

## Evidence of Interleukin Genes in the Sea-Star: *Asterias rubens* (Echinoderma)

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### ABSTRACT

The axial organ of the sea star *Asterias rubens* is a primitive immune organ the sea star T lymphocytes, when stimulated by various lectins, produce lymphokine-like substances with mitogenic properties as shown in earlier studies; they are correlated with Interleukin genes.

**Keywords:** Interleukin Genes, Sea-Star Axial Organ, *Asterias Rubens*

### 1. INTRODUCTION

A large number of investigations performed in the last few years, in our Laboratory, have provided evidence that the sea star *Asterias rubens* (echinoderma) possesses a primitive immune system with cellular and humoral responses functionally similar to those of the immune system of vertebrates.

Non-adherent-nylon wool cells (Leclerc *et al.* (1986): Sea star T lymphocyte subpopulation) can release soluble lymphokine-like mediators, after stimulation with P.W.M sepharose 6 MB beads, with mitogenic properties (Leclerc *et al.*, 1981). Some interleukins were discovered in 1997 (Legac *et al.*, 1996) such as IL1, IL6. In the present work (C) « control » sea-stars and (HRP): Immunized sea-stars (using Horse-radish Peroxidase, as antigen) were studied to research Interleukin genes, in their genomes.

### 2. MATERIALS AND METHODS

Sea stars *Asterias rubens* were obtained from the Biology Institute (Gothenburg University). Immunizations were performed on 20 animals, in an aquarium with sea water at 10°C by using Horse-Radish Peroxydase (HRP) (Sigma Products) as antigen at a concentration of 1 mg mL<sup>-1</sup>. 20 non-injected animals were used as controls. The axial organs were removed;

RNA was extracted, using Trizol (Invitrogen) according to manufacturer instructions, from immunized sea stars (HRP) and Controls (C). cDNA was normalized using double strand specific nuclease essentially as described by Zhulidov *et al.* (2004). cDNA was fragmented using DNA Fragmentase (New England Biolabs), according to the manufacturer's instructions. After ligation of adapters for illumina's GSII sequencing system, the cDNA was sequenced on the illumina GSII platform sequencing 1x 100 bp from one side of the approximately 200 bp fragments. Sequences were assembled using Velvet (Zerbino and Birney, 2008).

### 3. RESULTS AND DISCUSSION

We describe first « interleukin genes » then « interleukin receptor genes » as compared to mammals.

#### 3.1. Control- « Nuclear Factor Interleukin-3-Regulated Protein »

The results of our BlastX (Blast Version 2.2.20, Parameters: -e 0.001 -F F -b 3 -v 3 -I T -a 16 -m 7) were the following.

One contig (NODE\_3558\_length\_451\_cov\_24.356985) could be annotated via BLASTX to mouse "Nuclear factor interleukin-3-regulated protein" from the SWISSPROT database, with an e-value of 2.87689e-10. On an aligned

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region of 62 amino acids, 42 positive and 28 identical amino acids were found.

### 3.2. HRP-« Nuclear factor interleukin-3-regulated protein »

One contig (NODE\_48893\_length\_689\_cov\_15.156749) could be annotated via BLASTX to mouse “Nuclear factor interleukin-3-regulated protein” from the SWISSPROT database, with an e-value of 1.49652e-09. On an aligned region of 57 amino acids, 40 positive and 26 identical amino acids were found.

We study now the interleukin 1 and more specially interleukin 1 receptors.

### 3.3. Control-« X-linked Interleukin-1 Receptor Accessory Protein-Like 2 »

One contig (NODE\_21299\_length\_1163\_cov\_23.764402) could be annotated via BLASTX to mouse “X-linked interleukin-1 receptor accessory protein-like 2” from the SWISSPROT database, with an e-value of 2.64154e-07. On an aligned region of 278 amino acids, 110 positive and 60 identical amino acids were found.

### 3.4. HRP-« X-linked Interleukin-1 Receptor Accessory Protein-like 2 »

One contig (NODE\_23687\_length\_300\_cov\_7.280000) could be annotated via BLASTX to mouse “X-linked interleukin-1 receptor accessory protein-like 2” from the SWISSPROT database, with an e-value of 7.2295e-05. On an aligned region of 77 amino acids, 38 positive and 27 identical amino acids were found.

### 3.5. And Now the Interleukin-1 Receptor-Associated Kinase 4

#### 3.5.1. Control-« Interleukin-1 Receptor-Associated Kinase 4 »

One contig (NODE\_45921\_length\_271\_cov\_7.907749) could be annotated via BLASTX to mouse “Interleukin-1 receptor-associated kinase 4” from the SWISSPROT database, with an e-value of 0.000100449. On an aligned region of 96 amino acids, 37 positive and 22 identical amino acids were found.

### 3.6. HRP- « Interleukin-1 Receptor-Associated Kinase 4 »

In sea star (HRP) especially, we have found also three new transcripts about IL12, IL6 and IL18 with sequences producing significant alignments as compared to Mouse (Blasts against Mouse in Swissprot system):

- Query = Locus97 transcript 2 3 confidence 0,667 length 5159
- Interleukin-12 receptor (e-value: 4e-08),bits = 58.2 sp/P97378.1
- Interleukin-6 receptor( e-value: 3e-05),bits = 48.5 sp/00560.2
- Interleukin-18 receptor (e-value: 3e-16),bits = 83.6 sp/Q9Z2B1.1

### 3.7. We Finish with the Interleukin-17 Receptor B as Compared to Mammals

#### 3.7.1. Control-« Interleukin-17 Receptor B »

One contig (NODE\_31878\_length\_179\_cov\_15.167598) could be annotated via BLASTX to mouse “Interleukin-17 receptor B” from the SWISSPROT database, with an e-value of 0.000617054. On an aligned region of 42 amino acids, 24 positive and 14 identical amino acids were found.

#### 3.8. HRP - « Interleukin-17 Receptor B »

One contig (NODE\_46091\_length\_308\_cov\_16.399351) could be annotated via BLASTX to mouse “Interleukin-17 receptor B” from the SWISSPROT database, with an e-value of 0.000279731. On an aligned region of 49 amino acids, 28 positive and 16 identical amino acids were found.

### 3.9. As Shown, it Exists Also an Interleukin-17 Receptor A

#### 3.9.1. Control - « Interleukin-17 receptor A »

#### 3.9.2. HRP - « Interleukin-17 receptor A »

One contig (NODE\_13602\_length\_446\_cov\_23.168161) could be annotated via BLASTX to mouse “Interleukin-17 receptor A” from the SWISSPROT database, with an e-value of 0.000671577. On an aligned region of 81 amino acids, 38 positive and 24 identical amino acids were found.

#### 4. CONCLUSION

The immunization leads to modifications in the case of « Interleukin-1 receptor-associated kinase 4 » and in the case of « Interleukin-17 receptor A », « Interleukin-18 receptor », « Interleukin-12 receptor » where these last appear only, in immunized animals. Furthermore interleukin genes (most of them corresponding to interleukin receptor genes) such as IRPL2, IRAK 4, IL-17RB, IL-17RA, they are present in the sea-star *Asterias rubens*.

These observations would indicate that certain interleukins and particularly « receptors » recalling mammal ones are present in the immune system of the sea star.

We note specially, in mammals that, the interleukin 17 receptor B is implicated in the immune response by mediating the activation of NF-Kappa B present also in the genome of *A. rubens*; that the interleukin 17 receptor A belongs to a novel family of inflammatory cytokines. As for the IRAK 4, it is required for various responses induced by IL-1R and toll-like receptor signals (To note that various toll-like receptors have been found in the sea-star genome).

As for the IL6-receptor: it plays an important rôle in the immune response in mice. Embryological, anatomical and biochemical evidences seem to indicate that the « sea-star phylum » is an ancestor of the vertebrates. Since the typical immune system is not present in invertebrates, it can be suggested that it was developed at this point in evolution. We recall that the main acquisition of Echinoderms seems: to be the cellular differentiation in two subpopulations of cells, ancestral to T and B lymphocytes and their interplay with phagocytes resulting in the synthesis of specific humoral primitive antibody (Leclerc, 2012) in correlation with the Complement to be expressed (Leclerc *et al.*, 2013) at the difference of another Echinoderma: the sea-urchin (Hibino *et al.*, 2006; Rast *et al.*, 2006).

#### 5. REFERENCES

Hibino, T., M. Loza-Coll, C. Messier, A.J. Majeske and A.H. Cohen *et al.*, 2006. The immune gene repertoire encoded in the purple sea urchin genome. *Dev. Biol.*, 300: 349-365. DOI: 10.1016/j.ydbio.2006.08.065

Leclerc, M., 2012. Humoral immune responses to various antigens in the asterids: *A. gibbosa* and *A. rubens*. *Am. J. Immunol.*, 8: 196-199. DOI: 10.3844/ajisp.2012.196.199

Leclerc, M., C. Brillouet and G. Luquet, 1986. The immune system of invertebrates: The sea star *Asterias rubens* (Echinoderma) as a model of study. *Bull. Institut. Pasteur.*, 84: 311-330.

Leclerc, M., C. Brillouet, G. Luquet, P. Agogue and R.A. Binaghi, 1981. Properties of cell subpopulations of starfish axial organ: In vitro effect of pokeweed mitogen and evidence of lymphokine-like substances. *Scand. J. Immunol.*, 14: 281-284. DOI: 10.1111/j.1365-3083.1981.tb00565.x

Leclerc, M., N. Kresdorn and B. Rotter, 2013. Evidence of complement genes in the sea-star *Asterias rubens*. Comparisons with the sea urchin. *Immunol. Lett.*, 151: 68-70. DOI: 10.1016/j.imlet.2013.02.003

Legac, E., G.L. Vaugier, F. Bousquet, M. Bajelan and M. Leclerc, 1996. Primitive cytokines and cytokine receptors in invertebrates: The sea star *Asterias rubens* as a model of study. *Scand. J. Immunol.*, 44: 375-380. DOI: 10.1046/j.1365-3083.1996.d01-322.x

Rast, J.P., L. C. Smith, M. Loza-Coll, T. Hibino and G.W. Litman, 2006. Genomic Insights into the Immune System of the Sea Urchin. *Science*, 314: 952-956. DOI: 10.1126/science.1134301

Zerbino, D.R. and E. Birney, 2008. Velvet: Algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res.*, 18: 821-829. DOI: 10.1101/gr.074492.107

Zhulidov, P.A., E.A. Bogdanova, A.S. Shcheglov, L.L. Vagner and G.L. Khaspekov *et al.*, 2004. Simple cDNA normalization using kamchatka crab duplex-specific nuclease. *Nucl. Acids Res.*, 32: e37-e37. DOI: 10.1093/nar/gnh031