able to mount an active response to the threat.

In the end, Metchnikoff's theory carried the day, and all other competing concepts became only ancillary contributors to one or another special case of inflammation. This "crazy outsider", an embryologist who could not even find a job with the German pathologists, would be proven not so crazy after all and would win a Nobel Prize in the bargain.

Finally, there are the remarkable heuristic benefits that may stem from such scientific controversies. This is especially true when, as so often happens, both sides are partly right; the two blind men of the old allegory, one holding a leg and the other a tail, are both describing different parts of the same elephant. In the battle between the Metchnikovian cellularists and the (mostly German) humoralists [6], each scanned the monthly literature to see what the opposition was up to, and each carefully designed experiments to nurture its own cause and to cast doubt on that of the other side. The result was that novel experiments were performed and new concepts advanced that would otherwise not have been stimulated—at least not so rapidly. Indeed, it is interesting (and instructive) that each side chose a substrate favorable to its own theory; Metchnikoff did much with anthrax (in which phagocytosis is prominent), whereas the Germans favored experiments with diphtheria and tetanus organisms (where the disease is prevented or neutralized by humoral antitoxins). But, both sides made significant contributions to more than one scientific discipline; thus, does science advance.

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Editorial: **The double life of M-ficolin: what functions when circulating in serum and tethered to leukocyte surfaces?**

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Ficolins are lectin-like proteins that are members of the soluble-defense collagens family, which are part of the arsenal used by the host innate immune system to establish a first line of antimicrobial defense. Human L- and H-ficolins are serum PRRs that share with MBL the ability to trigger complement activation through association to MASP-2 and facilitate phagocytosis of opsonized targets. The paper by Kjaer et al. [1] in this issue of the *Journal of Leukocyte Biology* focuses on the third human ficolin, M-ficolin, which has raised renewed interest in recent years, lending further credence to its special status within the defense collagens family.

M-ficolin was initially shown to localize at the cell surface of circulating monocytes (hence, its name). Later studies revealed its presence in secretory vesicles/granules of peripheral blood monocytes and granulocytes and its association with the cell surface after neutrophil activation [2, 3]. Given that the protein sequence contains no transmembrane or membrane anchor domain, it appears plausible that it binds to yet-unknown membrane constituents. During the past years, expression of recombinant M-ficolin allowed characterization of its recognition specificity for acetylated ligands and revealed a marked preference for N-acetylneuraminic or sialic acid, a property not shared with L- and H-ficolins. In addition, recombinant M-ficolin, bound to immobilized acetylated albumin, was shown to trigger activation of the lectin pathway, although less efficiently than L- and H-ficolins [4]. However, we observed no significant difference in the affinity constants for binding of MASP-2 to each of the three recombinant ficolins (unpublished results). This probably means that the complement-activating efficiency depends mainly on the binding strength of M-ficolin for its targets. Recently, independent studies by the authors' group [5] and Honoré et al. [6] characterized M-ficolin in serum, thus raising the possibility of it being an authentic pattern recognition molecule. However, nothing was known until now

Abbreviations: CR=collectin receptor, MASP-2=mannan-binding lectin-associated protease-2, MBL=mannan-binding lectin, PTX3=long pentraxin-3

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about the microbial ligands of serum M-ficolin and whether they would be able to trigger M-ficolin-dependent activation of the lectin complement pathway.

Kjaer et al. [1] show here for the first time that human serum M-ficolin binds to capsulated isolates of a pathogenic bacterium, namely Group B Streptococcus, and identify sialic acid as the bacterial ligand. Interestingly, this pathogen is recognized neither by L- and H-ficolins nor by MBL. Moreover, they demonstrate that binding of M-ficolin to the bacteria triggers complement activation, which strongly suggests that serum M-ficolin acts as a soluble PRR similar to L-ficolin and MBL. This clearly opens the way to the search for other pathogenic bacteria, fungi, or parasites that express sialic acids on their surfaces [7] as potential M-ficolin targets. The possible collaboration of serum M-ficolin with other soluble PRRs, such as PTXs (PTX3, C-reactive protein, serum amyloid protein), should also be considered, as described recently in the case of L-ficolin and MBL [8, 9].

In the second part of the study, the authors quantified the total contents of M-ficolin in monocytes and granulocytes and evaluated its surface expression from the amount released by N-acetylglucosamine treatment of the cells. Comparable expression on both cell types was observed, corresponding to ~7% and 14% of the M-ficolin content of monocytes and granulocytes, respectively, whereas no significant M-ficolin was found at the lymphocyte surface, confirming previous observations. The total content in neutrophils was estimated to 330 ng/10^6 cells, a much larger value than previous estimates (10–30 ng/10^6 cells) [10].

What comes next? The most puzzling issue regarding cell-bound M-ficolin resides in the fact that it is tethered to cell surfaces through binding of its pattern recognition domain to sialic acid, a typical marker of healthy cells [10]. This precludes the possibility that the novel role described for serum M-ficolin applies to its cell-associated counterpart, as unwarranted complement activation would have dramatic consequences for host cells. An equally hazardous outcome would be the consequences of

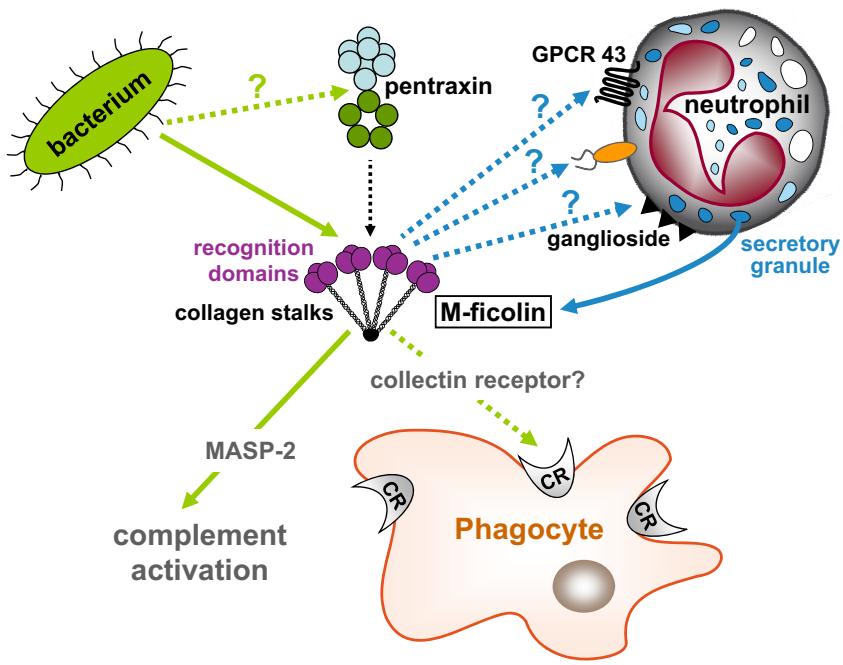

Figure 1. Sensor and effector mechanisms of M-ficolin. Serum M-ficolin senses bacteria through its globular recognition domains, directly or possibly through other soluble immune proteins such as PTXs. This multivalent binding initiates the complement cascade through activation of the collagen stalks-associated protease MASP-2. M-ficolin may also act as a bridging molecule through interaction with CRs, thus facilitating opsonophagocytosis of its targets. In addition, M-ficolin is localized in secretory granules of leukocytes and monocytes and appears as a marker of these cells by binding to the sialylated moieties of yet-unidentified cell surface ligands (as illustrated in the neutrophil case). The physiological role of cell-attached M-ficolin remains to be determined.

opsonization of healthy cells by M-ficolin, favoring their uptake by phagocytes through interaction of the ficolin collagen stalks with complement receptors. The homology of M-ficolin with the other members of the defense collagens indeed strongly suggests that it likely interacts with CRs, such as CD91 and/or calreticulin, although this remains to be demonstrated.

It should also be kept in mind that a common hallmark of the soluble defense collagens resides in their capacity to interact with and enhance phagocytosis of apoptotic cells, independently of their ability to activate complement, as shown for C1q, MBL, L- and H-ficolins, as well as pulmonary surfactant proteins SP-A and SP-D. Should soluble M-ficolin recognize apoptotic cells, their recognition would still differ from what is observed with L- or H-ficolins, as sialic acid is a marker of healthy self-cells known to decrease at the surface of apoptotic cells. In this respect, it will be of interest to analyze the fate of endogenous M-ficolin during neutrophil apoptosis, which is an essential step in the resolution of inflammation. Notably, other soluble proteins secreted by neutrophil granules and released upon stimulation, such as PTX3 and proteinase 3, have been shown to translocate to the surface of apoptotic neutrophils and proposed to serve as "eat-me" and "don't-eat-me" signals for their phagocytosis, respectively [11, 12]. It would also be worthwhile investigating the possibility that M-ficolin associates with apoptotic cell-bound PTX3, given its recently reported capacity to interact with sialylated glycans of PTX3 [13].

Undoubtedly, the key to decipher the biological functions of cell-bound M-ficolin resides in the identification of cell-surface partners, which may be lipid or protein membrane constituents. Gangliosides are possible glycolipid candidates, considering the recognition specificity of M-ficolin. Indeed, the recombi-

nant protein recognizes sialylated glycans typical of mammalian gangliosides, known constituents of membrane lipid microdomains. This hypothesis needs to be investigated on resting, activated, and apopoptotic neutrophils. Regarding the search for membrane-anchored partner proteins, a recent study identified GPCR43 as the M-ficolin cognate receptor on monocytes using yeast two-hybrid library screening [14]. This receptor for short-chain fatty acids, also present at the surface of neutrophils, actually interacted with the recognition domain of M-ficolin. However, M-ficolin did not bind to the region containing the two N-linked carbohydrates of GPCR43. Furthermore, the fact that the receptor also bound L-ficolin casts doubt on its identification as a specific ligand of M-ficolin. It can be anticipated that M-ficolin does not bind to a unique receptor and that different partners will be identified depending on the state of neutrophils (resting, stimulated, apoptotic) and on the cell type (neutrophils vs. monocytes). The nature of the cellular partners of M-ficolin will undoubtedly serve as a basis for the discovery of novel physiological functions of M-ficolin in modulation of the immune response.

A future challenge to further understand the biological functions of M-ficolin will depend on the availability of gene-targeted animal models. Homologues of M-ficolin have been characterized in rodents (ficolin B) and in pig (ficolin β), and they have not been detected yet in serum. Murine ficolin-B has been localized in lysosomes of activated macrophages, whereas porcine ficolin β is expressed and secreted by neutrophils. Interestingly, mouse ficolin B is unable to activate complement [15], and the sequence of porcine ficolin β lacks a conserved lysine residue of the collagen-like region that is required for association with MASP-2. This reinforces the idea that cell-associated human ficolin exerts yet-unknown functions, independently of its role in serum as a complement-activating PRR (**Fig. 1**). Finally, the role of polymorphisms of the M-ficolin gene in the predisposition to infectious or autoimmune pathologies will clearly need further investigation.

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