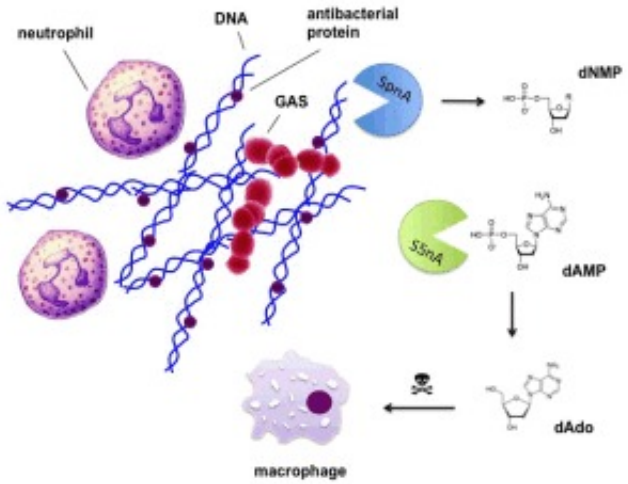
# S5nA – a novel group a streptococcal immune evasion factor

*Streptococcus pyogenes* (aka Group A Streptococcus, GAS) is a pathogenic bacterium that causes disease in humans, ranging from pharyngitis (‘strep throat’) and skin infections to severe invasive diseases, such as necrotizing fasciitis (‘flesh-eating disease) and life-threatening toxic shock syndrome. To achieve this, the bacteria produce a large arsenal of virulence factors, including molecules that interfere with the host immune response (immune evasion factors).




We have identified a novel GAS enzyme (streptococcal 5’-nucleotidase, S5nA) that generates the immunomodulatory molecule adenosine from AMP, thereby mimicking the host regulator CD73 (also a 5’-nucleotidase). A computer-generated model shows that S5nA and CD73 have a similar protein structure and a conserved catalytic site, which was further validated by mutational analysis. Adenosine interferes with the host’s immune system and suppresses a pro-inflammatory response. Furthermore, S5nA converts dAMP to deoxy-adenosine, a molecule that has previously been shown to kill macrophages (an important immune cell that removes bacteria from the body). Therefore, S5nA can be considered as a novel GAS immune evasion factor. Our study also showed that S5nA works synergistically with *Streptococcus pyogenes* nuclease A (SpnA), an enzyme with DNA-degrading activity. We have recently shown that SpnA is able to degrade neutrophil extracellular traps (NETs), a net-like structure consisting of DNA spiked with antimicrobial proteins. NETs are released by activated neutrophils (a type of white blood cell) and can trap and kill invading bacteria, including GAS. The enzymatic activity of SpnA is used to cleave the long DNA strands to prevent bacterial trapping and killing. This reaction also results in the production of the DNA building blocks (dNMPs). This includes dAMP, which can then serve as substrate for S5nA to generate the macrophage-toxic deoxy-adenosine (dAdo). This synergy provides a ‘double-hit’ to the host immune response to increase bacterial survival during infection (Fig. 1). A similar scenario has previously been shown by Olaf Schneewind and colleagues for *Staphylococcus aureus*, another important pathogenic bacterium.

In our study, we show the importance of Sn5A for bacterial survival in an in-vitro bactericidal assay

where fresh human blood was mixed with *Lactococcus lactis* and recombinant S5nA. In contrast to GAS, *L. lactis* is a harmless food-grade bacterium that gets quickly killed when incubated in human blood due to immune mechanisms. We showed that addition of recombinant S5nA protein significantly increases survival of *L. lactis* in human blood (p less than 0.001) and this effect was not observed when S5nAN99A (an inactive catalytic site mutant) was added. These results demonstrate that S5nA facilitates immune evasion due to its enzymatic activity and the production of immunomodulatory molecules.

Finally, our study shows that antibodies against S5nA can be found at higher frequency and titer in serum samples from GAS disease patients compared to healthy donor samples indicating the production of the enzyme during infection. Therefore, S5nA might be a suitable target for vaccine or drug development.

***Thomas Proft***
*School of Medical Sciences, The University of Auckland,*
*Auckland, New Zealand*

## Publication

Streptococcal 5'-Nucleotidase A (S5nA), a Novel Streptococcus pyogenes Virulence Factor That Facilitates Immune Evasion.
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