Image: R. Kidmose

Structural immunology in Aarhus, Denmark

Complemental Structure

Crystals of the MASP2·C4 complex

The complement system is named after its ability to complement antibodies and phagocytic cells in their quest to dispose of unwanted cells. But how does it switch from inactive 'lazy bum' to powerful executioner? Gregers Rom Andersen and his team from Aarhus University have some structural answers.

When we talk about our immune system, most of us will think immediately of T-cells and B-cells, the acquired immune system. But that's not all our body has in store for those nasty little bugs that try to take control of us. The pathogens first have to get past the innate immune system. "Detection of molecular patterns associated with danger and pathogens, is an essential mechanism of innate immunity. One tool that humans and other animals have for this is the complement system," says Gregers Rom Andersen, Associate Professor of the Centre for Structural Biology at the University of Aarhus.

Danger detection

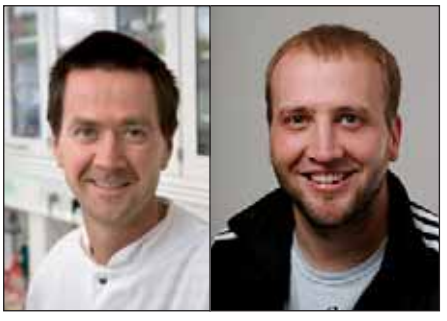
At its core, the complement system is a collection of proteins that circulate in the bloodstream as inactive precursors. Upon stimulation, say by a pathogen, specific proteases cleave these precursors to initiate a well-coordinated amplifying cascade, which culminates in the creation of pores in the target pathogen, leading to its death and ultimate clearing from our body.

Andersen is most interested in the early events in this cascade, particularly in how the complement system is activated. It is known that pattern detection can take place via three different pathways. "In two of these pathways, one of the first things to happen is the cleavage of the protein C4," he tells us. In a recently published paper, Andersen, together with his PhD student Rune Kidmose, and others managed to crystalise the C4 protein, also in complex with its cleaving partner (*PNAS*, 18;109(38):15425-30).

"Our structural work shows in detail how C4 is cleaved by the proteolytic enzyme MASP-2 in one of these pathways that recognise glycans on pathogens. Based on our functional work, we believe that C4 is cleaved in almost the same fashion in the second pathway, which is typically dependent on antibody-antigen complexes," he elaborates. Interestingly, not only do these findings provide, "an essential structural framework" of these pathways of complement activation, they also possess therapeutic significance. Uncontrolled complement activation can lead to myocardial infarction, rheumatoid arthritis and sepsis, among other conditions; learning how to inhibit proteases like MASP-2 is hence of great value.

The birth of an idea

The decision to embark upon this scientific journey was taken at a conference in New York. Kidmose reminisces, "Nick



Complementing each other's work: Gregers Andersen (l) and Rune Kidmose (r)

Laursen [back then a PhD student in Andersen's lab] and I had already decided to begin work on C4 and we also had a couple of ideas as to what we could do besides solving the structure of C4 alone. But all that changed when we attended an excellent talk by Robert N. Pike from Monash University, Australia. There, he basically identified the minimal requirements for complex formation between MASP-2 and C4, and showed that they had high affinity for each other. And, just like that, the C4·MASP-2 project was born."

"We knew if we succeeded with the project, it would give us vast amounts of information concerning how C4 is recognised specifically by MASP-2 and how MASP-2 facilitates cleavage of one specific peptide bond in C4. The project was easily pitched to Gregers, our common supervisor and he quickly established collaboration with leading experts in the MASP-2 field in Hungary and Denmark," he adds.

Working without windows

It would be two years before Kidmose and Laursen solved the structures of C4, alone and in association with the MASP-2 protease. Long hours were spent optimising C4 crystals and testing them in France and Sweden on synchrotrons producing intense X-ray radiation. "Each time we improved the quality of the diffraction obtained from our crystals, not major improvements but enough to maintain the incentive to keep on optimising and testing," he recalls.

This hard work would fade in comparison to what was to follow: the construction of detailed atomic models. "The model building was mainly carried out sitting alone for months in a windowless graphics room basement in a building the depart-

ment had just vacated," describes Kidmose. Ironically, the graphics room was moved to a brand new office (with windows) at almost the same time that the duo had finished their structure!

Nightmare before crystals

Obtaining the first batch of C4·MASP-2 crystals was quite easy but reproducing them was a bit of a nightmare. Kidmose explains, "Upon initial screening of crystallisation conditions for C4·MASP-2, we identified a lot of promising crystals. And after only one round of optimisation, we had one single drop with a handful of extremely beautiful single crystals of a fairly good size. When our Hungarian collaborators informed us that MASP-2 alone crystallised fairly easy and even at extremely low concentrations (<1 mg/ml), we assumed that our beautiful crystals were composed of MASP-2 alone. Therefore, we didn't proceed with reproducing the crystals until we had tested them with X-rays."

It was at this point that the scientists ran into some bad luck. "During transportation we lost the largest of the five useful crystals, something that otherwise rarely happens to us. But we didn't think much of it because we were pretty sure it was just the MASP-2 alone. However, when we collected a full X-ray diffraction data set, we realised that the unit cell of the crystal was too big to only accommodate MASP-2," says Kidmose. Even though this data set was sufficient to determine the structure of the C4·MASP-2 complex, they decided to attempt to reproduce the crystals to obtain even better diffraction data.

"We were quite confident that the reproduction would be a walk in the park, considering how easily we got the first crystals. Well it wasn't, actually. Quite the contrary. Numerous C4 purification runs and optimisation trials later, we were just beginning to see crystals of the same quality that were far from the same size. As of this day, we still don't know what the decisive factor was but one thing was clear, we had been extremely lucky with our first crystallisation trials," he recounts.

Putting the puzzle together

For Rune Kidmose, the most significant part of any crystal structure is being able to explain previously published data and observations, in combination with making novel observations that lead to new theories or models. And their team has managed to achieve just that. "We were able to identify two MASP-2 exosites – contact points between the protease and the substrate not occurring at the catalytic site – and substantiate them with new functional data, together with prior published functional data. Furthermore, the structure of C4·MASP-2 was the last piece needed in understanding at the structural level, how glycans on a pathogen result in the cleavage of C4. With the C4·MASP-2 structure, it was for the first time ever possible to put together a model of the large complement complex because crystal structures of all the constituents of the complex were now known," he concludes triumphantly.

The team now plans to focus on gaining a deeper structural understanding of complement activation via the three different pathways in light of these results, and will have more to talk about in the near future.

Kidmose reminisces about how he entered this field of research, "I first began working with complement factors during my PhD, despite the fact I have been under the supervision of Gregers Rom Andersen since my Bachelor. Prior to my PhD I had worked on protein synthesis, solving the structure of a viral RNA replicase in complex with two host proteins. As many other members in our group worked on complement, I already knew a lot about the field, so the transition to complement was smooth."

Having your cake and eating it

And how does the scientist keep himself busy outside the lab? "Acquiring a PhD in molecular biology is more than a full time job, so I don't have as much spare time to manage as I would like. But most of what I have is spent together with my girlfriend, friends, colleagues and family, though I am sure my mom would argue I don't spend enough, and my colleagues the opposite!

Furthermore, I suffer from a work-related injury; in that one of my latest interests is molecular gastronomy, a pompous name for experimental cuisine but fitting in this context. I guess my obsession with details and control, which I exploit in my everyday work, have evaded my private life and found molecular gastronomy to be an adequate substitute. With this, I get to play with and taste the chemicals, and as an added bonus I get to eat the finished product, which normally never happens in the lab," adds Kidmose humorously.

Well, we do hope that all future projects in the lab and kitchen will bear fruit, so that this scientist may have his cake and eat it too!

Latika Bhonsle