

## Innate immunity in Dublin

# From ugly Duckling to Swan

Luke O'Neill and his group at Trinity College Dublin are unravelling the signalling pathway of Toll-like Receptor 4. Just recently, they identified a new splice variant of a TLR4 adaptor protein that shows promise as a drug target.

The 20th century saw several successful vaccine developments and smallpox was even eradicated in the 1970s. Although not all diseases can be prevented, HIV and Malaria still being areas without successful vaccines, the overall success story of vaccines has sparked an immense interest in the adaptive immune system.

At the same time, the innate immune system has spent a long time in the shadows, mostly viewed as the less interesting half of the immune system. "The innate immune system was considered to be cruder, non-specific, less sophisticated and without much scope to modulate the responses," as Luke O'Neill explains.

The task of the immune system is to fight intra- and extracellular pathogens and to eliminate them before the attack progresses to full blown disease. The innate immune system is the first line of defence in the body against invaders. It is an effective way of protecting the body because it provides an immediate response; however, there is no long-lasting or protective effect. Receptors of the innate immune system are called pattern recognition receptors (PRRs) because they are not specific to a certain molecule, like antigen receptors, but have a broad specificity. The receptors can recognise many related molecular structures called pathogen associated molecular patterns (PAMPs) that are present on a range of attacking pathogens. Examples for PAMPs are flagellin, peptidoglycan on Gram-negative bacteria and lipopolysaccharides (LPS) on Gram-positive bacteria.

### Multitasking macrophages

The innate immune system includes the anatomical barriers like the epithelial surfaces and bacterial flora on the skin and in the gut. However, if mechanical barriers like the skin are breached, inflammation sets in. This response enables the body to remove the pathogen and initiates healing of tissue at the same time. Molecules like interferon (limits virus replication) and IL-1 (induc-

es fever) are released to aid the work of the cellular components of the innate immune system that are recruited to the area of inflammation – eosinophiles and macrophages. Eosinophiles release proteins out of their granules that are able to kill certain parasites. Macrophages are not only responsible



A macrophage recognizes *E. coli* (red) flagellin ... and attacks.

for phagocytosis of pathogens but also for antigen presentation to the cells of the adaptive immune systems and tissue repair.

### The cross talks of Toll-like Receptors

Toll had been identified in the fruit fly in genetic screens for embryonic development in 1985 and was shown to contain a cytoplasmic domain similar to an IL-1 receptor domain. The signalling pathway of Toll in *Drosophila* also included a member of the NF- $\kappa$ B family. This suggested that a conserved pathway was used to determine dorsoventral polarity in *Drosophila* and inflammation in humans.

But then followed what Luke considers to be "the most important discovery in the field of innate immunity in the last 10 years." The group of Jules A. Hoffmann described in 1996 how Toll has a role in the defence against fungal pathogens (*Cell* vol. 86(6):973-83). This initiated the search for human Toll-like receptors (TLRs). "The developing field of bioinformatics was the key to identification of homologous proteins and the discovery of TLRs." The first one was described only a year later and so far 13 TLRs have been found in humans,

mice and other mammals. "Interest in TLRs was so immense because such a highly conserved protein that is found in plants, fruit flies and humans must be of importance!" Luke points out.

The study of TLRs led to a renewed interest in the field of innate immunity because links and crosstalk between innate and adaptive immune systems were discovered. The innate immune system was found to be able to initiate more specific responses than originally thought.

TLRs, their adapters and the Interleukin-1 receptors form a receptor superfamily, the 'Interleukin-1 Receptor/Toll-Like Receptor Superfamily'; all members have a TIR (Toll-IL-1 receptor) domain in common. TLRs recruit adapter proteins and kinases after being activated through ligands of microbial origin to mediate downstream signalling. Four adaptors have been discovered, called MyD88, Mal (TIRAP), TRIF and TRAM. Transcription factors like interferon regulatory factor 1 and 3 (IRAK1 and 3) amplify the signal and lead to changes in gene expression that induce an inflammatory response. TLRs are expressed on the cell membrane of innate immune cells like macrophages, cells of the adaptive immune system like T-cells and on non-immune cells like epithelial cells. That is why the effects of activation of TLRs are spread across the innate and adaptive immune system and provide a link between the innate and adaptive immune system.

### "It's good to share"

Luke O'Neill started off as a post-doc in Cambridge on IL-1 and established his own lab at Trinity College Dublin in the School of Biochemistry and Immunology in 1992. Here, he worked on IL-1, NF $\kappa$ B and since the late nineties on TLRs. "The research atmosphere is really productive and a lot of undergraduates are coming through. We have plenty of funding as well, which is a big bonus in these times. We are working in joint projects, in a Strategic Research Cluster funded by the Science Foundation Ire-

land.” Luke enjoys not only the research but also lecturing, “It is a good thing to be able to inspire students, to share your knowledge with them. You should never forget that they are the next generation of researchers!”

### Boosting the ‘right guys’

Luke’s aim is to unravel the signalling pathway of TLR4. “It was exciting to find the adaptor molecule Mal. Then progressively, we found more complexity in the system than originally expected, and many more components.” TLR4 was identified as the receptor for LPS but heat shock proteins and other molecules can also trigger its activation. TLR4 is able to signal from two distinct places in the cell. TLR4 is present on the plasma membrane and, if the ligand LPS activates it, TLR4 recruits Mal and MyD88. This triggers the activation of the transcription factor NF- $\kappa$ B and results in host defence and inflammation. TLR4 then traffics to the early endosome where TRAM engages with TLR4. TRIF is recruited and activates interferon regulatory factor 3 (IRF3) at the end of the cascade.

Luke’s group has recently published a splice variant of the adaptor TRAM which is called TAG and modulates TLR4 signalling

hibited, the immune stimulation is boosted but not the inflammation because TAG only inhibits one arm of the pathway.

This is a very elegant approach but as Luke points out, “The only problem is that there is no TAG in mice. We cannot use mouse models for our experiments and rely on human tissue culture instead. The ultimate goal is to design drugs that specifically inhibit TAG and then enable an adjuvant to boost the immune response by preventing inflammation at the same time.” An adjuvant enhances the immune response to a vaccine by mimicking molecules that are typical for pathogens. The immune system is trained to recognise these patterns and becomes more strongly activated than by sole stimulation with the antigen that is the basis for the vaccine. Adjuvants are safe to use because they have no pathogenic effect themselves. TLR activation leads to adaptive immune response and that is why many adjuvants today are developed to mimic TLR ligands.

### A bit ‘Star Trek’-like

Luke sees the biggest challenge in the area of innate immunity in entangling the cross talk. “Signalling pathways are not linear, it is not that simple. If you want to un-



First and last author: Eva Palsson-McDermott and Luke O’Neill

by inhibiting TRAM dependent signalling to IRF3 (*Nat Immun* vol. 10 (6), 579-587). This, as first author Eva Palsson-McDermott found out, happens by sequestering TRAM from TRIF in the late endosome. When TLR4 is activated by a ligand, two things happen at the same time. The positive effect is the induction of a strong immune enhancing effect through the TRAM and TRIF pathway. But at the same time, a negative effect occurs, i.e. inflammation. This response is mediated by Mal and MyD88. If TAG is in-

derstand the intricacies of the TLR4 signalling for example it develops quickly into systems biology.” To study the complex interactions in biological systems, with the goal of discovering new emergent properties, is a challenging and exciting field of research. And if that is not enough to get you out of bed in the morning, Luke adds, “Nothing beats getting paid to do your hobby! It is an adventure and a bit like in Star Trek: the final frontier – to go where no man has gone before.”

ANDREA HERB