

## INTERVIEW about

Zinc finger protein  
Gfi1 and inflammation  
with

Tarik Möröy, Prof. Dr., President and Scientific Director, Institut de recherches cliniques de Montreal, I R C M, 110, Avenue des Pins West, Montreal, QC, H2W 1R7, Canada

Tarik.Moroy@ircm.qc.ca



## What is known about Gfi1?

**Tarik Möröy:** Gfi1 is a small nuclear protein of about 55 kD that acts as a transcriptional repressor (1,2). At its C-terminus, it bears six typical C<sub>2</sub>H<sub>2</sub> type zinc-finger domains that mediate sequence specific DNA binding but can also serve as a platform for the interaction with other proteins. At its N-terminus, Gfi1 has a 20 amino acid stretch that was named SNAG domain since it is also found in the proteins Snail and Slug which have similar repressor functions (3). When these 20 amino acids are removed, Gfi1 loses its activity as a repressor which under-

scores the importance of this small domain (3). Gfi1 exerts a number of functions in early hematopoietic differentiation and mature immune cells (2). Most strikingly, Gfi1 is required for the formation of mature granulocytes, the self renewal of hematopoietic stem cells and the proper maturation of thymic pre T-cells (4,5). Interestingly, Gfi1 is also a critical factor for the maturation and development of inner ear hair cells, a role that became apparent when the Gfi1 gene was knocked out in mice and when it was observed that the mice were deaf (6).

## How was Gfi1 originally discovered?

**Tarik Möröy:** The Gfi1 gene was discovered as a proviral insertion site in a retroviral screening experiment designed to find pathways rendering T-cells independent of IL-2 (1). A few years later, retroviral tagging experiments in transgenic mice that were predisposed for T-cell lymphoma by transgenic expression of Myc or Pim oncogenes were conducted in order to find new oncogenes with the ability to cooperate with Myc or Pim-1

(7-9). The Gfi1 gene turned out to be one of the most frequent insertion sites selected in T-cell lymphomas that arose in retrovirally infected Myc or Pim-1 transgenic mice (7-9). From these experiments, we concluded that Gfi1 has the activity of a dominant oncogene. Indeed, we could confirm this later by generating transgenic mice that over express Gfi1 in T-cells and which showed a low incidence of T-cell lymphomas (10).

## How does Gfi1 function at the molecular level?

**Tarik Möröy:** The evidence obtained so far suggests that Gfi1 as well as its closely related paralogue Gfi1b can bind to histone modifying enzymes such as histone methyl transferases (11,12) and histone deacetylases (12) and induce changes in chromatin after being recruited to target genes. In particular, Gfi1 and Gfi1b can induce the methylation of Histone H3 at lysine 9, a modification that is associated

with gene silencing. However, it is clear that Gfi1 can have other functions that are independent of DNA binding. One example is the interaction of Gfi1 with PIAS3 which is an inhibitor of STAT3 signaling. Through this interaction PIAS3 is being sequestered and STAT3 is relieved from its inhibition (13). This indirect activation of STAT3 by Gfi1 does not require a DNA binding activity of Gfi1.

## What is the role of Gfi1 in endotoxin-mediated inflammation?

**Tarik Möröy:** Gfi1 attenuates inflammatory responses induced by endotoxins or by bacterial infection. We became aware of this activity when we studied Gfi1 deficient mice and had to notice that in some cases, these animals showed symptoms of septic shock and general inflammation (4). When we looked at this phenomenon more closely, we observed that after being stimulated with lipopolysaccharide (LPS), a component of bacterial cell walls that is highly toxic, Gfi1 KO mice responded very heavily even to low doses that were still well tolerated by wild type mice (4,14). Also, when we used live bacteria, in our case *P. aeruginosa*, in low doses that have no immediate adverse effect in healthy mice, Gfi1 KO mice

succumbed very rapidly with symptoms of septic shock and general inflammation (15). We suspected an overproduction of inflammatory cytokines such as TNF or IL-1  $\beta$  to be the cause for this. Indeed, when we deleted the TNF gene in Gfi1 KO mice or when we treated Gfi1 KO mice with anti-TNF antibodies or antibodies against IL-1  $\beta$ , typical inflammatory cytokines, we could either eliminate or strongly attenuate the inflammatory reaction to both LPS or infection with *P. aeruginosa* (14,15). We concluded that Gfi1 may well be a novel negative regulator of the endotoxin mediated innate immune response, and that it acts very likely by preventing the overproduction of inflammatory cytokines.

## Have you identified the cellular sources of the expression of TNF alpha or Il-1 beta?

**Tarik Möröy:** Yes. We isolated macrophages from lung and bone marrow but also epithelial cells and fibroblasts from the lung or lymphocytes from the spleen. We found that when stimulated with LPS, only lung or bone marrow macrophages derived from Gfi1 KO mice produce significantly higher levels

of TNF or IL-1  $\beta$  than the corresponding cells from wild type mice. None of the other cell types showed this difference or did not produce any cytokine at all. We concluded that Gfi1 has a particular role in macrophages where it regulates cytokine production after these cells have encountered endotoxin (4,14).

## Do you know by which mechanism Gfi1 exerts this negative regulatory function?

**Tarik Möröy:** This is still under investigation. We know however, that in resting non-stimulated macrophages Gfi1 is not expressed but that it is very rapidly induced within minutes after contact with LPS or bacteria. Since LPS is recognized by the Toll like receptor 4, we speculate that Gfi1 is a downstream effector of this type of receptors. It will be of interest to see, whether Gfi1 is also induced by other Toll like receptor (TLR) ligands such as CpG DNA or peptidoglycans and whether it is part of the known signaling pathways that negatively regulate TLR activity for instance the pathway initiated by Phosphatidylinositol 3-Kinase. Since septic shock and an overshooting systemic inflam-

matory response are such important problems in the clinic, it will be of interest to decipher how this new negative regulator works and in which way it interferes with the often deadly effects of endotoxins.

## REFERENCES

1. Gilks et al. Mol Cell Biol 13, 1759, 1993
2. Möröy T Int J Biochem Cell Biol 37, 541, 2005
3. Zweidler-Mckay et al. Mol Cell Biol 6, 4024, 1996
4. Karsunky et al. 30, 295, 2002
5. Yücel et al. J Exp Med 197, 831, 2003
6. Wallis et al. Development 130, 221, 2003
7. Zörnig et al. Oncogene 12, 1789, 1996
8. Schmidt et al. Nucleic Acids Res 24, 2528, 1996
9. Scheijen et al. J Virol 71, 9, 1997
10. Schmidt et al. Oncogene 17, 2661, 1998
11. Duan et al. Mol Cell Biol 25, 10338, 2005
12. Vassen et al. EMBO J 25, 2409, 2006
13. Rödel et al. EMBO J 19, 5845, 2000
14. Jin et al. Eur J Immunol 36, 421, 2006
15. Grasse et al. Cell Microbiol 8, 1096, 2006