Hycult Scope

Pentraxins, Innate immunity and Inflammation

Innate immunity is a first line of host defense against pathogens, it plays a key role in the activation of adaptive immunity and in the maintenance of tissue repair. Recognition of pathogens and damaged tissues is mediated by pattern recognition receptors (PRRs) that are part of both the cellular and humoral arms of innate defense mechanisms. Toll-like receptors (TLRs), scavenger receptors, and lectin receptors are cellular PRRs, whereas the humoral arm of innate immunity includes collectins (mannose-binding lectin, surfactant proteins A and D, C1q), ficolins, and pentraxins.

Pentraxins are a superfamily of proteins which are characterized by a structural motif, the pentraxin domain and are divided into short and long pentraxins. The acute phase proteins, C-reactive protein (CRP) and serum amyloid P component (SAP) are prototypic short pentraxins: they are mainly produced in the liver in response to inflammatory signals, most prominently IL-6. Pentraxin 3 (PTX3) is the prototype of the long pentraxin family; produced in a variety of cell types and similarly to CRP and SAP it binds to complement component C1q. CRP and SAP show optimal interaction with C1q only after chemical cross-linking, whereas PTX3 binds to surface immobilized C1q and does not require aggregation.

Interaction of PTX3 with surface immobilized C1q results in the activation of the classical complement cascade, measured as deposition of C3 and C4 products. However fluid-phase binding of PTX3 to C1q actually inhibits complement activation by blocking relevant interaction sites indicating that PTX3 may exert a dual role in complement activation, depending on how C1q is presented.

CRP can modulate the alternative pathway of complement activation through interaction with complement factor H (CFH), the main soluble regulator of the alternative pathway. In accordance, PTX3 can also interact with complement factor H, suggesting a more general and complex role of PTX3 in the control of complement functions. ***Read more on page 2.***

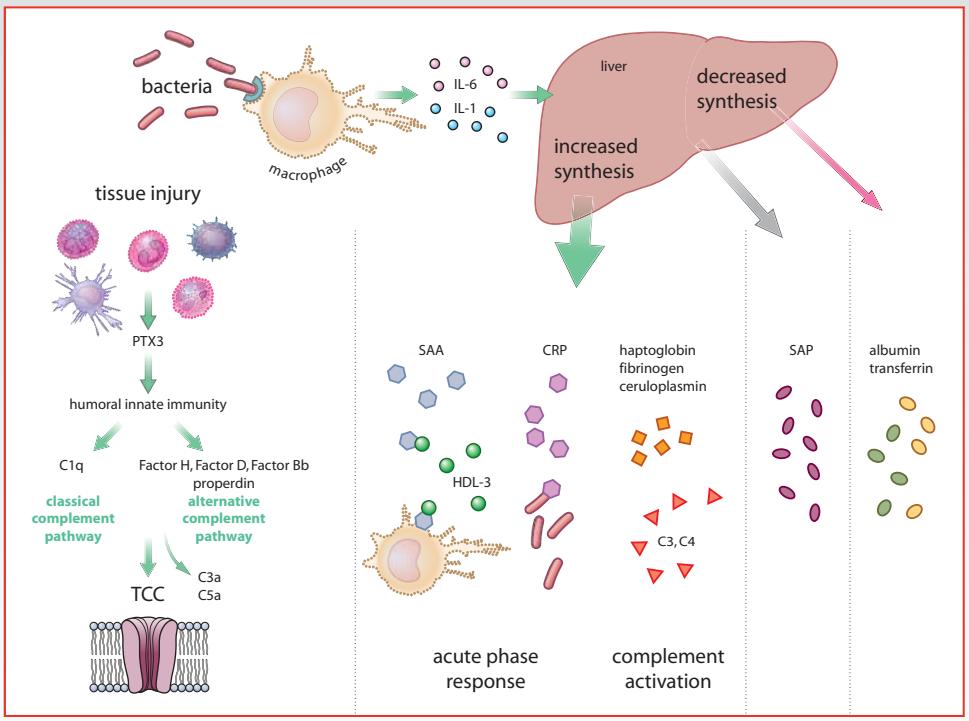

Figure 1 The role of innate immunity mechanisms: complement and acute phase proteins in inflammation. Figure designed by Rogier Trompert Medical Art.

Complement and Acute phase response

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Continued from page 1. Under conditions of tissue damage (e.g. myocardial infarction) or infection (e.g. sepsis), PTX3 levels increase more rapidly than CRP. For instance, in acute myocardial infarction with ST elevation (a finding on an electrocardiogram), PTX3 reaches a peak in 6–8 h, compared to 36– 48 h for CRP. SAP is constitutively present in serum.
Increased levels of PTX3 have been observed in diverse infectious disorders, including sepsis, *A. fumigatus* infections, tuberculosis, and dengue. In some of these disorders, PTX3 levels correlated with disease activity or severity.
SAP is a universal constituent of the amyloid deposits that are characteristic of systemic amyloidosis, Alzheimer's disease, and prion diseases. SAP binds to amyloid fibrils and stabilizes the deposits, participating in the pathogenesis of the disease.
The effects of CRP are not limited to protection against pathogens. It also offers protection against allergic encephalomyelitis, and systemic lupus erythematosus (SLE).

References
1. Manfredi, AA et al; Curr Opinion Immunol 2008, *20*:1
2. Garlanda, C et al; Annu Rev Immunol 2005, *23*:337
3. Mantovani, A et al; J Clin Immunol 2003, *33*:465

ASSAYS COMPLEMENT PROTEINS AND ACUTE PHASE PROTEINS

PRODUCT	QUANTITY	CAT.#
Pentraxin 3, Human, ELISA	2 x 96 det.	HK347
SAA, Human, ELISA	2 x 96 det.	HK333
SAP, Human, ELISA	2 x 96 det.	HK331
C1q, Human, ELISA	2 x 96 det.	HK356
Factor H, Human, ELISA	2 x 96 det.	HK342
Factor D, Human, ELISA	2 x 96 det.	HK343
C3a, Human, ELISA	2 x 96 det.	HK354
C5a, Human, ELISA	2 x 96 det.	HK349
TCC, Human, ELISA	2 x 96 det.	HK328
Factor H variant, Human, ELISA	2 x 96 det.	HK353
Hycult Dx MBL, ELISA CE	1 x 96 det.	DH001

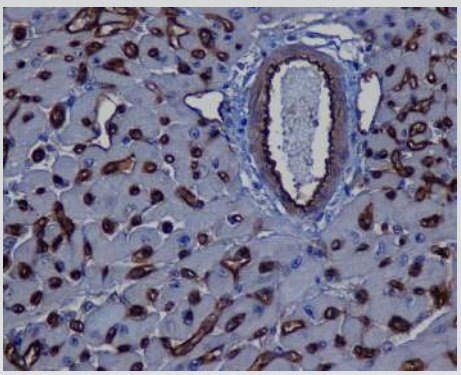

Figure 2a C4d in rat heart transplant. Staining of paraffin section with antibody to rat C4d (Cat.# HP8034).
From Dr. J. Werner and Dr. W. Baldwin, unpublished data

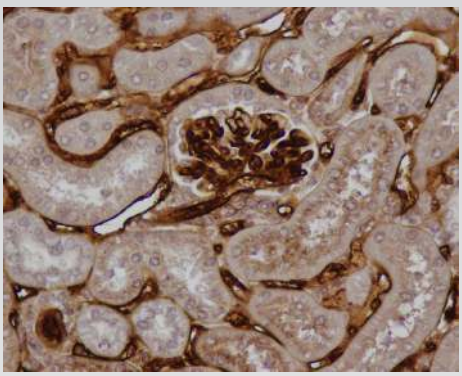

Figure 2b C4d in mouse kidney transplant. Staining of paraffin section with antibody to mouse C4d (Cat.# HP8033).
From Dr. J. Werner and Dr. W. Baldwin, unpublished data

MOUSE / RAT PRODUCTS

PRODUCT	APPLICATIONS	CAT.#
C1q, Mouse, mAb 7H8	F FC IA IP W	HM1044
C1q, Mouse, mAb JL-1	F, FC, FS, IA, W	HM1096
C3, Mouse, mAb 11H9	F FC IA IP W	HM1045
C3/C3b, Rat, mAb 2B10B9B2	IA W	HM3031
C3a, Mouse, mAb 3/11	IA W	HM1072
C3aR, Rat, mAb 74	F FC	HM3028
C3b/iC3b/C3c, Mouse, mAb 2/11	FS F FC IA W	HM1065
C3b/iC3b/C3c, Mouse , mAb 3/26	FS F FC IA W	HM1078
C4, Mouse, mAb 16D2	F FC IA IP W	HM1046
C5, Mouse, mAb BB5.1	F FS IA IP	HM1073
C5aR, Mouse, mAb 20/70	FS FC	HM1076
C5aR, Mouse, mAb 10/92	F FC	HM1077
C5aR, Rat, mAb R63	F FC	HM3017
C5b-9, Rat, mAb 2A1	F FC IA W	HM3033
C6, Rat, mAb 3G11	F IA W	HM3034
CD14, Mouse, mAb Sa14-2	FS FC IP W	HM1060

Binding of acute phase proteins PTX3 and CRP to complement factor H

Complement factor H (CFH) is a regulator of the alternative pathway, binding to C3b thereby inhibiting the binding of factor B to C3b, acting as a cofactor for the factor I-mediated cleavage of C3b to iC3b (cofactor activity) and accelerating the decay of C3bBb, the alternative pathway C3 convertase (decay-accelerating activity). Lack of CFH activity results in activation of the alternative pathway which may lead to potentially hazardous conditions such as membranoproliferative glomerulonephritis type II, age-related macular degeneration (AMD), or atypical hemolytic uremic syndrome.
PTX3 shares with CRP and SAP the capacity to bind to C1q and activate the classical complement pathway. In this study, the direct interaction of CFH with surface-bound PTX3 has been shown to enhance CFH recruitment and iC3b deposition. Furthermore, PTX3-bound CFH retains its activity as a cofactor for factor I-mediated C3b cleavage. In contrast, surface-bound CRP has been shown to decrease the alternative pathway C3-convertase activity and to inhibit the alternative pathway amplification loop.
Mutations and polymorphisms of CFH have also been linked with certain pathological conditions. One polymorphism has been associated with AMD, 402HH. It is known that CFH 402HH genotype has reduced CRP binding capacity that could result in excessive inflammation in individuals who carry this CFH variant and develop AMD. In contrast, the 402HH polymorphism does not affect binding to PTX3.

References
1. Deban, L et al; J Immunol 2008, *181*:8433
2. Laine, M et al; J Immunol 2007, *178*:3831

Polymorphism CFH-Y402H and cardiovascular disease

Volcik, K et al; Am J Hypertens 2008, *21*:533

The 402HH homozygous genotype was a significant predictor of incident ischemic stroke in Caucasians. Significant interaction effects between genotype and hypertension were observed for coronary heart disease (CHD) in Caucasians and for carotid artery wall thickness (cIMT) in Caucasians and African Americans. In further analyses of incident CHD, genotypes carrying the 402H allele were a significant predictor of incident CHD in Caucasians who had hypertension. The 402H allele was also associated with higher cIMT measures for Caucasians in the overall cohort, and for Caucasians with hypertension.

Serum amyloid A, a non-invasive biomarker for lung cancer

Cho, W et al; Brit J Cancer 2010, *102*:1731

Lung cancer, a top cancer killer, is usually diagnosed at a relatively late stage, meaning that despite remarkable progress in treatment, most patients still die. Therefore, a biomarker that could aid the early diagnosis or recurrence of cancer after treatment could increase patient survival rates.

This study showed that the level of SAA is highly elevated in lung cancer patients with poor prognosis, which makes SAA a promising marker for lung cancer diagnosis.

Complement and CD14 in inflammation

Knut Tore Lappegård and Tom Eirik Mollnes, Norway.

The complement system and the Toll-like receptors are two main danger recognition systems of innate immunity, playing crucial roles in host defence as well as in inflammation-induced tissue injury and disease. These systems cross-talk, as described by several groups (1,2) and recently reviewed (3). To study the role of complement in the inflammatory reaction, we have described a whole blood model, anticoagulated with the thrombin-specific inhibitor lepirudin, enabling mutual interaction between the inflammatory systems (4). Selective inhibition of complement and CD14, a key recognition molecule in the Toll-like receptor system, attenuated different inflammatory reactions to Gram-negative bacteria to various degrees, whereas combining complement- and CD14-inhibitors efficiently blocked CD11b expression (5) and release of cytokines (6).

Based on these observations we performed a comprehensive study using fresh whole blood from two individuals with complete, genetic deficiencies of complement factors 2 and 5, respectively, where we delineated in detail the complement-dependent and the complement-independent inflammatory reactions induced by the Gram-negative bacteria *E. coli* or *N. meningitidis* (7). By reconstitution with the missing component as well as addition of specific inhibitors it was possible to demonstrate that numerous inflammatory responses were crucially dependent on complement, and for the majority of them on factor C5 (see figure). This was true for expression of tissue factor on the monocyte surface, expression of CD11b on the granulocyte surface, granulocyte oxidative burst and killing of bacteria. Release of enzymes from granulocytes was also complement dependent, but in contrast to previous views it was shown to depend on C3 and not C5. Blocking of CD14 attenuated in particular monocyte activation and release of cytokines, whereas all the inflammatory responses studied were completely blocked by combined complement- and CD14 inhibition. Such a combined strategy might be a therapeutic alternative to attenuate systemic inflammatory responses in sepsis.

References
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2. Zhang, X, et al; Blood 2007, *110*: 228
3. Hajishengallis, G.,et al; Trends Immunol 2010, *31*:154
4. Mollnes, T. E., et al; Blood 2002, 100:1869
5. Brekke, O. L. et al; J Leukoc. Biol. 2007, *81*:1404
6. Brekke, O. L. et l; Mol. Immunol 2008, *45*:3804
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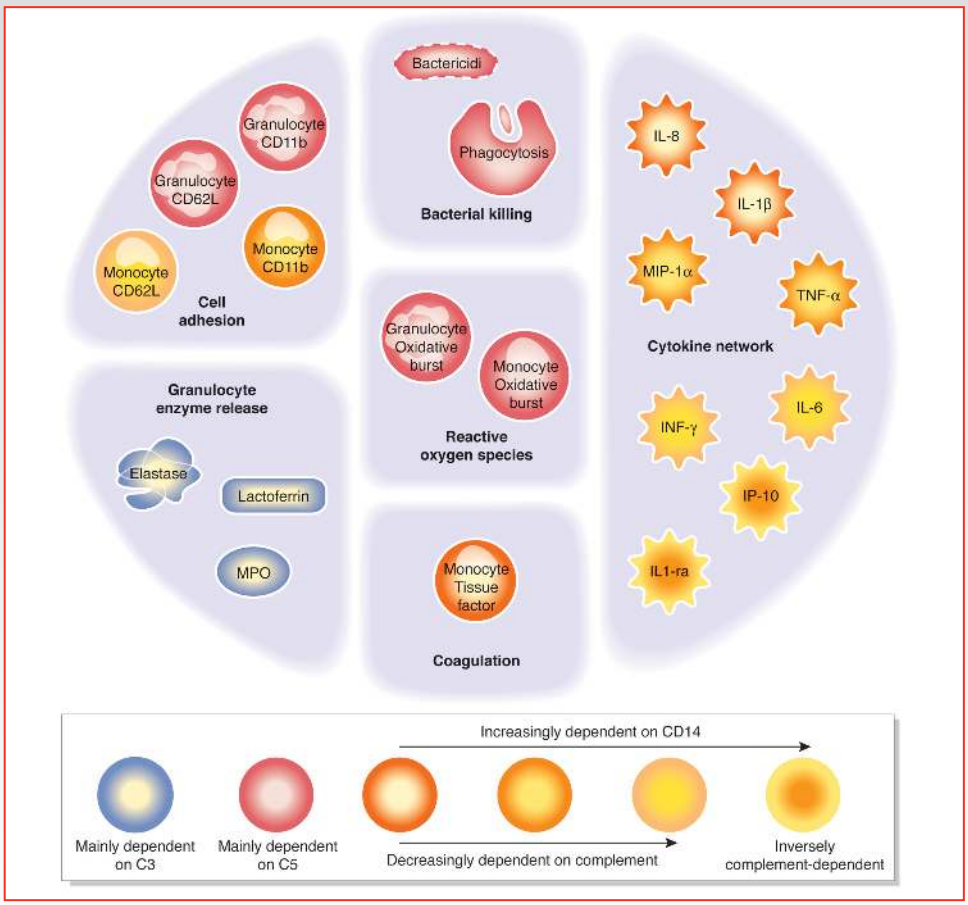

Figure 3
Schematic description of the role of the complement system in bacterial-induced inflammatory response. Dependence on C3, C5 and CD14 is shown in blue, red and yellow, respectively. Relative contribution of C5 vs CD14 is shown with merging colors of yellow and red. (From Lappegård K et al. Proc Natl Acad Sci U S A 2009, 106:15861)

HUMANE MAB

PRODUCT	APPLICATIONS	CAT. #
C3/C3a, mAb 474	IA W	HM2073
C3/C3a, mAb 2898	IA W	HM2075
C3/C3b, mAb 755	IA IP W	HM2072
C3a/C3a desArg, mAb 2991	IA W	HM2074
C3aR, mAb 17	F FC	HM2195
C4d, mAb 12D11	F IA W	HM2229
C4d, mAb 7H4	F IA W	HM2230
C5/C5a, mAb 557	FS IA	HM2077
C5/C5a, N-terminus, mAb 561	FS IA IP W	HM2076
C5/C5b, mAb 568	IA W	HM2080
C5a/C5a des-Arg, mAb 2942	FS IA W	HM2078
C5a/C5a des-Arg, 2952	FS IA W	HM2079
C5aR, mAb S5/1	FS FC P W	HM2094
C5aR, mAb W17/1	F FC P	HM2095
C6, mAb WU6-4	FC FS IA IP P W IF	HM2276
C7, WU 4-15	FC IA IP W IF	HM2277
C9 neoantigen, mAb WU13-15	F FC IA P W	HM2264
C9, mAb X197	FS F FC IA P W	HM2111
Factor H, mAb C18/3	IA W	HM2248
Factor H, mAb L20/3	IA W	HM2249
Factor D, mAb I8/1	IA	HM2259
SAA, mAb Reu86.1	F IA P	HM2100
SAA, mAb Reu86.5	F IA P	HM2101
PTX3, mAb MNB1	F IA IP P W	HM2241
PTX3, mAb MNB4	F FC IA IP P W IF	HM2242
CD14, Human, mAb MEM-15	FC W	HM2060
CD14, Human, mAb 18D11	FC FS	HM2224

Serum lipopolysaccharide activity is associated with the progression of kidney disease in Finnish patients with type 1 diabetes

Nymark, M et al. Diabetes Care 2009, *32*:1689

Diabetic nephropathy is one of the leading causes of death in patients with type 1 diabetes worldwide. However, whilst such renal dysfunction is often associated with a number of factors, the etiology of the complication is largely unknown.
This study made use of Hycult Biotech's LAL Chromogenic Endpoint assay (Cat.#. HIT302) to measure the serum LPS activity in 477 patients with type 1 diabetes in order to investigate whether serum LPS activity is associated with the progression of kidney disease.
It was hypothesized that as bacterial infections are often associated with other forms of kidney disease and Lipopolysaccharides (LPS) from gram negative bacteria induce a systemic inflammatory response, which may in turn cause organ damage that bacterial infections may also trigger the process that leads to diabetic nephropathy.
As HDL is one of the key factors involved in the detoxification of endotoxins and is known to decline with the severity of diabetic nephropathy the level of HDL was also measured and the ratio of LPS to HDL was calculated.
This study found that a high LPS to HDL ratio was associated with the development of kidney disease in type I diabetes patients with a normal albumin excretion rate and with progression of kidney disease in type I diabetes patients with microalbuminuria, showing that high serum LPS activity is indeed associated with the development of diabetic nephropathy in patients with type I diabetes.

References:
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5. Kuula, H et al; Infect Immun 2009, *77*:850
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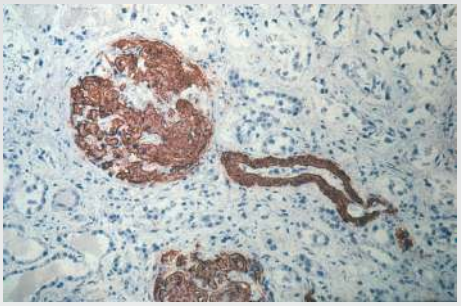

Figure 4 Serum Amyloid A deposits in glomerulus and arteriole of a kidney from a patient with amyloidosis. Staining with antibody Reu 86.1 (Cat.# HM2100).

PRODUCTS

PRODUCT NAME	CONCENTRATION RANGE	SENSITIVITY	QUANTITY	CAT.#
LAL Chromogenic Endpoint assay	0.01 -10 EU/ml	0.01 EU/ml	3 x 96 det.	HIT302
ENDOBLOCK LBP ELISA	3 to 2000 ng/ml	3 ng/ml	1 x 96 det.	HIT301
EndoCab, Human, ELISA	IgG: 0.13 – 8 GMU/ml IgM: 0.05 – 3.5 MMU/ml IgA: 0.16 – 10 AMU/ml	0.13 GMU/ml 0.05 MMU/ml 0.16AMU/ml	1 x 96 det.	HK504
EndoClear (EndoTrap + mini LAL)	0.01 -10 EU/ml	0.01 EU/ml	1 x 1ml column	HIT305 - 310
LL-37, Human, ELISA	0.1 to 100 ng/ml	0.1 ng/ml	2 x 96 det.	HK321
Pentraxin 3, Human, ELISA	78-5000 pg/ml	78 pg/ml	2 x 96 det.	HK347
SLPI, Human, ELISA	20 to 5,000 pg/ml	20 pg/ml	2 x 96 det.	HK316
SAA, Human, ELISA	3.1 to 200 ng/ml.	3.1 ng/ml	2 x 96 det.	HK333

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