Hycult Scope

Complement activation in susceptibility to neonatal sepsis.

Luregn Schlapbach, MD. Department of Pediatrics, University of Bern

Incidence of bacterial sepsis during the neonatal period:

The incidence of bacterial sepsis during the neonatal period is high. Innate immunity plays an important role in the defense of neonates against invasive infections. Mannan-binding lectin (MBL), L-ficolin, and H-ficolin recognize microorganisms and activate the complement system via MBL-associated serine proteases (MASPs). However, the entire lectin pathway has never been studied in patients with sepsis.

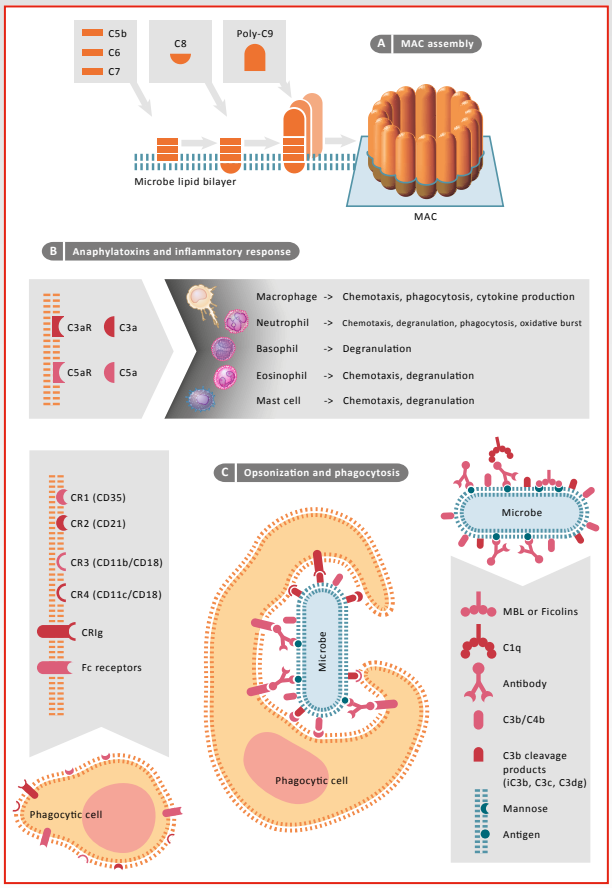
Cord blood concentrations of the lectin proteins:

This study investigated whether cord blood concentrations of the lectin pathway proteins are associated with neonatal sepsis. This case-control study includes 47 infants with culture-proven sepsis during the first month of life and 94 matched controls. Concentrations were measured in cord blood with use of enzyme-linked immunosorbent assay (**MBL, L-ficolin, MASP-2**) and time-resolved immunofluorometric assay (H-ficolin, MASP-3).

Different results Infants with gram-positive and gram-negative sepsis:

Infants with gram-positive sepsis had significantly lower H-ficolin concentrations than controls, whereas infants with gram-negative sepsis had lower MBL concentrations.

When excluding patients with postoperative sepsis, multivariate analysis confirmed that low H-ficolin was associated with a significantly higher risk of gram-positive sepsis and late-onset sepsis. In contrast, low MBL was associated with a significantly higher risk of gram-negative sepsis and early-onset sepsis. The concentrations of all the lectin pathway proteins increased with gestational age ($p < 0.01$).



Conclusions:

These results indicate that low MBL concentrations are a susceptibility factor for gram-negative sepsis, and low H-ficolin concentrations indicate susceptibility to gram-positive sepsis. The decreased expression of lectin pathway proteins in neonates must be considered to be an additional form of neonatal immunodeficiency.

Reference:

Schlapbach, L et al. **CID 2010**, *51*:153

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www.hycultbiotech.com

Pathogen Recognition and Interaction

ASSAYS COMPLEMENT

PRODUCT	QUANTITY	CAT.#
MBL, Human, ELISA	2 x 96 det.	HK323
L-ficolin, Human, ELISA	2 x 96 det.	HK336
H-ficolin, Human, ELISA	2 x 96 det.	HK340
MASP-2, Human, ELISA	2 x 96 det.	HK326
MASP-3, Human, ELISA	2 x 96 det.	HK339
MBL/MASP-2, Human, ELISA	2 x 96 det.	HK327

In this issue

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Variant-specific quantification of factor H identifies null alleles associated with aHus.

S. Hakobyan, PhD. Department of Infection, Immunity & Biochemistry, Cardiff University

Complement factor H mutations responsible for aHUS:

The complement system is a major mechanism of innate immunity that serves to link recognition of microbes to effector functions and to the development of inflammation. Complement is implicated in numerous diseases; for example, atypical hemolytic uremic syndrome (aHUS) is associated with complement alternative pathway defects in over 50% of cases. Mutations in factor H (fH) gene (CFH), the major regulator of complement alternative pathway, are most common in aHUS. In some aHUS cases, null mutations in CFH are found, resulting in heterozygous deficiency of fH. Although patients with null mutations in heterozygosity will usually have low plasma levels of fH, the large variability in fH concentrations in normal individuals often makes it impossible to identify cases simply by measuring fH levels in plasma.

Polymorphism Y402H useful marker for individual CFH alleles:

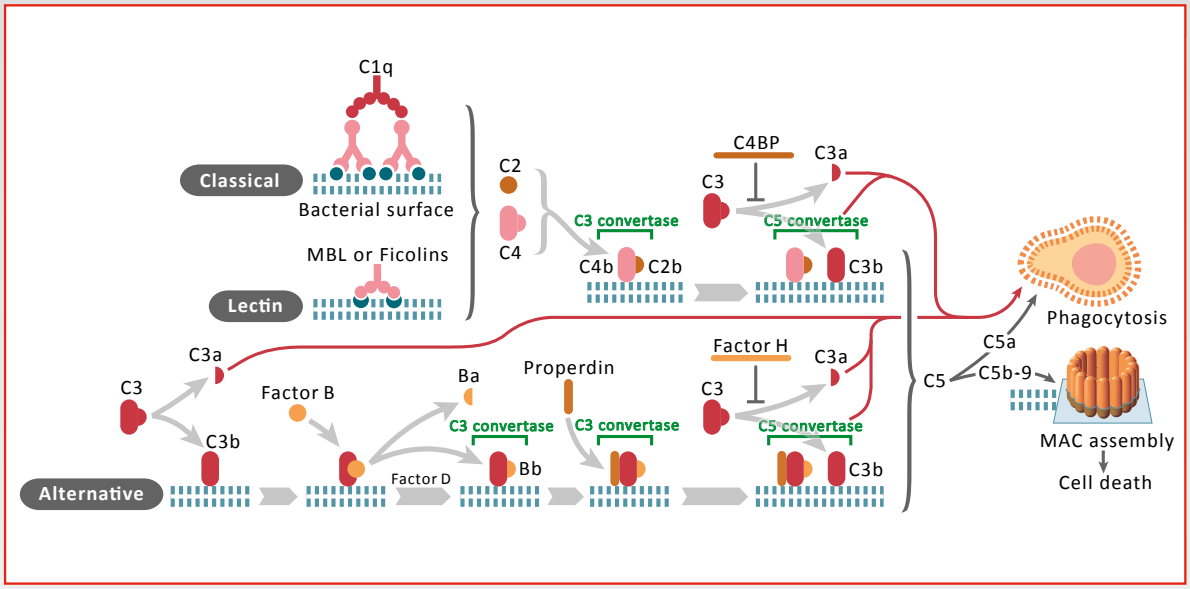
A common polymorphism in fH, Y402H, is strongly associated with another disease, macular degeneration. We have generated two novel anti-fH monoclonal antibodies MBI-6 and MBI-7, specific to Y402 and H402 alleles respectively. We have developed robust assays based on these antibodies that allow us to individually quantify Y402 and H402 alleles. Y402H is the most common polymorphism in fH and over 40% of Caucasians are Y402H heterozygous. This polymorphism therefore represents a useful "marker" for individual CFH alleles. Although the Y402H polymorphism has no apparent direct link to aHUS, application of the new assays to aHUS families enabled us to identify, characterize and confirm new CFH alleles associated with low or no expression of fH and show that these low/no expression alleles conferred strong predisposition to aHUS. These novel tools will not only help identify the molecular basis of disease in patients with aHUS, but also aid prediction of risk in their relatives.

Conclusion:

The complement system plays an important role in diverse diseases; these novel reagents and assays will be useful in unambiguous and rapid identification of occult low/no expression CFH alleles.

Reference:

S. Hakobyan, A. Tortajada, C. L. Harris, S. Rodriguez de Cordoba and B. P. Morgan. **Kidney International 2010** *78*:782.



ASSAYS COMPLEMENT

PRODUCT	QUANTITY	CAT.#
Complement factor H, Human, ELISA	2 x 96 det.	HK342
Factor H, 402H/Y variant detection, Human, ELISA	1 x 96 det.	HK353
C1q, Human, ELISA	2 x 96 det.	HK356
Complement 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

ANTIBODIES COMPLEMENT

PRODUCT	QUANTITY	APPLICATIONS	CAT.#
Complement factor B/Ba, Human, mAb M20/6	100 µg	IA IP W	HM2255
Complement factor B/Ba, Human, mAb P21/15	100 µg	IA IP W	HM2254
Complement factor B/Bb, Human, mAb M13/12	100 µg	IA IP W	HM2256
Complement factor D, Human, mAb I8/1	100 µg	IA	HM2259
Complement factor H, Human, mAb C18/3	100 µg	IA W	HM2248
Complement factor H, Human, mAb, L20/3	100 µg	IA W	HM2249
MASP-1/3, Human, mAb 1E2	100 µg	IA IP W	HM2092
MASP-1/3, Human, mAb 2B11	100 µg	IA IP W	HM2093
MASP-2, Human, mAb 8B5	100 µg	IA W	HM2190
MASP-2/MAp19, Human, mAb 6G12	100 µg	IA W	HM2191
MASP-3, Human, mAb 38:12-3	100 µg	IA W	HM2216
MBL, Human, mAb 3E7	100 µg	F FC IA W	HM2061
MBL, Human, mAb 3E7, FITC	100 µg	F FC IA W	HM2061F
MBL, Human, mAb D8.18	100 µg	FC IA W	HM2081
MBL, Human, mAb D8.18, biotinylated	50 µg	FC IA W	HM2082
MBL-A, Mouse, mAb 2B4	100 µg	IA W	HM1036
MBL-A, Mouse, mAb 8G6	100 µg	F IA W	HM1035
MBL-C, Mouse, mAb 14D12	100 µg	F IA IF W	HM1038
MBL-C, Mouse, mAb 16A8	100 µg	IA W	HM1037

PROTEINS COMPLEMENT

PRODUCT	QUANTITY	CAT.#
C5L2, Human, Peptide	10 µg	HC2103
C5a des Arg, Human, Recombinant	50 µg	HC2102
C5a, Human, Recombinant	50 µg	HC2101
C1, Human, Natural	200 µg	HC2122
C1q, Human, Natural	1 mg	HC2123
C2, Human, Natural	50 µg	HC2124
C3, Human, Natural	250 µg	HC2125
C3a, Human, Natural	50 µg	HC2126
C3a desArg, Human, Natural	50 µg	HC2127
C4, Human, Natural	250 µg	HC2128
Complement factor B, Human, Natural	250 µg	HC2129
Complement factor H, Human, Natural	250 µg	HC2130
Complement factor I, Human, Natural	250 µg	HC2131

IgG glycan hydrolysis in glomerulonephritis.

MM van Timmeren, PhD, Department of Pathology and Medical Biology, University Medical Center Groningen

Elimination of pathogenic IgG in autoimmune patients:

Rapidly progressive glomerulonephritis (RPGN) is acute glomerulonephritis (GN) in association with a 50% or more decrease in glomerular filtration rate within a 3-month period. If left untreated, RPGN rapidly progresses into acute renal failure and death within months. Auto antibodies as a constituent of immune complexes play a pivotal role in triggering inflammatory processes. Elimination of pathogenic IgG from the circulation in these autoimmune patients is pivotal for disease remission. Plasmapheresis and immunoadsorption are commonly used methods but have their limitations.

EndoS causes complete hydrolysis of circulating IgG:

The recently discovered enzyme Endoglycosidase S (EndoS) is secreted by Streptococcus pyogenes and has specific endoglycosidase activity on native IgG by hydrolyzing the conserved asparagine-linked glycans on the heavy chains of IgG.

Cleavage of glycan groups inhibits development of (auto)immune-mediated diseases:

We hypothesized that cleavage of glycan groups from the Fc heavy chain of IgG by the bacterial enzyme EndoS inhibits the development of (auto)immune-mediated renal diseases. As a model for (auto)immune-mediated glomerulonephritis we employed a well described mouse model for anti-myeloperoxidase (MPO) IgG-mediated GN.

EndoS treatment of ANCA IgG

In vitro studies demonstrated that EndoS treatment of ANCA IgG significantly attenuated ANCA-mediated neutrophil activation without affecting antigen binding capacity. More specifically, ANCA IgG mediated neutrophil oxygen radical production and degranulation of lactoferrin and elastase were diminished to a large extent upon ANCA IgG treatment with EndoS. In vivo, EndoS pretreatment of anti-MPO IgG reduced hematuria, leukocyturia, and albuminuria in the mouse model of anti-MPO IgG-induced GN. Early glomerular neutrophil influx was diminished and glomerular crescent formation was markedly attenuated. Furthermore, systemic treatment with EndoS after 3h reduced albuminuria and glomerular crescent formation, while EndoS treatment after 24h did not attenuate disease parameters.

Conclusion:

These results suggest that modulation of IgG glycosylation by EndoS is a promising strategy to interfere with the early ANCA-mediated inflammatory processes.

Reference:

van Timmeren MM, van der Veen BS, Stegeman CA, Petersen AH, Hellmark T, Collin M, Heeringa P. **J Am Soc Nephrol. 2010** *21*:1103.

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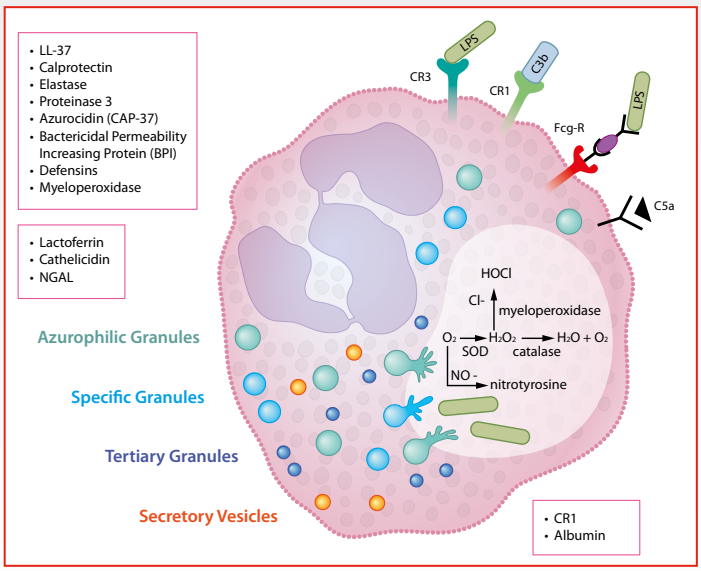


ANTIBODIES ANTIMICROBIAL PEPTIDES

PRODUCT	QUANTITY	APPLICATIONS	CAT.#
BPI, Human, mAb 3F9	100 µg	IA	HM2041
BPI, Human, mAb 4E3	100 µg	FC FS IA	HM2170
BPI, Human, mAb 4H5	100 µg	IA	HM2042
BPI, Human, pAb	100 µg	IA IP	HP9022
Calprotectin, Human, mAb 27E10	100 µg	F FC IA IP P W	HM2156
Calprotectin, Human, mAb 27E10, biotinylated	50 µg	F FC IA P W	HM2156BT
Calprotectin, Human, mAb 27E10, FITC	100 µg	F FC IA P W	HM2156F
Defensin 5, Human, mAb 8C8	100 µg	F IA W	HM2228
Elastase, Human, mAb 265-3K1	100 µg	IA W	HM2174
Elastase, Human, pAb	100 µg	IA	HP9027
HNP1-3, Human, mAb D21	100 µg	F FC FS IA IF IP P W	HM2058
HNP1-3, Human, mAb D21, biotinylated	50 µg	F FC FS IA IF IP P W	HM2059
Lactoferrin, Bovine, mAb a-bC-lobe	100 µg	IA W	HM4013
Lactoferrin, Bovine, pAb	100 µg	IA IP W	HP7001
Lactoferrin, Human, mAb 265-1K1	100 µg	F IA W	HM2173
Lactoferrin, Human, pAb	100 µg	IA IP W	HP9034
LL-37/CAP-18, Human, mAb 1-1C12	100 µg	IA IF P W	HM2071
LL-37/CAP-18, Human, mAb 3D11	100 µg	FS IF P W	HM2070
MPO, Human, mAb 266-6K1	100 µg	IA W	HM2164
MPO, Human, mAb 266-6K1, biotinylated	50 µg	IA W	HM2164BT
MPO, Human, mAb 266-6K1, FITC	100 µg	IA W	HM2164F
MPO, Mouse, mAb 8F4	100 µg	F FC IA	HM1051
MPO, Mouse, mAb 8F4, biotinylated	50 µg	F FC IA	HM1051BT
MPO, Mouse, mAb 8F4, FITC	100 µg	F FC IA	HM1051F
MPO, Rat, mAb 2D4	100 µg	F FC IA IF	HM3030
MPO, Rat, mAb 2D4, biotinylated	50 µg	F FC IA IF	HM3030BT
MPO, Rat, mAb 2D4, FITC	100 µg	F FC IA IF	HM3030F

PROTEINS ANTIMICROBIAL PEPTIDES

PRODUCT	QUANTITY	CAT.#
Calprotectin, Human, recombinant	50 µg	HC2120
Elafin, Human, Recombinant	50 µg	HC4011
HNP1-3, Human, Natural	>100 µg	HC4014
LBP, Human, Peptide	0.5 mg	HC4030-05
LBP, Human, Peptide	1 mg	HC4030-10
LBP, Human, Peptide	100 µg	HC4030-01
LBP, Human, Purified, Natural	10 µg	HC4010
LL-37/CAP-18, Human, Peptide	50 µg	HC4013

ASSAYS ANTIMICROBIAL PEPTIDES

PRODUCT	QUANTITY	CAT.#
alpha-Defensin 1-3, Human, ELISA	2 x 96 det.	HK317
Arginase-I, Human, ELISA	2 x 96 det.	HK322
BPI, Human, ELISA	2 x 96 det.	HK314
Calprotectin, Human, ELISA	2 x 96 det.	HK325
Elastase, Human, ELISA	2 x 96 det.	HK319
Lactoferrin, Human, ELISA	2 x 96 det.	HK329
LL-37, Human, ELISA	2 x 96 det.	HK321
MPO, Human, ELISA	2 x 96 det.	HK324
MPO, Mouse, ELISA	2 x 96 det.	HK210
MPO, Rat, ELISA	2 x 96 det.	HK105

Bacterial antigen RpL7/L12 in early stage colorectal cancer.

Harold Tjalsma, PhD, Nijmegen Institute for Infection, Inflammation & Immunity (N4i), Radboud University Nijmegen Medical Centre.

Humoral immune response to bacterium during different stages of CRC:

Intestinal bacteria have been implicated in colorectal cancer (CRC) pathology for a long time and a large number of reports point to a close linkage between Streptococcus gallolyticus infections and tumors of the human colon. This study aimed to investigate the humoral immune response to this bacterium during different stages of CRC.

Presence of IgGs against ribosomal protein (Rp) L7/L12 from S. gallolyticus:

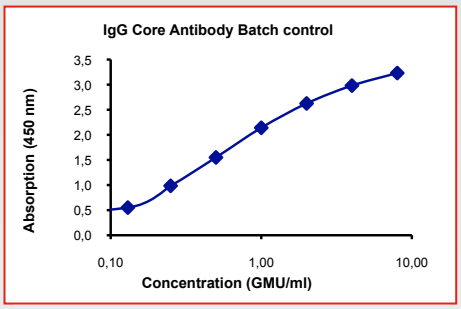
To this purpose, the presence of serum IgGs against ribosomal protein (Rp) L7/L12 from S. gallolyticus was evaluated in Dutch and American populations by an in-house developed ELISA assay. RpL7/L12 was previously identified as a candidate diagnostic antigen for CRC. The data consistently showed that an immune response against this antigen was increased in polyp patients and stage I/II CRC patients as compared to asymptomatic individuals. As a control, the humoral immune response to endotoxin was determined by the EndoCab assay from Hycult Biotech in the same study populations.

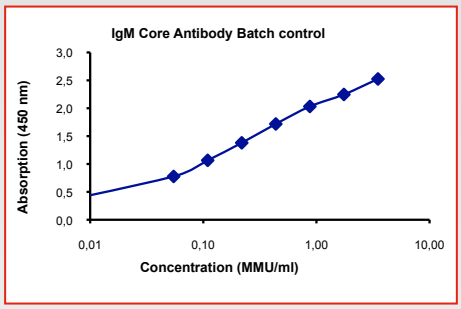
Endotoxin associated with loss of intestinal barrier function:

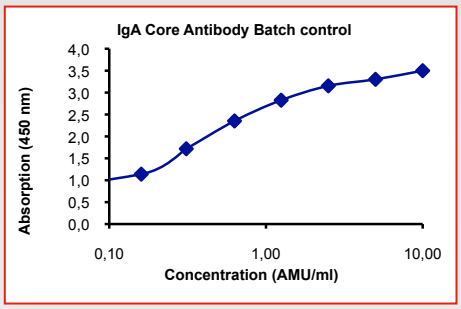
Endotoxin is an intrinsic component of the cell wall from the majority of Gram-negative intestinal bacteria and increased serum IgG levels against endotoxin have been associated with an impaired colonic barrier function. The latter analyses showed that anti-endotoxin IgG levels displayed a similar tendency as the anti-RpL7/L12 levels. However, in contrast to anti-RpL7/L12, the relative endotoxin antibody expression in patients with stage I/II tumors was markedly lower than in polyp patient. This suggests that increased anti-RpL7/L12 levels in early CRC patients cannot be fully attributed to a general loss of intestinal barrier function. Together, these findings are indicative for an increased exposure to antigen RpL7/L12 during early stages of colon carcinogenesis and may suggest that intestinal bacteria, such as S. gallolyticus, constitute a risk factor for the progression of pre-malignant lesions into early stage carcinomas. Clearly, the current findings emphasize the necessity for further studies on the possible etiologic relationship between intestinal bacteria and human CRC.

Reference:

A Boleij, R Roelofs, R M.J. Schaeps, T Schülin, P Glaser, D W. Swinkels, I Kato, H Tjalsma **Cancer 2010**; *116*:4014-22.







Determination of endotoxin core antibodies with EndoCab® ELISA.

ASSAYS LPS, MICROBIAL TOXINS		
PRODUCT	QUANTITY	CAT.#
EndoCab®, Human, ELISA	1 x 96 det.	HK504
LAL Chromogenic Endpoint Assay	3 x 96 det.	HIT302
EndoClear, blue, small (EndoTrap blue 1/1 + mini LAL)	1 x 1 ml column	HIT305
EndoClear, red, small (EndoTrap red 1/1 + mini LAL)	1 x 1 ml column	HIT306
ENDOBLOCK LBP ELISA	1 x 96 det.	HIT301
LBP, Human, ELISA	2 x 96 det.	HK315
LBP, Mouse, ELISA	2 x 96 det.	HK205
LBP, various species, ELISA	1 x 96 det.	HK503

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