See related article on page 2254

*The American Journal of Pathology, Vol. 174, No. 6, June 2009*

*DOI: 10.2353/ajpath.2009.081138*



# Commentary

## Hyaluronan, Platelets, and Monocytes

### *A Novel Pro-Inflammatory Triad*

Gustavo B. Menezes, Erin F. McAvoy, and Paul Kubes

*From the Immunology Research Group, Department of Physiology and Biophysics, Institute of Infection, Immunity and Inflammation, University of Calgary, Alberta, Canada*

Proper regulation of the immune response is essential for a positive outcome during inflammation. Controlled inflammation leads to effective clearance of infection, immune resolution, and tissue repair. In sharp contrast, uncontrolled inflammatory responses have been implicated in many chronic diseases including, but not limited to, arthritis, lung fibrosis, and inflammatory bowel diseases. Although there has been significant improvement in our understanding of the underlying cellular and molecular mechanisms involved in chronic inflammatory diseases, effective targets and therapies necessary for disease prevention and treatment still remain elusive. This is most likely due to the sheer number of mechanisms (some of which are well-defined, and others that remain unclear) involved in the initiation and propagation of complex inflammatory diseases. Therefore, it is essential that we broaden our understanding of the numerous underlying mechanisms occurring in the pathogenesis of chronic inflammatory diseases to develop effective therapies, or preventions. In this issue of *The American Journal of Pathology*, a novel study by de la Motte et al[1] have proposed a new role for platelets and hyaluronan (HA) in activating monocytes, cells known to contribute to numerous chronic inflammatory states. Additionally, this study has revealed potential therapeutic enzymatic targets that could reduce the inflammation associated with inflammatory bowel disease and perhaps other inflammatory conditions.

Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, affects about 1.4 million people in the United States and 2.2 million in Europe.[2] Currently, it is believed that IBD results from inappropriate and continuous activation of the mucosal immune system by the flora found within the intestinal lumen.[3] Studies using murine models of IBD have suggested that prototypical Th1 cytokines, including interferon–$\gamma$, activate macrophages to produce various mediators, which further stimulate the Th1 response and result in a self-sustaining inflammatory cycle.[3]

There is a growing body of evidence to support roles for various cytokines, such as those produced by activated macrophages, in IBD pathogenesis. TNF–$\alpha$, an important pro-inflammatory cytokine produced by cells of the innate immune system, has been shown to play several key roles in the pathogenesis of IBD. TNF–$\alpha$ stimulates intestinal endothelial cells to express numerous adhesion molecules and HA structures on their luminal surface, allowing for monocyte adhesion.[4,5] These findings led de la Motte and colleagues to test whether HA structures present on the endothelial surface could be involved in monocyte activation. Additional cytokines, such as interleukin-1$\beta$ and interferon-$\gamma$, have also been shown to contribute to the pathogenesis of inflammatory diseases, in part by potentially modulating the synthesis of HA.[6] However, the use of TNF–$\alpha$ as an initiating molecule in the *in vitro* system used by de la Motte and colleagues is certainly justified since anti-TNF–$\alpha$ is a viable therapy for IBD and other inflammatory diseases.

Platelets are the new players on the inflammatory scene. Although platelets were originally considered non-immune cells whose primary physiological role was homeostasis, it is becoming increasingly more obvious that these anuclear fragments may play crucial roles in several inflammatory processes. Firstly, platelets are often found at sites of inflammation and are involved in amplifying the inflammatory response by releasing many different pro-inflammatory molecules.[7] In addition, upon binding to the endothelium, platelets can express adhesion molecules that allow for direct binding of leukocytes, thereby bringing platelets and leukocytes into intimate contact. This is not inconsequential as platelets and leukocytes participate in transcellular synthesis of inflamma-

Accepted for publication January 15, 2009.

Address reprint requests to Paul Kubes, Ph.D.: University of Calgary, Calvin, Phoebe & Joan Snyder Institute for Infection, Immunity & Inflammation, HRIC 4AA16, University of Calgary 3280 Hospital Drive N.W. Calgary, Alberta, T2N 4N1 Canada. E-mail: pkubes@ucalgary.ca.

tory molecules such as certain lipid mediators that each cell cannot produce on its own.[8]

Inflammatory bowel disease is consistently associated with abnormalities of platelet number and function. Patients with IBD are commonly characterized by reactive thrombocytosis, an increased state of platelet activation in the peripheral circulation, as well as spontaneous aggregation and increased susceptibility to aggregating agents.[9] Increased leukocyte-platelet interactions in IBD have been described by numerous investigators.[10–12] Additionally, mucosal biopsies of patients with IBD revealed the presence of intravascular microthrombi due to an increase of pro-coagulant behavior in inflamed tissues.[13] Therefore, the newly proposed role for platelets in IBD could be of significant importance.

HA is essential for numerous physiological functions, including cell migration and tissue remodeling during the morphogenesis of organs, and in malignant tumor behavior.[14] Additionally, HA influences the hydration status of tissues and is capable of interacting with extracellular matrix macromolecules and cell surface receptors, such as CD44, receptor for hyaluronic acid-mediated motility, and lymphatic vessel endothelial HA receptor 1.[15] Fragmentation of HA by hyaluronidase (HYAL) type 2, in concert with CD44, can generate lower molecular weight fragments (10 to 20 kDa) that function biologically as pro-inflammatory mediators.[16] It has been shown that HA fragments can activate the inhibitor of nuclear factor-$\kappa B\alpha$/nuclear factor-$\kappa B$ pathway in murine macrophages, which can then up-regulate the expression of interleukin-1$\beta$ and TNF-$\alpha$[17] and, as de la Motte and colleagues demonstrate here, numerous other potential inflammatory mediators.

HA synthases and HYALs have been linked to inflammatory diseases. Moreover, HA fragment formation is observed under inflammatory conditions such as arthritis[18] and in the intestinal mucosa of patients with IBD, as well as in mouse models of IBD.[19] In this study, de la Motte et al[1] have shown that inflamed colon tissue fragments from mice fed with dextran sodium sulfate, a murine model of IBD, express HYAL1 and 2. Specifically, the authors convincingly demonstrate that HYAL2 is expressed predominantly by platelets. In addition, image co-localization revealed that HYAL2 is associated only with platelet membrane. Conversely, HYAL1 (required for further degradation of HA oligosaccharides into tetrasaccharides) was found mainly on leukocytes and not on platelets. The authors clearly demonstrate that platelets are able to bind HA and generate pro-inflammatory fragments, which can potently activate monocytes leading to production of interleukins–6 and –8. These intriguing observations provide insight into a new mechanism for promoting inflammation.

Previous studies have demonstrated that inflammatory cells, such as mononuclear cells, can bind to HA structures on colonocytes in IBD.[4] As previously mentioned, these mononuclear cells may well play an important role in the development of IBD by producing various cytokines that propagate the disease. In the present study, de la Motte et al[1] suggest that HA fragments produced by platelet HYAL2 are, at least in part, responsible for activating monocytes to produce pro-inflammatory cytokines. These HA fragments are thought to act in a paracrine fashion by activating monocytes that are in close proximity to platelets. Recent studies have reported that low molecular weight fragments of HA activate monocytes by binding not just CD44, but also toll-like receptor (TLR) 4. This is quite intriguing as the potential importance of TLRs in IBD is being avidly studied.[20]

Although lipopolysaccharide is the first molecule that comes to mind when thinking about TLR4, it is becoming increasingly more obvious that TLR4 is a promiscuous receptor and has several exogenous as well as endogenous ligands. If HA fragments can indeed activate this potent pathway, this would certainly be worth further exploration. A very recent study has shown that platelets stimulated via TLR4 will bind and deliver a very potent signal to neutrophils, which causes neutrophils to release almost all of their granular constituents. In fact, the lipopolysaccharide-stimulated platelets triggered the production of neutrophil extracellular traps (NETs), novel DNA structures covered with granular proteins that are released in the vasculature and have the capacity to injure surrounding host cells.[21]

The use of lipopolysaccharide is criticized because its concentration in human diseases never reaches levels used in most experimental studies. In this context, it is intriguing that de la Motte and colleagues demonstrate high local levels of inflammatory HA fragments, which could activate monocytes and possibly platelets via TLR4. One could imagine that stimulation of TLR4 by HA fragments during platelet-leukocyte interactions could lead to the production of these highly toxic NETs. This is underscored by the very recent report of NETs in IBD.[22] However, whether mononuclear cells such as monocytes are able to produce NETs through a platelet-TLR4 dependent mechanism remains unknown. The contribution of pro-inflammatory HA fragments in NET production would be an interesting topic for future study.

The present study raised the possibility that HYAL2 may play an important role in IBD initiation and progression. Consequently, HYAL inhibitors may be of great therapeutic value by inhibiting the formation of inflammatory HA fragments. In addition, HA has been demonstrated to be an adhesion molecule that promotes lymphocyte and neutrophil accumulation in sites of inflammation.[23,24] Whether these adhesive properties are also dependent on HYAL2 remains unclear, but inhibition of the enzyme to reduce both activation of monocytes as well as adhesion could potentially be a very potent anti-inflammatory therapy. Finally, HA has been shown to bind and be modified by serum-derived HA-associated protein, which enhances HA adhesivity.[25] It has also been suggested that serum-derived HA-associated protein has hyaluronidase inhibitory properties, raising important questions about its potential role in HA regulation and inflammation.

Since several different isoforms of HYALs are expressed, elucidating the role played by each of these different enzymes will be crucial to address the relevance of individual enzymes during inflammatory diseases. Interestingly, the gene for human HYAL1 is also a tumor

suppressor gene[26] whereas HYAL2 may be important in development,[27] suggesting that these different isoforms do play distinctive physiological roles. De la Motte et al[1] have provided the rationale to investigate how different HYAL inhibitors can impact monocyte activation and intervene in the progression of IBD.

Like any intriguing study, this work raises many more important questions. Imaging of platelet-monocyte interactions and the formation of HA inflammatory fragments might be possible with the new intravital microscopy techniques available. Moreover, the role of HA in animal models of IBD should certainly be pursued in light of the fact that certain variants of CD44 have been shown to be important in models of IBD.[28] Using inhibitors of HYALs as potential therapeutic intervention during IBD may also have the benefit of inhibiting the formation of inflammatory fragments of HA without affecting the homeostatic physiological roles of HA. This would minimize untoward effects of blocking the day-to-day function of HA and its various receptors.

## *References*

1. de la Motte CA, Nigro J, Vasanji A, Rho H, Kessler S, Bandyopadhyay S, Danese S, Fiocchi C, Stern R: Platelet-derived hyaluronidase 2 cleaves hyaluronan into fragments that trigger monocyte-mediated production of proinflammatory cytokines. Am J Pathol 2009, 174:2254–2264
2. Loftus EV Jr: Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. Gastroenterology 2004, 126:1504–1517
3. Podolsky DK: Inflammatory bowel disease. N Engl J Med 2002, 347:417–429
4. Bandyopadhyay SK, de la Motte CA, Kessler SP, Hascall VC, Hill DR, Strong SA: Hyaluronan-mediated leukocyte adhesion and dextran sulfate sodium-induced colitis are attenuated in the absence of signal transducer and activator of transcription 1. Am J Pathol 2008, 173: 1361–1368
5. Ducale AE, Ward SI, Dechert T, Yager DR: Regulation of hyaluronan synthase-2 expression in human intestinal mesenchymal cells: mechanisms of interleukin-1beta-mediated induction. Am J Physiol Gastrointest Liver Physiol 2005, 289:G462–G470
6. Campo GM, Avenoso A, Campo S, Angela D, Ferlazzo AM, Calatroni A: TNF-alpha, IFN-gamma, and IL-1beta modulate hyaluronan synthase expression in human skin fibroblasts: synergistic effect by concomital treatment with FeSO4 plus ascorbate. Mol Cell Biochem 2006, 292:169–178
7. Weyrich AS, Zimmerman GA: Platelets: signaling cells in the immune continuum. Trends Immunol 2004, 25:489–495
8. Li N: Platelet-lymphocyte cross-talk. J Leukoc Biol 2008, 83: 1069–1078
9. Shen J, Ran ZH, Zhang Y, Cai Q, Yin HM, Zhou XT, Xiao SD: Biomarkers of altered coagulation and fibrinolysis as measures of disease activity in active inflammatory bowel disease: a gender-stratified, cohort analysis. Thromb Res 2008, 123:604–611
10. Irving PM, Macey MG, Feakins RM, Knowles CH, Frye JN, Liyanage SH, Dorudi S, Williams NS, Rampton DS: Platelet-leucocyte aggregates form in the mesenteric vasculature in patients with ulcerative colitis. Eur J Gastroenterol Hepatol 2008, 20:283–289
11. Irving P, Rampton D: Platelet-leucocyte aggregation in IBD. Am J Hematol 2007, 82:686
12. Danese S, Katz JA, Saibeni S, Papa A, Gasbarrini A, Vecchi M, Fiocchi C: Activated platelets are the source of elevated levels of soluble CD40 ligand in the circulation of inflammatory bowel disease patients. Gut 2003, 52:1435–1441
13. Danese S, Motte Cd Cde L, Fiocchi C: Platelets in inflammatory bowel disease: clinical, pathogenic, and therapeutic implications. Am J Gastroenterol 2004, 99:938–945
14. Heldin P, Karousou E, Bernert B, Porsch H, Nishitsuka K, Skandalis SS: Importance of hyaluronan-CD44 interactions in inflammation and tumorigenesis. Connect Tissue Res 2008, 49:215–218
15. Turley EA, Noble PW, Bourguignon LY: Signaling properties of hyaluronan receptors. J Biol Chem 2002, 277:4589–4592
16. Harada H, Takahashi M: CD44-dependent intracellular and extracellular catabolism of hyaluronic acid by hyaluronidase-1 and -2. J Biol Chem 2007, 282:5597–5607
17. Noble PW, McKee CM, Cowman M, Shin HS: Hyaluronan fragments activate an NF-kappa B/I-kappa B alpha autoregulatory loop in murine macrophages. J Exp Med 1996, 183:2373–2378
18. Poole AR, Dieppe P: Biological markers in rheumatoid arthritis. Semin Arthritis Rheum 1994, 23:17–31
19. de la Motte CA, Hascall VC, Drazba J, Bandyopadhyay SK, Strong SA: Mononuclear leukocytes bind to specific hyaluronan structures on colon mucosal smooth muscle cells treated with polyinosinic acid: polycytidylic acid: inter-alpha-trypsin inhibitor is crucial to structure and function. Am J Pathol 2003, 163:121–133
20. Sartor RB: Microbial influences in inflammatory bowel diseases. Gastroenterology 2008, 134:577–594
21. Clark SR, Ma AC, Tavener SA, McDonald B, Goodarzi Z, Kelly MM, Patel KD, Chakrabarti S, McAvoy E, Sinclair GD, Keys EM, Allen-Vercoe E, Devinney R, Doig CJ, Green FH, Kubes P: Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. Nat Med 2007, 13:463–469
22. Yousefi S, Gold JA, Andina N, Lee JJ, Kelly AM, Kozlowski E, Schmid I, Straumann A, Reichenbach J, Gleich GJ, Simon HU: Catapult-like release of mitochondrial DNA by eosinophils contributes to antibacterial defense. Nat Med 2008, 14:949–953
23. Siegelman MH, DeGrendele HC, Estess P: Activation and interaction of CD44 and hyaluronan in immunological systems. J Leukoc Biol 1999, 66:315–321
24. McDonald B, McAvoy EF, Lam F, Gill V, de la Motte C, Savani RC, Kubes P: Interaction of CD44 and hyaluronan is the dominant mechanism for neutrophil sequestration in inflamed liver sinusoids. J Exp Med 2008, 205:915–927
25. Zhuo L, Kanamori A, Kannagi R, Itano N, Wu J, Hamaguchi M, Ishiguro N, Kimata K: SHAP potentiates the CD44-mediated leukocyte adhesion to the hyaluronan substratum. J Biol Chem 2006, 281:20303–20314
26. Lokeshwar VB, Cerwinka WH, Lokeshwar BL: HYAL1 hyaluronidase: a molecular determinant of bladder tumor growth and invasion. Cancer Res 2005, 65:2243–2250
27. Camenisch TD, Spicer AP, Brehm-Gibson T, Biesterfeldt J, Augustine ML, Calabro A Jr, Kubalak S, Klewer SE, McDonald JA: Disruption of hyaluronan synthase-2 abrogates normal cardiac morphogenesis and hyaluronan-mediated transformation of epithelium to mesenchyme. J Clin Invest 2000, 106:349–360
28. Hoffmann U, Heilmann K, Hayford C, Stallmach A, Wahnschaffe U, Zeitz M, Gunthert U, Wittig BM: CD44v7 ligation downregulates the inflammatory immune response in Crohn's disease patients by apoptosis induction in mononuclear cells from the lamina propria. Cell Death Differ 2007, 14:1542–1551