

Immune evasion in Sandnes/Norway

# A Parasite's Invisibility Cloak

Norwegian veterinary researchers identified the nature of tick-borne *Anaplasma phagocytophilum* infection in sheep: It is cyclic and persistent due to highly variable expression patterns of outer membrane proteins rendering these pathogens almost invisible for the mammalian immune system.

Each year about 300,000 lambs in Norway suffer from tick-borne infection with *Anaplasma phagocytophilum*. However, this intracellular pathogen, which belongs to the order Rickettsiales, does not only cause tick-borne fever in ruminants but can also be responsible for human anaplasmosis by infecting granulocytes. The main drawback of the disease is the subsequent suppression of the immune system after infection, which usually paves the way for secondary infections such as pneumonia, septicemia or arthritis.

Lambs may carry and suffer from the pathogens for many years. The early stadium can be detected as cytoplasmic inclusions in phagocytes, particularly neutrophils. To date, however, not much is known about how *Anaplasma* manages to persist in the animals' cells for such a length of time. Now, a group of scientists from the Norwegian School of Veterinary Medi-

cine in Sandnes and from the College of Veterinary Medicine at the University of Florida have, for the first time, been showing how the disease progresses in experimentally infected lambs (*Infection and Immunity*, 2008; vol. 76(1):120-6).

## Variations on a protein

For this purpose, PhD student Erik Granquist and his colleagues raised four lambs, two of which were infected with a specific "Norwegian" strain of *Anaplasma phagocytophilum* as determined by its typical 16S rRNA variant. The other two lambs were kept as negative controls.

From the third day on, the infected lambs ran a fever for about a week with maximum temperatures of more than 41°C. In addition, blood samples showed neutropenia for ten days and cytoplasmic inclusions in neutrophil granulocytes. Neutropenia, which means that neutrophil granulocytes are rare, represents the most severe problem for the potential immunological response. The usual amount of about 60% of all white blood cells is much lower in patients with neutropenia, leading to a suppressed inflammatory response. Thus, without "first aid" from neutrophils, the sick individuals are more prone to secondary infections.

Applying RT-PCR, Granquist and his colleagues observed a cyclic pattern over the three months where peaks of the *Anaplasma*-caused rickettsemia alternated with negative samples. Altogether, both lambs showed five clearly distinct peaks of rickettsemia.

"Former studies had already shown that *Anaplasma* may persist in hosts for many years due to the variation of outer membrane proteins (OMPs)," Granquist explains. In particular, the diversity of the major surface protein MSP2(P44) was already known to be responsible for the highly variable antigenic variation of *Anaplasma* causing the low level of immune response in sick individuals.



The vector: a tick

For this reason, the Norwegians and their US colleagues also focussed their attention on a single expression site of MSP2(P44). They analysed blood samples from the animals immediately after infection as well as every following week during the whole experimental period of three months, in order to determine the quantity and potential variations in MSP2(P44) expression.

"*Anaplasma* contains more than one hundred related genes and gene fragments encoding partial or apparently full-length outer membrane protein MSP2(P44), which are dispersed along the single chromosome," says Granquist. "These pseudogenes are considered to be inserted into the *msp2(p44)* expression site by gene combination." The group, therefore, followed previous suggestions that, in particular, segmental gene conversion in the genomic expression sites of *msp2(p44)* should be taken into account for further investigation.

## Diverse surfaces

Through their experiments with the infected lambs, Granquist *et al.* could finally prove this suggestion true: Cycling of *A. phagocytophilum* in the sheep's blood samples goes together with sequence variation in the *msp2(p44)* expression site. "This diversity of the antigen's surface very probably is the reason why the mammalian immune system cannot sufficiently attack the pathogen," Granquist concludes. "An 'unfair' competition between host and parasite, which usually the latter wins."

By RFLP analysis the veterinarians could roughly estimate the diversity of the *msp2(p44)* expression site. Interestingly, the first peak of the cycle – and one of the later ones – contained the most diverse patterns and, subsequently, the most variants of expression sites coding for outer membrane proteins. The highly variable clones were sequenced to further analyse these different patterns. The most striking variability appeared in a hypervariable region of



First authors Snorre Stuen (l.) and Erik G. Granquist (r.) together with their US colleague and last author Anthony F. Barbet in Florida.

A particular target for *Anaplasma* infection: Norwegian lambs



msp2(p44), which, for example, contained more than ten different sequence variants in the first fever peak from one of the infected animals.

In addition to the diversity, Granquist *et al.* also detected different quantities of those distinct sequences. Hence, they were also able to show that the clones could either vary enormously or be quite similar.

#### Success with a loose programme

Up until now, most experiments had been performed with *Anaplasma marginale*, which infects cattle. Infections of sheep by the related “Norwegian” strains of *Anaplasma phagocytophilum*, however, are also very common. By comparing the “Norwegian” sequences with the ones of *A. phagocytophilum* strains from the US, the scientists found very little similarity in the highly variable regions of the msp2(p44) expression site, whilst the different strains shared rather conserved sequences throughout the rest of their genomes.

Since the rickettsemias in the Norwegian study showed a very apparent cyclic nature, it would be logical to assume that

the pathogens probably escape from their hosts’ immune systems by altering their surface antigens. That way, they are able to remain unidentified by inflammatory mechanisms even at later stages of infection. Granquist confirms, “sometimes for years.”

Tick-borne pathogens may survive in the host for that long in order to be transmitted between sheep through feeding ticks. The Norwegian study now yields the suggestion that a loosely programmed order of outer membrane protein expression repeatedly makes the antigens “invisible”, since certain variants of the outer membrane expression sites sometimes occur in different individuals but at similar stadiums of infection. Interestingly, body temperature and occurrence of neutropenia did not depend on certain levels of rickettsemia during persistence of infection. That means that in the end the most dangerous period is the initial phase of infection because, as already said, this is the point at which secondary infection is most probable. “And usually these secondary infections cause much more damage than *Anaplasma* alone,” says Granquist.

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## ONE FINE DAY IN THE LAB...

BY LEONID SCHNEIDER

