

## The protein C anticoagulant pathway: Nexus between coagulation and inflammation

*There is growing evidence that the coagulation system co-evolved with the innate immune system. There is a remarkable degree of integration in their signaling pathways and regulatory circuits following tissue injury and microbial invasion: inflammatory mediators generate procoagulant signals and intravascular thrombosis activates multiple components of the innate immune system. The nexus between coagulation and inflammation is most obviously demonstrated by the successful use of recombinant activated protein C (APC) for the treatment of sepsis.*

Blood coagulation is an important mechanism against bleeding. The formation of a platelet plug provides the initial occlusion of a vascular lesion. Blood coagulation is controlled by several coagulant and anticoagulant mechanisms essential to maintain the fluidity of the blood. The protein C anticoagulant pathway is an important anticoagulant mechanism, that also controls inflammatory responses and potentially decreases endothelial cell apoptosis in response to inflammatory cytokines and ischemia.

The essential components of this pathway are thrombin, thrombomodulin (TM), the endothelial cell protein C receptor (EPCR), protein C (PC) and proteins S (PS). Protein C is the key component of this pathway. It circulates as a proenzyme that is activated by thrombin bound to the endothelial membrane protein TM. When bound to TM, thrombin has reduced procoagulant activity.

Activated protein C (APC) cleaves and inhibits several coagulation cofactors, hereby down-regulating the activity of the coagulation system. APC function is facilitated by its cofactor PS. APC also affects the fibrinolytic pathway by

neutralizing the plasminogen activator inhibitor 1 (PAI-1).

Components of the protein C pathway have a wide range of biological effects other than those strictly referred to as being anticoagulant. For example, the lectin domain of TM has anti-inflammatory properties, down-regulating NF kappa-beta and the MAP kinase pathways and decreases leukocyte adhesion and extravasation. Both protein C and APC directly inhibit the adhesion of neutrophils to the endothelial cell surface and the trans-migration of neutrophils.

Furthermore, APC plays an important role in the inhibition of inflammation in the gastric mucosa in patients with Helicobacter pylori infection. APC protects the vasculature by blocking p53-mediated apoptosis in ischemic cerebral vasculature. TM regulates the anti-inflammatory capacities of APC. On its turn, TM has additional physiological functions such as regulation of fibrinolysis, cell adhesion, embryonic development, and tumor growth. Soluble TM released from endothelial cell surfaces can be detected in plasma and urine and high soluble TM levels indicate injury and/or enhanced turnover of the endothelium. TM is expressed on both the endothelium and tumor cells in several cancers and loss of TM expression correlates with a more malignant profile with poorer prognosis.

Inflammation has an important impact on the protein C pathway since both TM and EPCR gene transcription can be down regulated by inflammatory cytokines.

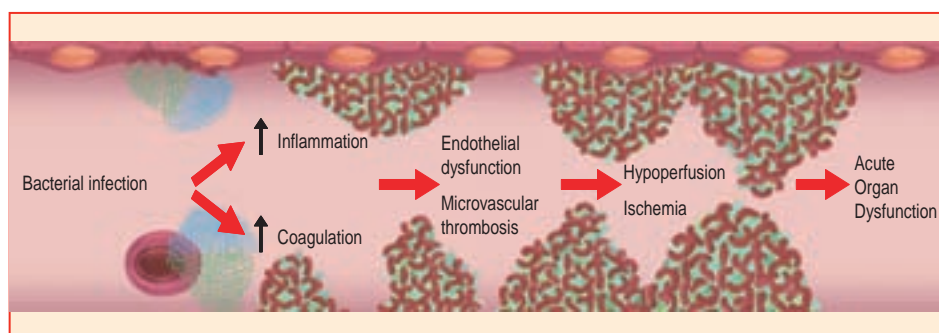
Protein S function is also down regulated by inflammation. The cross talk between blood coagulation and inflammation is well studied in severe sepsis where blood coagulation is activated and protein C is consumed. The drop in the plasma level of

### In this Issue:

The protein C anticoagulant pathway: the nexus between coagulation and inflammation 1/2

Oxidized phospholipids: Important mediators of chronic vascular inflammation 2

JAM-A: functional contribution to atherosclerosis 3



## TECHNICAL NOTE

### Oxidized phospholipids: Important mediators of chronic vascular inflammation

Oxidative stress and lipid peroxidation are characteristic features of atherogenesis.

Enzymatic or non-enzymatic oxidation of polyunsaturated fatty acids within phospholipid molecules generates oxidized phospholipids (OxPL) known to accumulate in vessel wall in vivo. OxPL demonstrate a variety of biological activities relevant to atherosclerosis such as stimulation of endothelial cells to bind monocytes but not neutrophils, which mimics mononuclear cell specificity of atherosclerosis. OxPL stimulate induction of genes related to atherothrombosis, such as MCP-1, KC/IL-8, MIP-1alpha, MIP-1beta, RANTES and tissue factor both in vitro and in vivo. In addition, specific OxPL are ligands for scavenger receptor CD36 or mimic inflammatory and pro-aggregant effects of platelet-activating factor. OxPL seem to activate cells via receptor-mediated and non-receptor mechanisms leading to elevation of cAMP and cytosolic Ca<sup>2+</sup> levels, activation of protein kinase C, ERK1/2 kinases, PI-3-kinase, c-Src, R-Ras, Rac and Cdc40-dependent pathways. Induction of specific protein synthesis by OxPL is mediated by several transcription factors, including EGR-1, NFAT, CREB, STAT3 and SREBP. These recent findings suggest that OxPL are not merely by-products but also important mediators of chronic vascular inflammation.

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#### OXIDIZED PAPC

Cat. #	Specificity	
HC4035	Oxidized PAPC (OxPAPC), 1 mg	<b>Unique</b>
HC4036	Oxidized PAPC (OxPAPC), 5 mg	<b>Unique</b>

Please inquire for other oxidized phospholipids and controls (PAPC and DMPC).

## The protein C anticoagulant pathway: the nexus between coagulation and inflammation

Continued from page 1

protein C is considered to contribute to the development of micro-vascular thrombosis. In addition, the expression levels of TM and EPCR on endothelium decrease during sepsis. APC can counteract the deleterious effects associated with sepsis. A study using recombinant APC treatment of severe sepsis led to a 19% reduction in the relative risk of death and an absolute reduction of 6%.

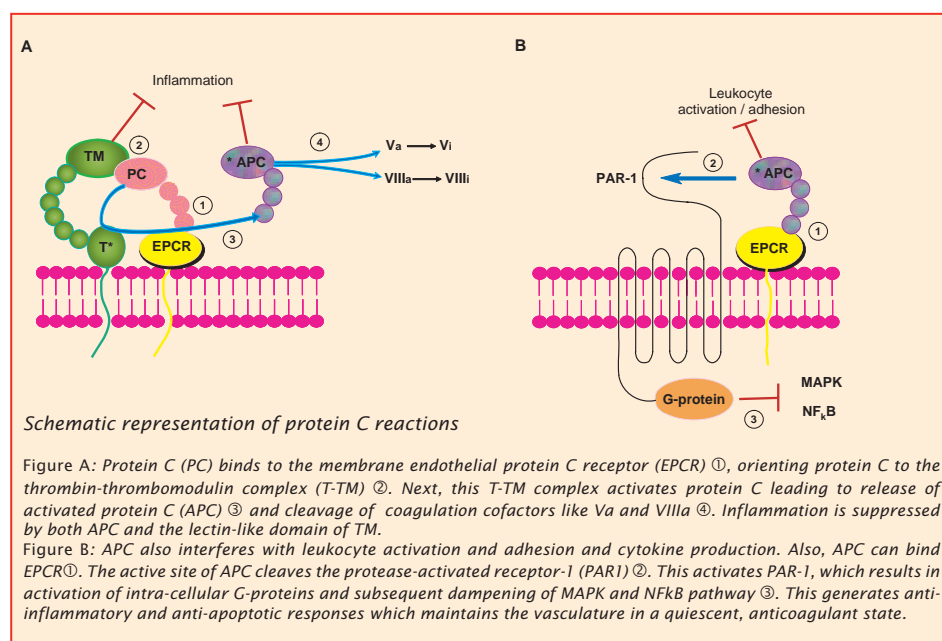
The innate immune system and the blood coagulation system originate from a common ancestor which explains the cross-talk between these two systems. Ficolins, a group of proteins that are involved in the complement mediated host defense through nonself-recognition

by vertebrates, are most probably intermediates in the evolution from invertebrate innate immunity to the vertebrate blood coagulation system.

Further understanding and unraveling of the link between coagulation and innate immunity may help to gain more insight in the study and treatment of severe infections.

TGM Lauterslager, Phd, Hycult biotechnology, Uden, The Netherlands.

For more information and references, please visit our website.



#### COAGULATION

Cat. #	Specificity		
HM2151	Activated Protein C (APC), Human	<b>Unique</b>	PC107
HM2145	EPCR, Human	<b>Unique</b>	PCR-252
HM2144	EPCR, Human	<b>Unique</b>	PCR-379
HM2180	PAI-1, AF epitope, Human (cross reactive Mouse, Rat)		MA-55F4C12
HM2179	PAI-1, hF epitope, Human (cross reactive Mouse, Pig, Rat)		MA-33H1F7
HM3026	PAI-1, E212/E220 epitope, Rat (cross reactive Mouse)		MA-124K1
HM2181	PAI-1, ts3BhG/RCL epitopes, Human (cross reactive Pig)		MA-56A7C10
HM2149	Protein C (PC), Human		PC50
HM2150	Protein C (PC), Human		PC98
HM2148	Protein S (PS), Human		PS7
HM2146	Thrombomodulin (TM), CD141, Human		RTM96
HM2147	Thrombomodulin (TM), CD141, Human		RTM98

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## JAM-A: functional contribution to atherosclerosis

The junctional adhesion molecule (JAM)-A is not only involved in maintenance of endothelial cell layer integrity via tight junctions, but is also involved in the mononuclear cell recruitment. The latter suggests a functional contribution of JAM-A to atherogenesis.

JAMs are proteins of about 30-40 kDa and members of the immunoglobulin superfamily. JAMs are important for a variety of cellular processes, including tight junction assembly, leukocyte transmigration, platelet activation, angiogenesis and virus binding. JAM-A (also known as JAM, JAM-1 or F11 R) is expressed by endothelial and epithelial cells, platelets, neutrophils, monocytes, lymphocytes and erythrocytes. The extracellular domains of JAM-A molecules are involved in the homophilic interaction linking adjacent endothelial or epithelial cells and thereby stabilizing intracellular junctions, especially around tight junction strands. JAM-A was first discovered in platelets as the receptor of the platelet aggregation stimulatory monoclonal antibody F11. Binding of F11 to human platelets caused granule secretion, fibrinogen binding and platelet aggregation. Interestingly, auto-antibodies against JAM-A have been detected in patients with thrombocytopenia.

JAM-A also plays an important role in leukocyte transmigration. Leukocyte transmigration can be blocked by antibodies and by soluble JAM-A/Fc fusion proteins. However, the precise mechanisms of JAM-A action during leukocyte transmigration are not yet fully understood. Homophilic JAM-A interactions between leukocytes and the endothelium but also heterophilic interactions of JAM-A with the  $\beta$ 2-integrin leukocyte function-associated antigen-1 (LFA-1) are considered to actively guide leukocytes during transmigration. Tumor necrosis factor is suggested to play an additional role by inducing disassembly of JAM-A from the junctions. This leads to junction loosening and redistribution of JAM-A

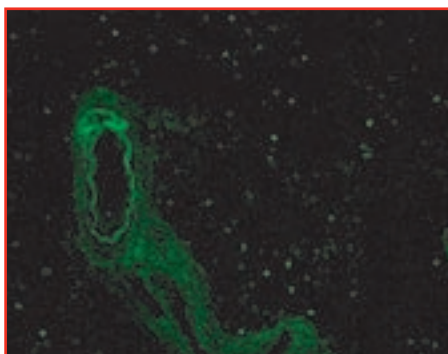
to the apical surface of endothelial cells thereby becoming available for adhesive interactions with leukocyte LFA-1.

Several studies imply a role of JAM-A in the initiation of atherosclerosis, since JAM-A is upregulated on early atherosclerotic endothelium and adhesion of activated platelets on activated endothelium is mediated by homophilic interactions of JAM-A. At atherosclerosis-prone sites, the intracellular adhesion molecule-1 (ICAM-1) is upregulated and inflammatory T lymphocytes are attracted. Soluble forms of JAM-A antagonize LFA-1/ICAM-1 interaction of LFA-1 expressing

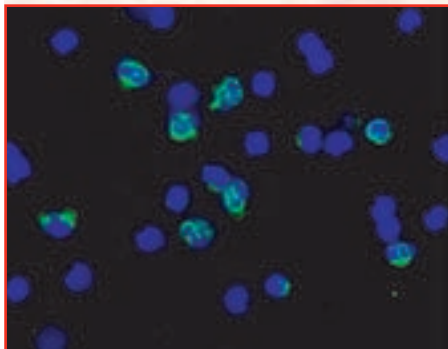
leukocytes to endothelial ICAM-1. Interestingly, JAM-A is also involved in neointimal lesion formation and monocyte infiltration after arterial injury in atherosclerosis-prone mice. In this context, hyperlipidemia upregulates JAM-A on atherosclerotic endothelium. Therefore, it would be very interesting to further investigate the role of JAM-A in blood vessel condition and to further elucidate the role of JAM-A in inflammatory thrombosis and atherogenesis.

For more information and references, please visit our website.

JAM & RELATED		
Cat. #	Specificity	mAb/pAb
HP9041	JAM-A (JAM-1) Domain 1, Human	<b>Unique</b> Rabbit
HP9042	JAM-A (JAM-1) Domain 2, Human	<b>Unique</b> Rabbit
HM2098	JAM-A (JAM-1), Human	BV16
HM2099	JAM-A (JAM-1), Human	M.Ab.F11
HM1050	JAM-A (JAM-1), Mouse	BV12
HM1057	JAM-C (JAM-2), Mouse (cross reactive Human)	CRAM-18 F26
HM1056	JAM-C (JAM-2), Mouse (cross reactive Human)	CRAM-19 H36
HM2102	Barmotin/7H6 antigen, Human	<b>Unique</b> 7H6
HM3013	L-afadin, Rat (cross reactive Dog, Human, Mouse)	<b>Unique</b> 3
HM1052	Nectin-2, Mouse (cross reactive Human)	<b>Unique</b> 502-57
HM1053	Nectin-3, Mouse	103-A1
ADHESION MOLECULES		
Cat.#	Specificity	Assays
HK305	sE-Selectin, Human	ELISA
HK304	sICAM-1, Human	ELISA
Cat.#	Specificity	mAb/pAb
HM2118	$\alpha$ -Catenin, Human	1G5
HM2034	$\alpha$ V $\beta$ 3-Integrin, Human	BV3
HM2035	$\beta$ 3-Integrin subunit, Human	BV4
HM2112	$\beta$ -Catenin, Human	9F2
HM2033	$\beta$ -Integrin, Human	BV7
HM2183	CD11/CD18, activated, Human	24
HM2113	Desmoglein 1, Human	27B2
HM2114	Desmoglein 2, Human	6D8
HM2115	Desmoglein 3, Human	5G11
HM4003	E-Selectin, CD62E, Fab2, Human	ENA2
HM4001	E-Selectin, CD62E, Human	ENA1
HP9017	E-Selectin, CD62E, Human, Biotin	Rabbit
HM4004	ICAM-1, CD54, Human	HM1
HP9018	ICAM-1, CD54, Human, Biotin	Rabbit
HM2207	MAdCAM-1, Human	314G8
HM2039	PECAM-1, CD31, Human	BV8
HM1013	PECAM-1, CD31, Mouse	MEC7.46
HM1084	PECAM-1, CD31, Mouse	ER-MP12
HM2116	Plakoglobin, Human	15F11
HM4006	VCAM-1, CD106, Human	1G11B1



Human kidney artery stained for JAM-A. Immunohistochemical staining of paraffin section with polyclonal antibody to JAM-A (Cat.# HP9041).



MPO detection in rat neutrophils and monocytes in rat white blood cells. Immunofluorescence with antibody 2D4 (Cat. # HM3030).

#### SCAVENGER RECEPTORS

Cat.#	Specificity	mAb/pAb
HM2122	CD36, Human	FA-152
HM1074	CD36, Mouse	CRF D-2712
HM3019	CD36, Rat	UA009
HM2177	CD38, Macrosialin, Human	KP1
HM1070	CD38, Macrosialin, Mouse	FA-11
HM3029	CD38, Macrosialin, Rat	ED1
HM2157	CD163, Human	RM3/1
HM3025	CD163, Rat	ED2
HM1061	CD204, SR-A, Mouse	2F8
HM1067	Dectin-1, Mouse	2A11
HM2138	LOX-1, Human	23C11
HM2056	Mannose Receptor, Human	15-2
HM1049	Mannose Receptor, Mouse	MR5D3
HM2208	MARCO, Human (cross reactive Bovine)	PLK1
HM1068	MARCO, Mouse	ED31
HM3027	SR-BI, Rat	3D12

#### ENDOTHELIUM

Cat.#	Specificity	Assays
HK305	MCP-1/CCL2, Human	ELISA
Cat.#	Specificity	mAb/pAb
HM1015	CD34, Mouse	MEC 14.7
HM2140	CD105, Endoglin, Human	E9
HM2185	EMAP II, Human (cross reactive Rat)	546-2
HM2188	Endostatin, Human	1837-46
HM3012	Endothelial Cell Antigen (RECA), Rat	RECA-1

#### ADHESION MOLECULES (CONTINUED)

Cat.#	Specificity	mAb/pAb
HM2032	VE-Cadherin, Human	BV9
HM2036	Vitronectin, Human	BV1
HM2126	VLA-5, Human	NKI-SAM1

#### NEUTROPHIL PROTEINS/MPO

Cat.#	Specificity	Assays
HK325	Calprotectin, MRP-8/MRP-14, Human	ELISA
HK319	Elastase, Human	ELISA
HK324	MPO, Human	ELISA
HK210	MPO, Mouse (cross reactive Rat)	<b>Unique</b> ELISA

Cat.#	Specificity	mAb/pAb
HM2154	Apoptotic Neutrophils, Human	<b>Unique</b> BOB93
HM2156	Calprotectin, MRP-8/MRP-14, Human	27E10
HM2186	Galectin-3, Human (cross reactive Mouse)	B2C10
HM2164	MPO, Human	266-6K1
HM1051	MPO, Mouse	<b>Unique</b> 8F4
HM3030	MPO, Rat	<b>Unique</b> 2D4
HM1039	Neutrophils, Ly-6G	NIMP-R14
HM2193	nGAL, Human	697
HM2172	Proteinase 3 (PR3), Human	PR3G-2
HM2171	Proteinase 3 (PR3), Human	WGM2

#### OTHER

Cat.#	Specificity	Assays
HIT304	Annexin V-FITC	reagent
HIT303	Lectin NPN early apoptosis detection	<b>Unique</b> kit
HK501	Nitrotyrosine	ELISA

Cat.#	Specificity	mAb/pAb
HP5003	Acetaminophen Protein Adducts	<b>Unique</b> Rabbit
HM2194	ADAMTSL-1, Punctin-1, Human	<b>Unique</b> 1B2-8-14
HM2169	ADAMTSL-1, Punctin-1, Human	<b>Unique</b> 1B2-8-2
HM2210	CD96, Tactile, Human	<b>Unique</b> NK92.39
HM1069	CD205, DEC-205, Mouse	NLDC-145
HM2209	CD209, DC-SIGN, Human	DCN47.5
HP5002	3-Chlorotyrosine, pAb	<b>Unique</b> Rabbit
HM1066	F4/80 - Macrophages, Mouse	BM8
HP5004	IDO, Indoleamine 2,3-dioxygenase, Human	Sheep
HM1082	Ly-6c, Mouse	ER-MP20
HM3007	Macrophage specific, Rat	F-6-J
HM2158	Mature Macrophages, Human	25F9
HM1088	Monocytes/Macrophages, Mouse	ER-HR3
HM5001	Nitrotyrosine	HM.11
HM1080	SIGN-R1, Mouse	ER-TR9
HM2119	Smoothelin, Human	C6G



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