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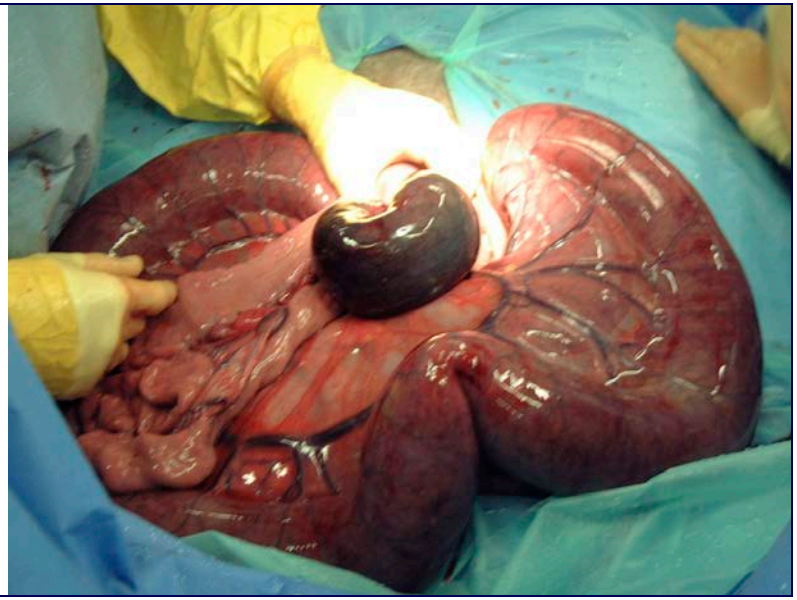
The behaviour of horse Toll-like receptors

A study designed to investigate the behaviour of specific horse immune receptors, and how we may change their activity with drugs.



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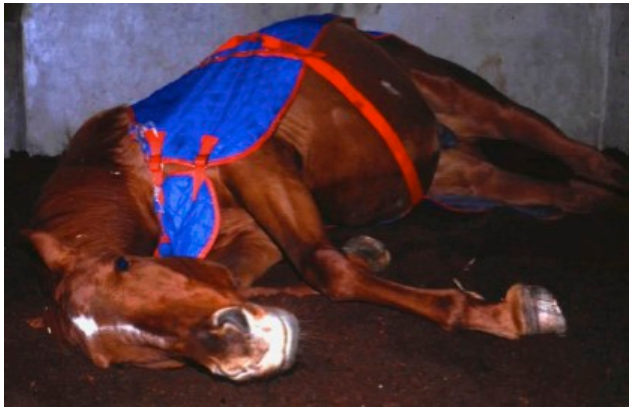
- Supervisor:Professor Claire Bryant
- University of Cambridge, Department of Veterinary Medicine

Dr Irvine was awarded a PhD for the work described in this report

Why study horse Toll receptors?

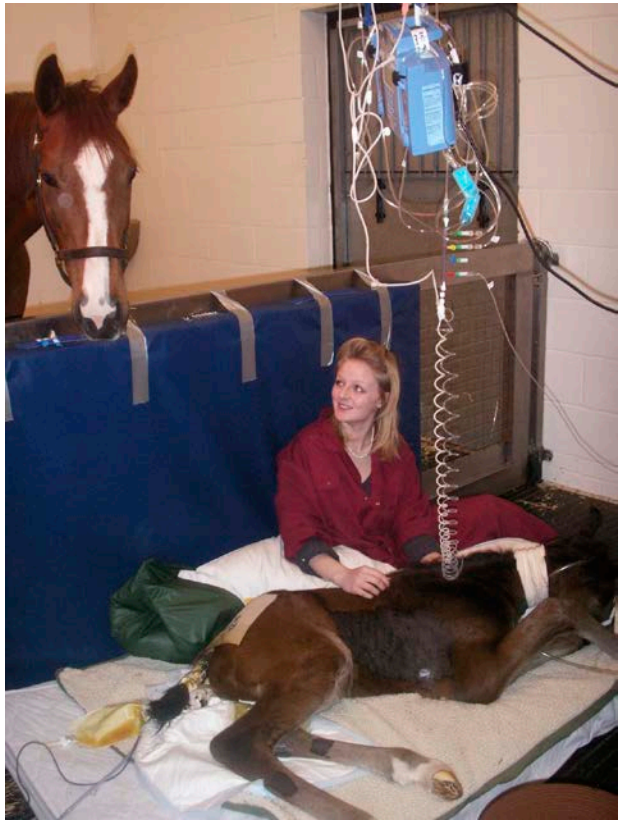
- Foals and adult horses are extremely sensitive to bacterial infection and bacterial toxins.
- Many conditions which involve bacteria entering the blood stream are fatal, for example neonatal sepsis and colic due to strangulation of the intestine.



Why study horse Toll receptors?

- Toll receptors are a critical component of the horse's natural defense mechanisms.
- When bacteria enter the blood stream, toxins released from bacteria are detected by the horse immune system by specialised proteins receptors.
- This detection is critical to fighting infection. Without it, the horse or foal would die. But, over-activation of the immune system can stimulate an inflammatory cascade, which if uncontrolled does more harm than good and can lead ultimately to organ failure.
- This process is known colloquially as blood poisoning, but its medical name is Systemic Inflammatory Response Syndrome, or SIRS.

Why study horse Toll receptors?



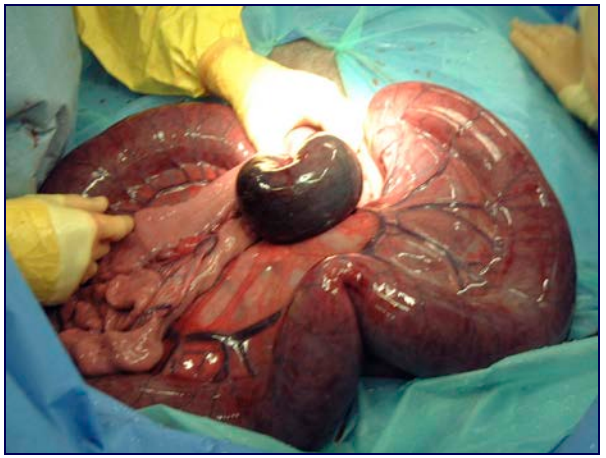
- One family of receptors involved in this bacterial detection system are the Toll-like receptors (TLRs).
- If we understood more about this important natural defense mechanism, we might be able to use drugs to support it and modify its effects to prevent activation of excessive inflammation.
- This would help prevent deaths associated with many bacterial infections like neonatal infection, colic, pneumonia, to name but a few

What was already known about horse Toll receptors?

- Toll-like receptor 4 (TLR4) is found on white blood cells and detects a specific bacterial toxin called lipopolysaccharide (LPS).
- A large number of bacteria capable of causing disease in horses carry the LPS toxin.
- Horses are more sensitive to LPS than other animals, but we do not know why this species difference exists.
- Horse TLR4 does not respond in the same way as human TLR4 to different types of LPS from different bacteria, or to different drugs.
- Human Toll-like receptor 2 (TLR2) detects bacterial toxins, one of which is called lipoteichoic acid (LTA).
- To recognise toxins, Human TLR2 pairs with TLR1 or TLR6.
- Much less is known about horse TLR2 than horse TLR4 so we set out to try to find out more.

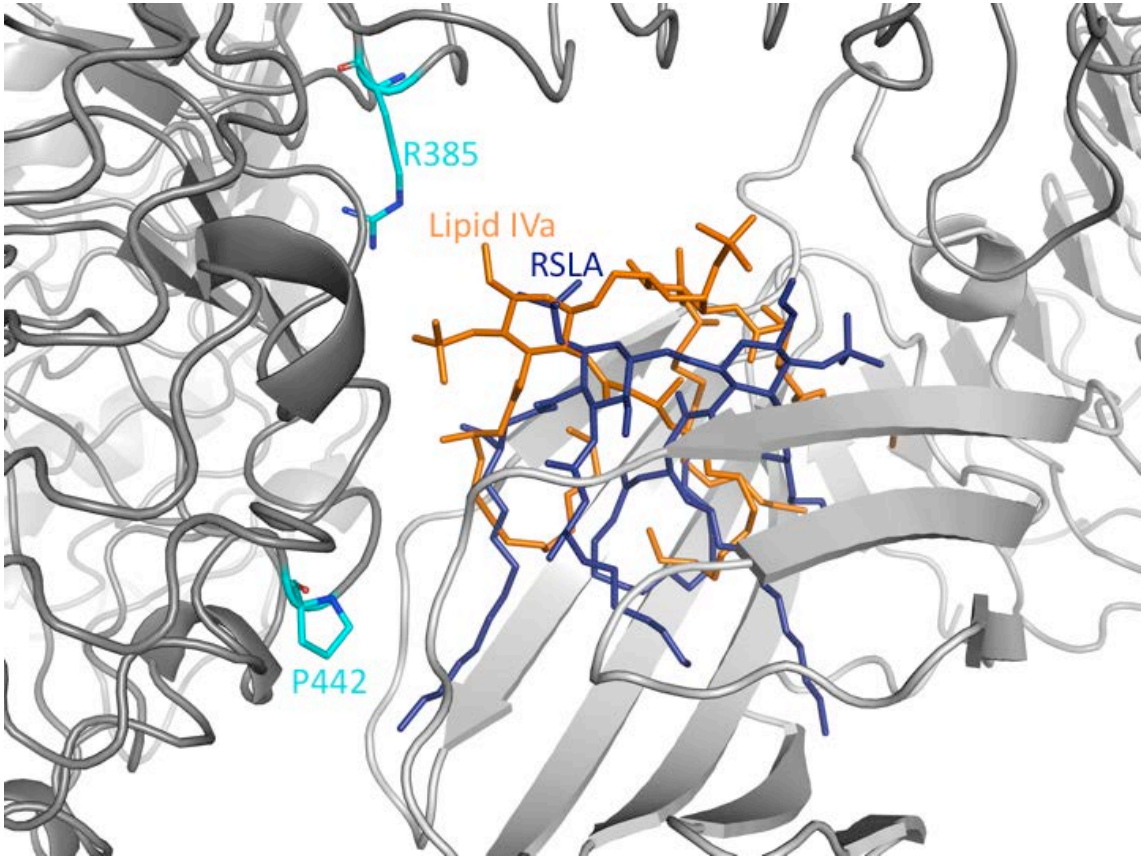
Objectives of this study

- To find out how horse TLR4 responds to a large number of synthetic and natural substances, some of which could be potential new drugs.
- To investigate why horse TLR4 is so sensitive to LPS.
- To study how horse TLR2 behaves in conjunction with horse TLR1 or TLR6.



Strangulated small intestine - aka “Twisted gut” - an example of a condition in which bacteria enter the blood stream. When the blood supply to the intestine is cut off by the twist, the gut begins to die and bacteria and their toxins are released.

Our findings: LPS activates horse and human TLR4. RSLPS, a closely related toxin, activates horse TLR4 less than LPS and does not activate human TLR4.



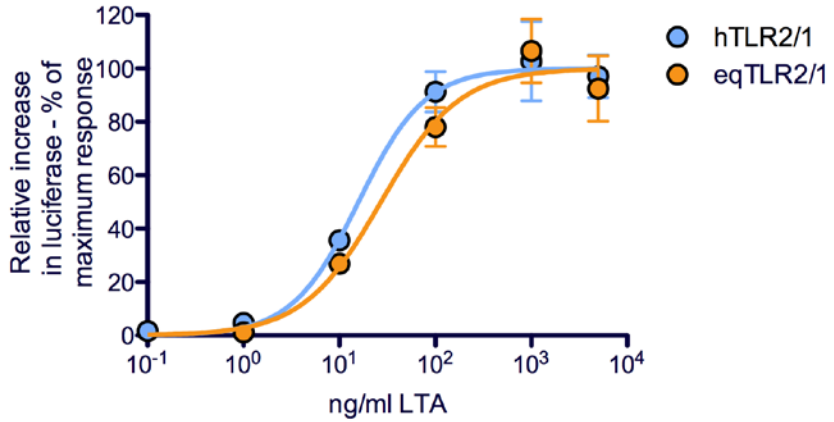
This model shows horse TLR4 recognising a part of RSLPS (in blue), called RSLA, and another toxin, called lipid Iva (in orange), in a slightly different way.

Our findings: we could change how TRL4 responded by changing its structure

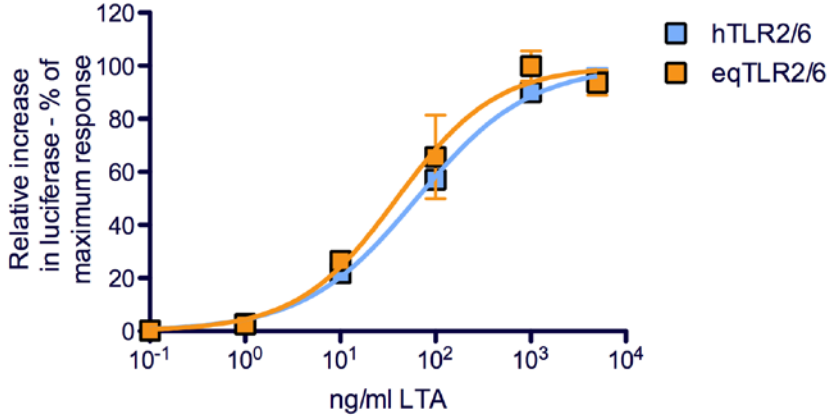
- Amino acids are the building blocks of proteins. Changing amino acids will alter the shape of the molecule, and if the protein receptor changes shape, it will interact with other molecules differently
- Changing one amino acid of horse TLR4 causes a large reduction in activation by RSLPS without affecting the response to LPS.
- The same amino acid change does not affect lipid IVa recognition.
- Changing a different amino acid in horse TLR4 also causes a reduction in activation by RSLPS without affecting the response to LPS.
- That amino acid change does not affect lipid IVa recognition.

Our findings: when different TRLs were combined we found horse and human TRLs shared some similarities in the way they reacted to bacteria

Horse and human TLR2, together with TLR1, respond to lipoteichoic acid (LTA) from the bacterium *Staphylococcus aureus* in the same way.

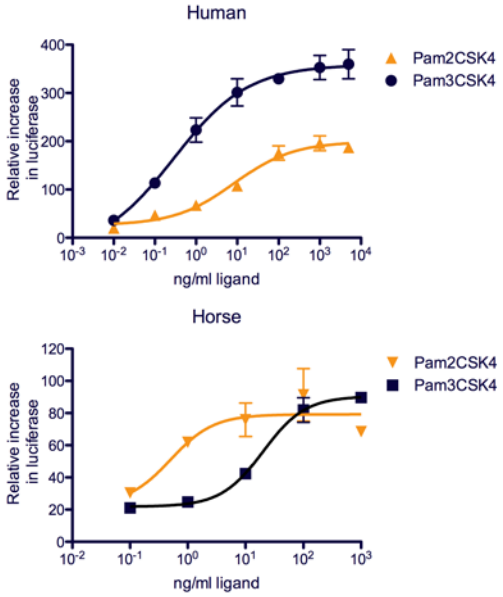


Horse and human TLR2, together with TLR6, respond to LTA from *S. aureus* in the same way also.

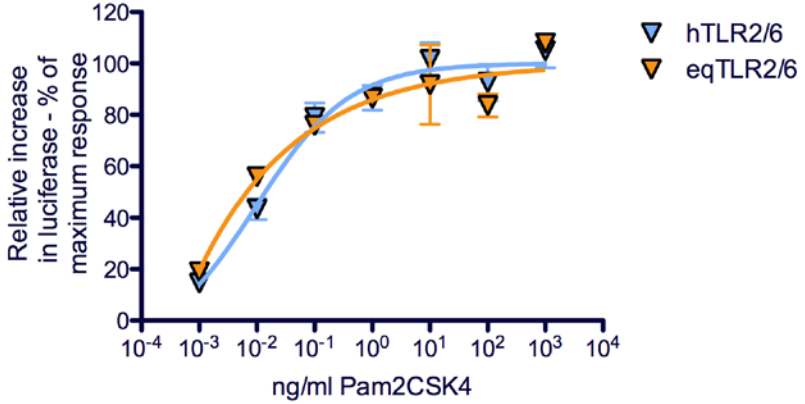


Our findings: when different TRLs were combined we found horse and human TRLs shared some similarities but had some differences in the way they responded to toxins

Horse and human TLR2+TLR1 do not recognise synthetic toxins in the same way.



Horse and human TLR2+TLR6 recognise a synthetic toxin in the same way.



Conclusions

- The structure of horse TLR4 affects how it recognises different toxins
- The toxin structure also influences how the TRL4 responds to it
- A model of horse TLR4 shows toxins with different structures interact with the receptor in different ways.
- Despite testing 24 molecules, we did not identify one which blocked TRL4
- Some of the molecules we tested, might have potential in the development of vaccines which would protect the horse from an excessive inflammatory response.
- TLR2-blocking drugs used to treat certain bacterial infections in humans may be of use for treating infections in the horse also, but drugs which act on the combination of human TLR2+TLR1 are unlikely to have the same effect in the horse.