrast to Ly6Chi cells, patrol arterial endothelial surface, it seems possible that CD16⁺ monocytes exert a specific role in the handling of oxidised LDL deposits on the vessel wall (19, 25). Indeed, monocytes isolated from patients with coronary artery disease also have increased CX3CR1 expression (26).

However, acknowledging substantial role of monocytes and their subsets in atherogenesis raises important issue of the interactions of these cells with recognised cardiovascular risk factors. At present it is commonly accepted that cardiovascular risk factors exert atherogenic properties mainly through the damage of vascular wall and modulation of lipid metabolism.

In this issue of *Thrombosis and Haemostasis*, Hristov et al. demonstrate for the first time that presence and number of cardiovascular risk factors is significantly and differentially associated with counts of monocyte subsets (27). They report significant correlation of the 'classic' inflammatory CD14⁺CD16⁻ monocytes with the number of risk factors present in patients with coronary artery disease suggesting novel mechanisms linking cardiovascular risk factors and chronic inflammation in atherosclerosis (27). Given that experimental data indicate CD14^{low}CD16⁺ monocytes may be specifically involved lipid accumulation in atherosclerosis, the observa-

tion of the reduced levels of this subset in those with numerous risk factors might appear surprising. However, recent experimental data demonstrates these cells are able to migrate promptly through arterial walls following their activation, but require prolonged time to be replenished (25). Consequently, one may speculate that a low count of CD14^{low}CD16⁺ cells in these settings may simply reflect their accelerated accumulation at atherosclerotic sites. However intrinsic impairment of mobilisation of this monocyte subset, known to have some reparative properties is another possibility which can not be currently be clarified.

Admittedly, whilst specific roles for different monocyte subsets in atherogenesis have been strongly suggested in animal studies, limited human data are available on this important issue. Clearly, much more research work is required to shed further light on the role of monocyte subsets in the formation of atherosclerotic plaques. Additionally, some methodological development is desirable to improve discrimination of monocyte subsets and perform their direct absolute count. More data on this interesting field are awaited with interest.

References

1. Eijgelhaar WJ, Heeneman S, Daemen MJ. The vulnerable patient: refocusing on the plaque? *Thromb Haemost* 2009; 102: 231–239.
2. Falk E. Pathogenesis of atherosclerosis. *J Am Coll Cardiol* 2006; 47 (8 Suppl): C7–12.
3. Santilli F, Davì G. Thrombin as a common downstream target blocking both platelet and monocyte activation. *Thromb Haemost* 2009; 101: 220–221.
4. Shantsila E, Lip GY. The role of monocytes in thrombotic disorders. Insights from tissue factor, monocyte-platelet aggregates and novel mechanisms. *Thromb Haemost* 2009; 102: 916–924.
5. Weber C. Frontiers of vascular biology: mechanisms of inflammation and immunoregulation during arterial remodelling. *Thromb Haemost* 2009; 102: 188–190.
6. Choudhury RP, Lee JM, Greaves DR. Mechanisms of disease: Macrophage-derived foam cells emerging as therapeutic targets in atherosclerosis. *Nat Clin Pract Cardiovasc Med* 2005; 2: 309–315.
7. Murphy JE, Tedbury PR, Homer-Vanniasinkam S, et al. Biochemistry and cell biology of mammalian scavenger receptors. *Atherosclerosis* 2005; 182: 1–15.
8. Kapinsky M, Torzewski M, Büchler C, et al. Enzymatically degraded LDL preferentially binds to

- CD14(high) CD16(+) monocytes and induces foam cell formation mediated only in part by the class B scavenger-receptor CD36. *Arterioscler Thromb Vasc Biol* 2001; 21: 1004–1010.
9. Nozaki S, Kashiwagi H, Yamashita S, et al. Reduced uptake of oxidized low density lipoproteins in monocyte-derived macrophages from CD36-deficient subjects. *J Clin Invest* 1995; 96: 1859–1865.
 10. Horne BD, Anderson JL, John JM, et al. Which white blood cell subtypes predict increased cardiovascular risk? *J Am Coll Cardiol* 2005; 45: 1638–1643.
 11. Ohashi K, Burkart V, Flohe S, et al. Cutting edge: heat shock protein 60 is a putative endogenous ligand of the toll-like receptor-4 complex. *J Immunol* 2000; 164: 558–561.
 12. Soehnlein O, Zernecke A, Weber C. Neutrophils launch monocyte extravasation by release of granule proteins. *Thromb Haemost* 2009; 102: 198–205.
 13. Schuett H, Luchtefeld M, Grothusen C, et al. How much is too much? Interleukin-6 and its signalling in atherosclerosis. *Thromb Haemost*. 2009; 102: 215–222.
 14. Hanriot D, Bello G, Ropars A, et al. C-reactive protein induces pro- and anti-inflammatory effects, including activation of the liver X receptor alpha, on human monocytes. *Thromb Haemost* 2008; 99: 558–569.
 15. Swirski FK, Libby P, Aikawa E, et al. Ly-6Chi monocytes dominate hypercholesterolemia-associated monocytoysis and give rise to macrophages in atherosclerosis. *J Clin Invest* 2007; 117: 195–205.
 16. Tacke F, Alvarez D, Kaplan TJ, et al. Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques. *J Clin Invest* 2007; 117: 185–194.
 17. Veillard NR, Steffens S, Pelli G, et al. Differential influence of chemokine receptors CCR2 and CXCR3 in development of atherosclerosis in vivo. *Circulation* 2005; 112: 870–878.
 18. Combadière C, Potteaux S, Rodero M, et al. Combined inhibition of CCL2, CX3CR1, and CCR5 abrogates Ly6C(hi) and Ly6C(lo) monocytoysis and almost abolishes atherosclerosis in hypercholesterolemic mice. *Circulation* 2008; 117: 1649–1657.
 19. Wu H, Gower RM, Wang H, et al. Functional role of CD11c+ monocytes in atherogenesis associated with hypercholesterolemia. *Circulation* 2009; 119: 2708–2717.
 20. Auffray C, Sieweke MH, Geissmann F. Blood monocytes: development, heterogeneity, and relationship with dendritic cells. *Annu Rev Immunol* 2009; 27: 669–692.
 21. Rothe G, Herr AS, Stöhr J, et al. A more mature phenotype of blood mononuclear phagocytes is induced by fluvastatin treatment in hypercholesterolemic patients with coronary heart disease. *Atherosclerosis* 1999; 144: 251–261.
 22. Schlitt A, Heine GH, Blankenberg S, et al. CD14+CD16+ monocytes in coronary artery disease and their relationship to serum TNF-alpha levels. *Thromb Haemost* 2004; 92: 419–424.
 23. Mosig S, Rennert K, Krause S, et al. Different functions of monocyte subsets in familial hypercholesterolemia: potential function of CD14+ CD16+ monocytes in detoxification of oxidized LDL. *FASEB J* 2009; 23: 866–874.
 24. Dresel HA, Via DP, Stöhr M, et al. Observations on leukocytes from patients with severe familial hypercholesterolemia. *Arteriosclerosis* 1986; 6: 259–264.
 25. Auffray C, Fogg D, Garfa M, et al. Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. *Science* 2007; 317: 666–670.
 26. Soehnlein O, Zernecke A, Weber C. Neutrophils launch monocyte extravasation by release of granule proteins. *Thromb Haemost* 2009; 102: 198–205.
 27. Hristov M, Leyendecker T, Schuhmann C, et al. Circulating monocyte subsets and cardiovascular risk factors in coronary artery disease. *Thromb Haemost* 2010; 104: 412–414.