

## Viral pathogens as novel activators of procoagulant signalling

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Toll-like receptors (TLR) constitute a protein family of 13 different mammalian subtypes of heterodimeric immune receptors which are activated by a diverse range of molecules specific to certain microbial hazards. Among them, TLR subtype receptor 3 (TLR3) is unique because it is activated by double-stranded RNA (dsRNA) of viral pathogens. Polyinosinic:polycytidylic acid (poly I:C) serves as a well-known activator of TLR3 in cell culture studies to mimic the agonistic effect of dsRNAs (1). In general, TLRs are expressed on immune cells of monoblastic or myeloblastic origin, such as dendritic cells, lymphocytes or monocytes and play a pivotal role in innate immunity. Moreover, certain TLRs have been described on certain epithelial cell types and in particular TLR3 was found to be present on epithelial and neuronal cells, in the kidney and also on mesothelial cells (2–5). In contrast to most TLRs, signalling of TLR3 is independent of the common TLR adaptor protein MyD88 but involves the adaptor protein Trif (TIR-domain-containing adapter-inducing interferon- $\beta$ , TICAM-1), which ultimately induces transcriptional activation of interferon- $\alpha$  and - $\beta$ , both of which play a substantial role in the inflammatory immune response following viral infections.

In this issue of *Thrombosis and Haemostasis*, Wörnle et al. describe a novel link between viral infection of serosal cavities and regulation of fibrin generation and fibrinolysis (6). In a cell culture model of mesothelial cells isolated from explanted human omental tissue, they show that stimulation with poly I:C leads to both a concentration-dependent increase of plasminogen activator inhibitor 1 (PAI-1) synthesis and a concentration-dependent decrease of tissue plasminogen activator (t-PA) synthesis. Aside from TLR3, mesothelial cells also express the helicas retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5), which may also act as sensors of viral infections through recognition of viral dsRNA and may up-regulate type I interferons. The specificity of the involvement of TLR3, RIG-I and MDA5 in these observations is confirmed by the use of RNA interference technology. These results indicate that the effect of poly I:C on increased PAI-1 but decreased t-PA synthesis is mainly mediated by TLR3. The authors interpret their findings to be of potential importance for the pathogenesis of viral diseases in serous tissue such as the me-

sothelium, pleura or pericardium because fibrin deposition at these sites could be promoted through the described mechanism. This could ultimately determine the course of the infectious disease e.g. by leading to pleural or pericardial fibrosis. In the mesothelium, similar mechanisms may be responsible for the failure of the peritoneal space to contribute to permeability changes. Hence, in this specific context, activation of mesothelial viral receptors followed by disturbance of the fibrinolytic system could explain why patients undergoing chronic abdominal peritoneal dialysis only profit from this type of treatment for a limited time span.

From the data obtained by Wörnle et al., such conclusions seem straightforward and comprehensible, yet another implication of the findings presented in this work could be related to the change in gene expression of adjacent vascular tissue. Since under quiescent conditions *in vivo* the ratio in gene expression between t-PA and PAI-1 in vascular endothelium is very high, poly I:C could dramatically shift this value resulting in the up-regulation of PAI-1. This would lead to a more prothrombotic cellular phenotype as recently documented by the poly I:C induced up-regulation of tissue factor and down-regulation of thrombomodulin in the vessel wall (7).

Indeed, increased levels of t-PA and PAI-1 have long been known to be present in the early stages of atherosclerosis and to be independent risk predictors for myocardial infarction (8, 9). However, according to present data, they seem to be markers of increased endothelial activation rather than playing an active role in promoting atherosclerosis (10). Intriguingly, besides being expressed in the above mentioned tissues, TLR3 also appears to be expressed on cells of the vascular endothelium, where it most likely acts as a receptor for viral dsRNAs. Thus, activation of endothelial innate immune receptors such as TLR3 could actually represent an active promoting mechanism in atherosclerosis and its thrombotic complications because it might not only result in dysregulation of the t-PA-PAI-1 system, but could also activate other signalling pathways within endothelial cells that directly promote endothelial dysfunction. Indeed, in a very recent report by Fischer et al., the authors describe endothelial activation through extracellular RNA (but not DNA) leading to binding of vascular endothelial growth factor (VEGF) to neuropilin-1,

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which then results in phosphorylation of the VEGF receptor sub-type 2, the activation of phospholipase C, in an intracellular calcium release, followed by von Willebrand factor secretion (11). Although blockade of TLR3 could not depress similar effects exerted by poly I:C in this study, such experiments underscore the need for clarification of the role of extracellular RNA species in vascular cell activation and thrombosis.

The present work by Wörnle et al. thus gives exciting stimuli for further investigation on the interaction between free nucleic acids and the signalling in cellular systems related to innate immunity. The respective sites are the mesothelium, the pleura, or the pericardium but also the vascular endothelium, where free nucleic acids may play a thus far underestimated role in the atherothrombotic disease.

## References

1. Alexopoulou L, Holt AC, Medzhitov R, et al. Recognition of double-stranded RNA and activation of NF- $\kappa$ B by Toll-like receptor 3. *Nature* 2001; 413: 732–738.
2. Zhou L, Wang X, Wang YJ, et al. Activation of Toll-like receptor-3 induces interferon-lambda expression in human neuronal cells. *Neuroscience* 2009; 159: 629–637.
3. Jang YJ, Wang JH, Kim JS, et al. Levocetirizine inhibits rhinovirus-induced ICAM-1 and cytokine expression and viral replication in airway epithelial cells. *Antiviral Res* 2009; 81: 226–233.
4. Ciferska H, Horak P, Kontinen YT et al. Expression of nucleic acid binding Toll-like receptors in control, lupus and transplanted kidneys—a preliminary pilot study. *Lupus* 2008; 17: 580–585.
5. Wörnle M, Sauter M, Kastenmuller K, et al. Novel role of toll-like receptor 3, RIG-I and MDA5 in poly (I:C) RNA-induced mesothelial inflammation. *Mol Cell Biochem*. 2009; 322: 193–206.
6. Wörnle M, Sauter M, Kastenmüller K, et al. Role of TLR3, RIG-I and MDA5 in Mesothelial t-PA und PAI-1 Synthesis. *Thromb Haemost* 2009; 101: 1128–1137.
7. Shibamiya A, Hersemeyer K, Schmidt Wöll T, et al. A key role for Toll-like receptor-3 in disrupting the hemostasis balance on endothelial cells. *Blood* 2009; 113: 714–722.
8. Salomaa V, Stinson V, Kark JD, et al. Association of fibrinolytic parameters with early atherosclerosis. The ARIC Study. *Atherosclerosis Risk in Communities Study*. *Circulation* 1995; 91: 284–290.
9. Thogersen AM, Jansson JH, Boman K, et al. High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. *Circulation* 1998; 98: 2241–2247.
10. Pradhan AD, LaCroix AZ, Langer RD, et al. Tissue plasminogen activator antigen and D-dimer as markers for atherothrombotic risk among healthy postmenopausal women. *Circulation* 2004; 110: 292–300.
11. Fischer S, Nishio M, Peters SC, et al. Signaling mechanism of extracellular RNA in endothelial cells. *FASEB J* 2009; in press.