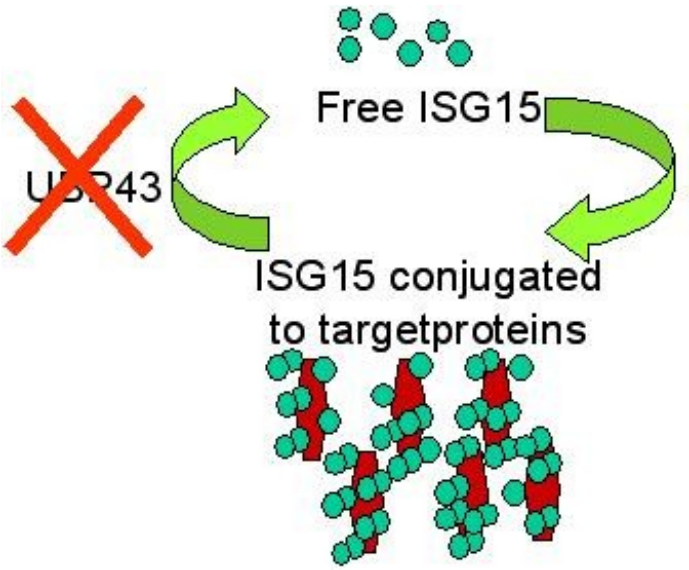
Healthcare industry BW

More than just waste removers

Cells need to get rid of misfolded proteins as quickly as possible, something that for a long time has appeared to be the major function of the enzyme ubiquitin and other similarly structured proteins. It has since become clear that ubiquitin and ubiquitin-like proteins also interfere considerably with the signalling networks of cells. Dr. Klaus-Peter Knobeloch and his colleagues at the Freiburg Neurocentre are investigating the molecular components of a ubiquitin-like system that has connections with the immune system. If parts of this structure are missing, then this can result in severe brain damage, amongst other things.

Ubiquitin is rather like a slip of paper that people moving into a new house attach to obsolete furniture that they want to get rid of. In the same way, ubiquitin labels defective proteins that are beyond repair. Ubiquitin-labelled proteins enter the proteosomes – the waste disposal units of cells – where they are shredded. But ubiquitin is capable of a lot more: some ubiquitin molecules can be covalently attached to target proteins in order to modify their function. Proteins that are similar to ubiquitin in the structure of some domains are also capable of similar actions. One such protein is the interferon-stimulated gene 15 (ISG15), which is produced by cells upon stimulation via an external interferon signal. Interferon is one of the most important alarm molecules of the human immune system and it induces cellular defence mechanisms, for example when a pathogen enters the cell. Interferon also plays a role in defending the body against viruses and tumours.

An important antagonist



The modification of proteins through ISG15 is a process that can also be reversed. However, if the mediator USP43 is missing, the reversal of this process is no longer possible.

Dr. Klaus-Peter Knobeloch and his team at the Department of Neuropathology working under the leadership of Prof. Dr. Marco Prinz of the Neurocentre at the Freiburg University Medical Centre have been focusing for some time now on research into the individual components of the ISG15 system. In contrast to many other groups of researchers, Knobeloch's team is not only focusing on the biochemical and cellular levels, but also on the entire organism. This is why the researchers use different mouse strains that are missing parts of the ISG15 system. There is a large number of molecular interaction partners because ubiquitin-like proteins are unable to conjugate to their target molecules on their own, instead the process is mediated by certain ligases. Normally, at least three different categories of these helpers are necessary in order for ubiquitin to conjugate to a protein. This is also similar for ISG15. This conjugation is a reversible process, in which ligase antagonists - the so-called deconjugases that are found in cells - can once again deconjugate ubiquitin or ISG15 from their target molecules. ISG15's antagonist is ubiquitin isopeptidase 43 (USP43), which is also activated by interferon, in the same way as all other components of the system.

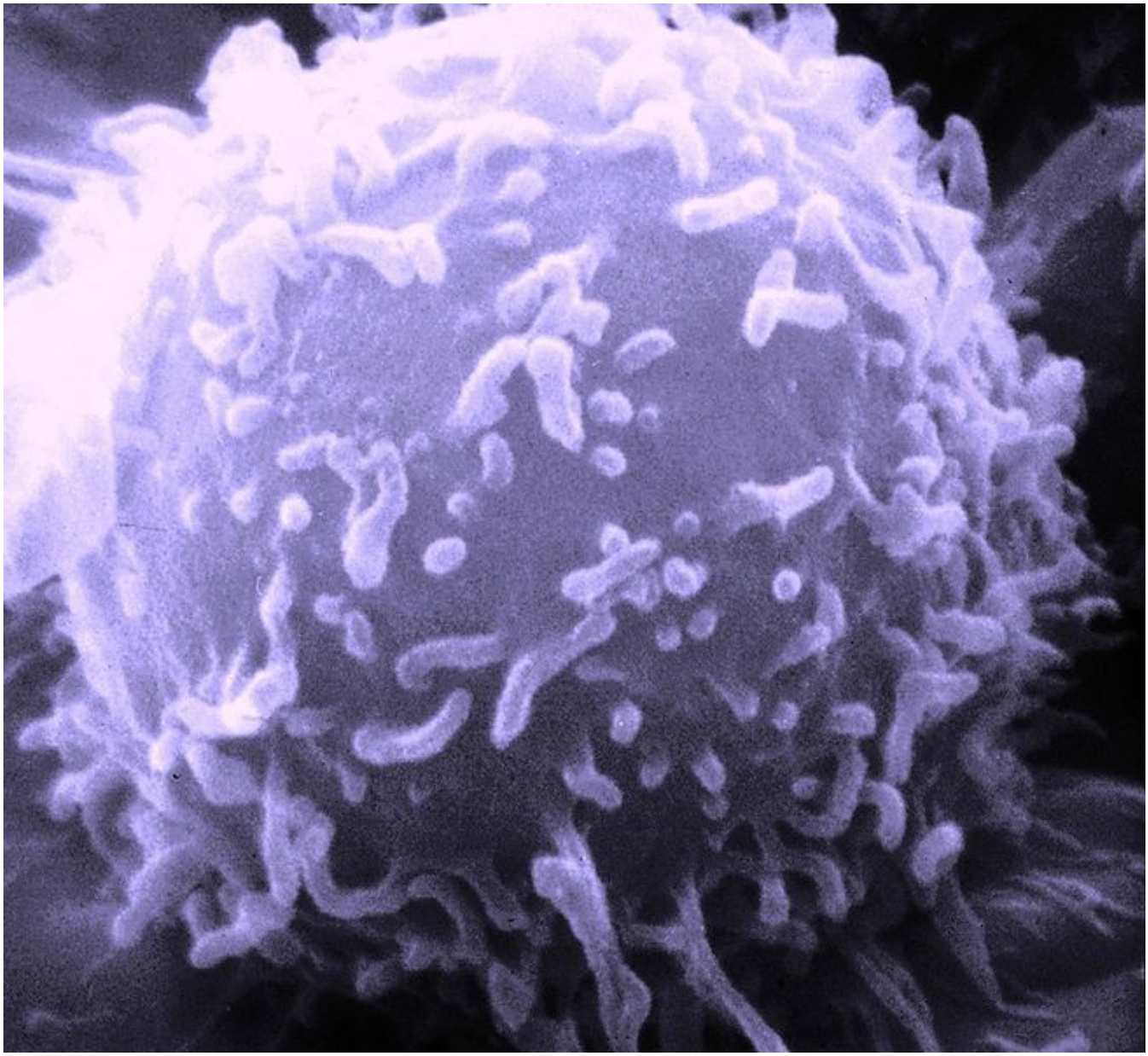
It was possible to demonstrate the key importance of USP43 in experiments involving mice that were lacking USP43. “These animals die relatively quickly,” said Knobeloch, “but before dying, they develop severe brain damage.” The most plausible explanation for this seemed to be that the damage occurred because USP43 is no longer able to fulfil its balancing function. Modified proteins accumulate in the mice because the ubiquitin-related protein ISG15 cannot be cleaved from its target.

Knobeloch and his team tested this hypothesis by breeding mouse strains which, in addition to lacking USP43, were also unable to produce ISG15. They assumed that such animals would be able to balance out the surplus of modified proteins and that the disorders would disappear. However, this was not the case at all. The mice still displayed brain anomalies and died very quickly. “This experiment shows that USP43 also has other functions that are independent from ISG15,” said Knobeloch. “But what are these functions?” Initial experiments suggested that USP43 had an important role in switching off interferon signals, thereby interfering with the molecular communication networks of immune cells and their target structures.

A role in autoimmune diseases?

Knobeloch’s team and their American cooperation partners have also been able to show that there is a link between the entire ISG15 system and the defence against viruses. They found that the lack of ISG15 reduced the ability of mice to effectively fight off influenza and herpes viruses. Future investigations will focus on the interplay of the individual proteins with the interferon system and on the cell types where this interplay is of particular importance. The team is part of a German Research Foundation (DFG) priority programme that focuses on research into ubiquitin-like proteins. The Freiburg researchers bring their know-how in the production of knock-out mice to the project.

Experiments involving a molecule that counteracts classical ubiquitin modifications also demonstrated the importance of using knock-out mice. This molecule is ubiquitin isopeptidase 8



T-lymphocyte under the scanning electron microscope: the Freiburg researchers led by Dr. Klaus-Peter Knobeloch switched off the ubiquitin isopeptidase 8 (USP8) enzyme.

(USP8), and it is able to deconjugate ubiquitin from certain target molecules. Knobeloch and his team produced conditional USP8 knock-out mice, in which they are able to switch off the molecule in certain cell types or at a specific point in time during the animals' development. For example, they switched off USP8 in the T-cells that play an important role in the immune system. Mice who carried this defect developed inflammatory intestinal diseases that were induced by the overreaction of the immune system in their own tissue. Similar intestinal diseases also occur in humans. It can therefore be assumed that a defect in this system might also play a role in the development of human autoimmune diseases. "However, potential therapies are still a long way off," said Knobeloch. "First, we need to gain a detailed understanding of many aspects." It takes around two years to create a specific knock-out mouse strain. Considering the time required to gain further insights, the cooperation between different research groups in the DFG-funded research project is certainly very advantageous.

Further information:

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