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Issue 23

Focus on Complement

A newsletter of the International Complement Society

EUROPEAN COMPLEMENT NETWORK

About this issue & More

What's inside?

<1>Two flash news, are presented by (a) Dr. Kinga Hosszu on "gC1qR as a novel receptor for HIV-gp41" and ***(b)*** Dr. Richard Kew presents on " the crosstalk between TLR and C5L2 in pro-inflammatory responses"

<2>No complement Teams are presented in this issue. However, the editor presents the second installment of groups working in the area of MBL/Ficolin who are being given a chance to present their views on the nomenclature of these groups of proteins.

<3>. Please also note that you can now get preliminary information about the XXIV ICS meeting in Crete **by** visiting the website at:
www.complement2012.org

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FLASH NEWS

gC1qR is a receptor for gp41 -Reporter: K.Hosszu

HIV gp41 engages gC1qR on CD4+ T cells to induce the expression of an NK ligand through the PIP3/H2O2 pathway.

Fausther-Bovendo H, Vieillard V, Sagan S, Bismuth G, Debre P. PLoS Pathog. 2010 Jul 1;6:e1000975

This paper uncovers the mechanism of how CD4+ T cells are depleted during the chronic phase of HIV-1 infection. Most of the dying CD4+ T cells during this phase of the infection are uninfected. Previously, data from this lab showed that an HIV-1 envelope protein, gp41, induces the expression of the stress molecule NKp44L on the surface of uninfected $CD4^+$ T lymphocytes, resulting in the NK cell mediated lysis of these cells. Here Hugues Fausther-Bovendo and colleagues use different techniques to identify gC1qR, as the receptor that induces the expression of NKp44L on $CD4^+$ T lymphocytes. The interaction site between gC1q-R and gp41 is the 3S motif of HIV-1 gp41. Binding of gp41 to gC1qR in turn activates the PI3K, the NADPH oxidase, and the p190A RhoGAP proteins and induces the translocation of pre-existing NKp44L molecules from the cytoplasm to the plasma membrane of $CD4^+$ T cells. This signaling mechanism and subsequent expression of NKp44L may identify new therapeutic strategies that could help in preventing death of uninfected CD4+ T cells during HIV infection.

TLR & C5L2 crosstalk -Reporter : R. Kew

TLR activation enhances C5a-induced pro-inflammatory responses by negatively modulating the second C5a receptor, C5L2

Raby A-C., B. Holst, J. Davies, C. Colmont, Y. Laumonnier, B. Coles, S. Shah, J. Hall, N. Topley, J. Köhl, B.P. Morgan, and M.O. Labéta Eur J Immunol 41:2741-2752, 2011

Complement and TLRs are the soluble and cellular microbial pattern recognition elements of innate immunity. Ligand recognition by either system induces rapid pro-inflammatory responses in a variety of immune cells. Several recent studies have demonstrated that C5a induces marked synergistic activation of TLR-mediated cellular responses. However, little is known concerning how treatment of cells with TLR agonists influences subsequent C-mediated responses. Using human peripheral blood mononuclear cells from healthy donors, the authors show that pretreatment of cells with TLR agonists significantly augments C5a-induced IL-8 generation. The in vitro enhancing effect was observed using agonists for TLR4 (LPS), TLR2/TLR1 (Pam_3-Cys-Ser-Leu_4), TLR2/TLR6 (zymosan), TLR5 (bacterial flagellin), and TLR7/TLR8 (imiquimod). Ex vivo analysis of mouse leukocytes showed that LPS had no effect on C5a-induced cytokine production in cells from TLR4-deficient mice but had a robust response in cells from wild-type animals. The enhanced C5a-mediated response to LPS was not due to increased expression or activity of the classic C5a receptor (C5aR1, CD88), but rather due to the second C5a receptor, C5L2, which negatively regulates the activity of C5aR1. This paper identifies a novel mechanism to enhance C5a-mediated functions via TLR inhibition of C5L2 and demonstrates clearly that the cross-talk between TLRs and C5a receptors is both bidirectional and synergistic.

SPOTLIGHT ON "MBL" TEAMS

CONTINUATION…….from previous issue

NOMENCLATURE FOR THE COMPONENTS OF THE LECTIN COMPLEMENT PATHWAY- III

Nomenclature issues in the lectin pathway of complement

Peter Garred, Laboratory of Molecular Medicine, Department of Clinical Immunology- 7631 Rigshospitalet and University of Copenhagen Copenhagen, Denmark

In recent years several soluble collagen-like pattern recognition receptors have been shown to be associated with a set of serine proteases that are involved in lectin complement activation pathway. However, little consensus about the nomenclature of these proteins exists. Thus a debate about this issue is highly appreciated. I have tried to review the names given to the human orthologs in the databases provided by National Center for Biotechnology Information (NCBI) and include some personal suggestion based on my experience over the years. An overall problem in this field is the lack of evolutionary consistency and that the fine specificity may be different in different species.

MBL

The *MBL2* gene encodes human mannose- or mannan-binding lectin (MBL). The *MBL1* gene is a pseudogene in humans. The original phrases used were mannan- or mannose-binding protein. None of these names are particularly good since the specificity is wider, but during the last 15 years the phrase mannose-binding lectin has gained momentum in the literature. A simple PubMed search provides 3616 hits for mannose-binding lectin and 2601 for mannan-binding lectin. Alternative names for MBL are COLLECTIN-1 and COLEC1. To avoid much confusion I think we should stick with the gene name *MBL2* and leave it up to each group to choose between mannose- or mannan-binding lectin abbreviated MBL.

The ficolins

The *FCN1* gene encodes ficolin-1, which is the name we use in my group. It is also called M-ficolin due to its expression in monocytes, but the primary reservoir is the granulocytes. Ficolin-1 is also synthesised in the lungs. An alternative name is COLLAGEN/FIBRINOGEN DOMAIN-CONTAINING LECTIN 1 P35-LIKE. I suggest that we stick with the name ficolin-1, which will link the protein to the gene and confusion about the cells of expression could be avoided.

The *FCN2* gene encodes ficolin-2, which is the name we use in my group. The protein is primarily expressed by hepatocytes. It is also called l-ficolin, because its origin in the liver. Alternative names are COLLAGEN/FIBRINOGEN DOMAIN-CONTAINING LECTIN 2 P35, opsonin P35, hucolin or elastin binding protein 37 (EBP-37). I suggest that we stick with the name ficolin-2, which will link the protein to the gene and confusion about the cells of expression could be avoided.

The *FCN3* gene encodes ficolin-3, which is the protein name we use in my group. It is also called H-ficolin derived from one of the original names, Hakata antigen. Hakata is a Japanese town and the phrase was used to designate an autoantigen found in some SLE patients

more than 30 years ago. Ficolin-3 is primarily synthesised by ciliated bronchial epithelial cells and Type II alveolar epithelial cells and in the liver it is synthesised by bile duct epithelial cells and hepatocytes. However, the expression profiles are much wider and the *FCN3* gene may be turned on in a variety of cells and tissues. Alternative names are COLLAGEN/FIBRINOGEN DOMAIN-CONTAINING LECTIN 3 P35, HAKA1, thermolabile β-2 macroglycoprotein, or thermolabile substance. I suggest that we stick with the name ficolin-3, which will link the protein to the gene and will make the ficolins names consistent.

The evolutionary gene structure is very complicated for the ficolins. The *FCN3* gene appeared to split as an independent branch of the ficolins before or at the appearance of frogs. There is no ortholog of *FCN2* below primates and based on sequence analysis *FCN2* appears to be a duplication of the *FCN1* gene. *FCN*1 appears to be the gene that has carried ficolin information through evolution. However, each species branches have created new ficolins, e.g. in rodents ficolin-A which in function resembles ficolin-2 is derived from ficolin-B, which in fact seems to be the rodent version of *FCN1*.

Collectin-11

There is a new kid on the block in the lectin pathway. Collectin-11 (originally named CL-K1) derived from the *COLEC11* gene. It is a collagen-like lectin and is found associated with at least MASP-1 and MASP-3. Collectin-11 probably plays a fundamental role during embryogenesis and is most likely important in innate immmunity in the context of the complement system. It is indeed the eleventh collectin discovered as far as I know.

MASPs

These proteins were originally named after their association with MBL. The first protein to be discovered was MASP-1, which was designated mannose-binding protein associated serine protease or MBP-associated serine protease. Subsequently MBL-associated serine protease 2 (MASP-2) was discovered. NCBI uses the names mannan-binding lectin serine protease 1 or 2 for these proteins without abbreviations. They originate from the *MASP1* and *MASP2* genes, respectively. However, both genes give rise to different alternative splice variants.

MASP1

MASP1 splice variants	protein names
MASP1_v1	MASP1 isoform 1 is MASP-1
MASP1_v2	MASP1 isoform 2 is MASP-3
MASP1_v3	MASP1 isoform 3 is MAP-1, which also is called Map44

We have chosen the name MAP-1 because it is a protein that lacks a serine protease (without S) and it originates from the MASP1 gene. Map44 is based on a theoretical prediction of MW. It is actual 43.6, but this includes the signal peptide. Thus the predicted MW of the mature protein would be around 41. MAP-1 is glycosylated and in SDS PAGE the protein comes with a MW of 45.

MASP2

MASP2 splice variants	protein names
MASP2_v1	MASP2 isoform 1 is MASP-2
MASP2_v2	MASP2 isoform 2 is sMAP or Map19

Both sMAP and Map19 are somewhat confusing names and I would like to suggest MAP-2 using the same argument as for MAP-1, since MASP2 isoform 2 is derived from the *MASP2* gene. However, sMAP and Map19 have been in the literature for many years, thus I think it would be difficult to reach a nomenclature consensus on this subject. Nevertheless, in general using MW in protein names are problematic since the correct MW tends to differ based on the technique employed used to measure the mass of the protein.

We try to avoid to spell out good abbreviations like MASPs or MAPs. Nevertheless since mannose-binding lectin associated serine proteases appear to be somewhat misleading we have begun to use the phrase MBL/ficolin associated serine protease 1, 2 or 3 or MBL/ficolin associated protein 1 or 2 for MASP-1 to -3 and MAP-1 and -2, respectively.

Ficolin-3 appears to be a key activator of the lectin pathway even more than MBL, but to avoid confusion the terms MASP and lectin pathway (even though, ficolins are not strictly lectins) still are good we think.

With the introduction of collectin-11 (CL-K1) to the complement field, we could use the phrase MBL/ficolin/collectin-11 associated serine proteases or in daily use just MASPs or MAPs for the non-serine protease versions. There may be more to come, but then we have a set of abbreviations to work with at least in the human situation.

I am quite sure that others in the field will have other strong opinions than we have here at my laboratory in Copenhagen, but at least it is an attempt to link genes and proteins together and also to make everything more transparent.

NOMENCLATURE FOR THE COMPONENTS OF THE LECTIN COMPLEMENT PATHWAY- IV

Nomenclature of the proteins of the lectin pathway

Tina Hummelshøj, Department of Clinical Immunology-7631, Rigshospitalet Blegdamsvej 9, 2100 Copenhagen Ø DENMARK

During the 1990s, the MASPs, MBL and Ficolin proteins were given their names that are used today. At that time the MASPs (MBL-associated serine proteases) were only found associated to the MBL (mannose-binding lectin). However, further research in recent years has demonstrated association of the MASPs to not only MBL, but also to other proteins such as the ficolins. The members of the ficolin family have been given many different names since their discovery and transcript variants of both the *MASPs* and *FCNs* genes have been identified making the nomenclature of the proteins even more confusing.

THE MASPs

Regarding the *MASPs*, two *MASPs* genes are identified: *MASP1* and *MASP2*.

As a result of alternative splicing, the *MASP1* gene produces two different serine proteases named "Mannan-binding lectin-associated serine protease 1" and "Mannan-binding lectin-associated serine protease 3" abbreviated MASP-1 and MASP-3, respectively. If the transcript is not spliced, the mRNA is translated into the "Mannan-binding lectin associated protein". This name is abbreviated to MAP-1 or MAp44 and this protein variant does not contain any serine protease domain.

The *MASP2* gene also generates a serine protease named "Mannan-binding lectin-associated serine protease 2" abbreviated MASP-2. Furthermore, a transcript variant without the serine protease domain is named "small MBL-associated protein" abbreviated sMAP or MAp-19.

In order to get a uniform nomenclature of all the transcripts of the MASPs, I suggest using the name "MASP" if the protein contains a serine protease domain, and "MAP" if the protein does not contain a serine protease domain. The serine proteases should thereby be named "MASP-1", "MASP-2" and "MASP-3" so that the abbreviation would become the unique protein name without having to spell it out each time. Possible new transcript variants containing other serine protease domain could be called "MASP-4" etc. keeping a simple chronological order of the protein names.

The protein name of the alternative transcript variant of *MASPs* genes without serine protease activity should thereby be "MAP-1" (related to the *MASP1* gene) and the former sMAP/Map19 should be named "MAP-2" (related to the *MASP2* gene). Thereby, the transcript variants correlate directly to their respective genes. If further transcript variants without the serine protease domain are found, they could be names "MAP-3" etc. keeping a simple chronological order of the protein names.

THE MBL

The *MBL2* gene encodes the human mannose binding lectin (MBL), also named mannose- or mannan-binding protein (MBP). However, MBL does not only bind mannose and mannan. Several other substances have been described as ligands for MBL. In human, the *MBL1* gene is a pseudogene and is not found as a protein and *MBL2* is thereby the only active *MBL* gene in human. I will suggest using the abbreviation "MBL" in the future as a unique name for the protein. Even though the correct protein name should have been "MBL-2", the "MBL" nomenclature has been well implemented in the literature and I think it will make unnecessary confusion to change this name.

THE FICOLINS

The protein-based nomenclature of the ficolins is also quite confusing where each of the three human ficolins have different names related to chronological numbering, expression, molecular weight or origin of discovery.

M-ficolin is named so because of its presence on monocytic surfaces, however this molecule is more abundant on granulocytes and is furthermore expressed in lung. L-ficolin was called so, since its gene is expressed in the liver. However, Ficolin-3 (or H-ficolin) is even higher expressed in the liver as well as the lung. H-ficolin was called so, since it was described as an autoantigen found in a patient from the city Hakata in Japan. Several other names for the ficolin proteins have also been suggested but these have not been used the latest years.

This protein nomenclature of the ficolins does not reflect to the genomic nomenclature described in many genome databases. All the major gene browsers such as Ensembl, NCBI and UCSC as well as most published papers agree to use the gene terms *FCN1*, *FCN2* and *FCN3*. I think that it is more straightforward to use the protein term Ficolin-1, Ficolin-2 and Ficolin-3 that directly relates to the gene names, instead of the alternative names, M-Ficolin, L-Ficolin and H-Ficolin. Supporting this suggestion, all the main gene browsers use the protein terms Ficolin-1, Ficolin-2 and Ficolin-3 for the respective gene names.

Furthermore, most orthologues proteins (except the rodents and pigs) are named Ficolin-1, Ficolin-2 and Ficolin-3. Apparently, the rodents seem to have a different ficolin profile than other mammals. They do not have any active *FCN3* gene (called *FCNc* in mice) and they may not

be any orthologous sequence to the human *FCN2*. Instead the mice have a unique *FCNA* gene and a *FCNB* gene that corresponds to the human *FCN1* gene. Therefore, the nomenclature of the rodent ficolins should be maintained as Ficolin-A and Ficolin-B since their ficolin profile are different compared to human and other mammals.

Additionally, three splicing variants of *FCN2* and two of *FCN3* have been described. Whether these transcript variants generated active proteins still remains to be investigated. However, identification of active transcripts variants of the ficolins would require naming. These may be named e.g. "Ficolin-2-V2" etc.

In conclusion, I suggest using the term "MASPs" for the family of *MASPs* derived serine proteases and "MAPs" for the non-serine proteases. MBL as well as the MASPs should be used as independent names and the ficolins should be named Ficolin-1, Ficolin-2 and Ficolin-3, corresponding to the actually gene names. This nomenclature accommodates genes and alternative transcripts and it offers the flexibility needed to incorporate additional molecules (see table below).

NOMENCLATURE OF THE PROTEINS OF THE LECTIN PATWAY			
Gene Name	**Suggested protein name***	**Transcript name**	**Alternative names**
MASP1	MASP-1	*MASP1_v1*	Mannan-binding lectin serine peptidase 1 isoform 1, Complement-activating component of Ra-reactive factor, CRARF, C4/C2 activating component of Ra-reactive factor, Mannan-binding lectin associated serine protease 1
MASP1	MASP-3	*MASP1_v2*	Mannan-binding lectin serine peptidase 1 isoform 2, Mannan-binding lectin associated serine protease 3
MASP1	MAP-1	*MASP1_v3*	Mannan-binding lectin serine peptidase 1 isoform 3, Mannose-binding lectin/ficolin-associated protein, MAP44
MASP2	MASP-2	*MASP2_v1*	Mannan-binding lectin serine peptidase 2 transcript variant 1, Mannan-binding lectin associated serine protease 2
MASP2	MAP-2	*MASP2_v2*	Mannan-binding lectin serine peptidase 2 transcript variant 2, MAP19, small mannan-binding lectin associated protein (sMAP)
MBL1	No protein (Pseudogene)	*MBL_v1*	
MBL2	MBL	*MBL_v2*	Mannose binding lectin, mannose binding protein, mannan-binding protein (MBP)

FCN1	Ficolin-1	*FCN1_v1*	M-ficolin, Collagen/fibrinogen domain containing lectin 1, P35 like protein
FCN2	Ficolin-2	*FCN2_v1*	L-ficolin, Collagen/fibrinogen domain containing lectin 2, Hucolin, P35, Opsonins p35, Elastin binding protein 37 (EBP-37)
FCN3	Ficolin-3	*FCN3_v1*	H-ficolin, Collagen/fibrinogen domain containing lectin 3, Hakata antigen, Thermolabile beta-2-macroglycoprotein, Thermolabile substance, HAKA1

* This name should be used as an independent name without the need for spelling it out.

NOMENCLATURE FOR THE COMPONENTS OF THE LECTIN COMPLEMENT PATHWAY- IV

Proposed nomenclature for components of the Lectin Pathway of Complement Activation

Malcolm W Turner, Emeritus Professor of Molecular Immunology Institute of Child Health University College London

Regarding the serum lectin now designated "MBL" by almost all immunologists it is important to recognise that the initial isolation and structural characterization studies were undertaken by biochemists who named it mannose-binding protein and consequently used the acronym "MBP". Indeed, I myself used this nomenclature for several years (1989-1996). However, functional studies increasingly showed that the protein was of major immunological significance and there was considerable scope for confusion since the term MBP was already widely used to denote two other immunologically relevant moieties ie. myelin basic protein and major basic protein of eosinophils. In addition molecular biologists also abbreviate maltose-binding protein to MBP.

To avoid the above confusion others and I began to advocate the use of "MBL" from about 1996 onwards (see box in Turner, MW (1996) Mannose-binding lectin: the pluripotent molecule of the innate immune system. Immunology Today 17: 532-540).

I have usually referred to the protein as "Mannose-binding lectin" but Jensenius has always preferred to call it "Mannan-binding lectin" since the early binding studies by Kawasaki made use of mannan as a substrate. In fact the sugar specificity of the binding is much wider and I would be content to see both "Mannose-binding lectin" and Mannan-binding lectin" approved for usage. Clearly the acronym would be the same for both.

For the above reasons I would strongly argue that MBP should not be approved as a currently acceptable alternative acronym by the International Complement Society – although a footnote to any Table could certainly be used to indicate that MBL was previously referred to as MBP.

Other points:

1) Some have argued that the serum collectins might be designated by a series of numbers eg. Collectin 1, Collectin 2 etc in the order of discovery. However this has never been widely supported and would make inter-species comparisons difficult eg. conglutinin would be the bovine Collectin 1 but has no human counterpart.
2) Several publications have now appeared in which recombinant human and rat MBL have been studied. The ICS committee should consider whether it wishes to make any recommendations in this area such as the usage of "rhMBL" to designate recombinant human MBL.

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