Toll-like Receptors and Inflammatory Responses

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Øystein Grimstad defended his PhD thesis at the Norwegian University of Science and Technology in Trondheim, on September 11, 2012. The title of his thesis was "Toll-like receptor-mediated inflammatory responses in keratinocytes". Main supervisor was Professor Terje Espevik. Opponents were Professor Lars Iversen, Aarhus University Hospital, Aarhus, Denmark and Senior Lecturer Caroline Jefferies,the Royal College of Surgeons, Dublin, Ireland. Dr Grimstad is presently at the University Hospital Northern Norway in Tromsø.

How do keratinocytes initiate repair mechanisms through activation of the innate immune system? We have studied Toll-like receptors (TLR), which are particularly important in innate immunity. Both foreign molecules from microbes and molecules from self can trigger inflammation through the innate immune system.

A main finding of our studies is that keratinocytes are particularly sensitive to the synthetic TLR3 ligand poly I:C. This ligand is an analog of double-stranded RNA, which can be present in some viruses. Other central TLR ligands triggered little or no release of these signaling molecules from skin cells. This discovery laid the foundation for further studies, where we tried to find out more about why cells are so sensitive to poly I:C, and how poly I:C stimulation leads to the release of signaling molecules important for the initiation of the inflammatory response.

We described how poly I:C stimulation seems almost exclusively to signal through TLR3 receptor, despite the fact that there are other potential receptors and signaling mechanisms for this ligand. TLR3 stimulation resulted in a dose-dependent cell death and concomitant release of the key cytokine CXCL-8. Stimulation with poly I:C and other nucleic acids protected against both toxicity and inflammatory responses.

We also described how TLR3-mediated responses depend on caspase-4 activation. Caspase-4 is a protein involved in programmed cell death and inflammation. TLR3 stimulation of cells led to a strong activation of genes associated with inflammation processes, including pro-forms of the important cytokine interleukin-1β and components of other innate immune defense, such as NLRP3 and caspase-1. By blocking caspase-4, we inhibited both IL-1β release and cell death. We found caspase-4 to be upstream of caspase-1 in the IL-1β-release cascade. Specific inhibition of the caspase-4, but not caspase-1, protected against cell death. TLR3 stimulation caused a pre-

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mature activation of both the inflammatory and apoptotic caspases. The poly I:C-mediated cell death of keratinocytes thus involves mechanisms in both apoptosis and pyroptosis.

In summary, our studies demonstrate mechanisms by which keratinocytes initiate inflammatory responses through TLR3 activation.

List of papers

1. Grimstad Ø, Sandanger Ø, Ryan L, Otterdal K, Damaas JK, Pukstad B, Espevik T. Cellular sources and inducers of cytokines present in acute wound fluid. Wound Repair Regen 2011; 19: 337–347.
2. Grimstad O, Pukstad B, Stenvik J, Espevik T. Oligodeoxynucleotides inhibit Toll-like receptor 3 mediated cytotoxicity and CXCL8 release in keratinocytes. Exp Dermatol 2012; 21: 7–12.
3. Grimstad Ø, Husebye H, Espevik T. TLR3 mediates release of IL-1β and cell death in keratinocytes in a caspase-4 dependent manner. Submitted manuscript.