

## Complement Genes in the Asterids Comparisons with the Echinids (Echinoderma)

Michel Leclerc

Immunologie Des Invertébrés Université d'Orléans 45100 Orléans Cedex 2, France

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### ABSTRACT

The axial organ of the sea star *Asterias rubens* (Asterid) is a primitive immune organ. The B-like cells, when stimulated by various antigens, produce antibody substances correlating with ig kappa genes. On the other hand, component complement genes were found. For each component, one or several contigs were analysed. It is said that *Asterias forbesi*, another sea-star, in earlier results, showed complement-like activity. A brief comparison with the complement system in sea urchin (Echinid) was performed, especially about the C3 component.

**Keywords:** Echinid, *Asterias Rubens*, Component, Echinoderma

### 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.

When isolated axial organ cells were stimulated “*in vitro*” by hapten-carriers, a soluble factor was secreted in the culture medium, that specifically lysed sheep red cells sensitized with the corresponding antigen (Brillouet *et al.*, 1986). This lytic reaction required the presence of thermolabile factor (complement) present in mammal serum and in the coelomic fluid of the sea-star. Moreover, it could be demonstrated that this antibody factor (Delmotte *et al.*, 1986), « unknown » in the sea urchin system (Hibino *et al.*, 2006) was produced by the B-like cells but only, when T-like and phagocytic cells were present during the antigenic stimulation (Leclerc *et al.*, 1986).

We have now observed that the antibody factor was correlated with IgKappa genes (Leclerc *et al.*, 2011) On the other hand, complement genes were found. In earlier studies (Leonard *et al.*, 1990) in *Asterias forbesi*, another sea star, complement-like activity was shown.

### 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 1mg 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 1×100 bp from one side of the approximately 200 bp fragments. Sequences were assembled using Velvet (Zerbino and Birney, 2008). Assembled nodes were used for further assembly including Beta vulgaris EST-Data from NCBI in MIRA.

### 3. RESULTS AND DISCUSSION

First, Components of the classical pathway as compared to mammals, are given with:

### 3.1. Control-Complement C1q Subcomponent Subunit A

One contig (NODE\_55223\_length\_184\_cov\_8.456522) could be annotated via BLASTX to mouse "Complement C1q subcomponent subunit A" from the SWISSPROT database, had an e-value of 8.55742e-06. On an aligned region of 72 amino acids, 29 positive and 25 identical amino acids were found.

### 3.2. HRP-Complement C1q Subcomponent Subunit A

No contig matched, no hit was found. Then,

### 3.3. Control-Complement C1q Subcomponent Subunit B

One contig (NODE\_48557\_length\_161\_cov\_19.316771) could be annotated via BLASTX to mouse "Complement C1q subcomponent subunit B" from the SWISSPROT database, had an e-value of 8.55742e-06. On an aligned region of 57 amino acids, 33 positive and 19 identical amino acids were found.

### 3.4. HRP-Complement C1q Subcomponent Subunit B

One contig (NODE\_68235\_length\_178\_cov\_7.724719) could be annotated via BLASTX to mouse "Complement C1q subcomponent subunit B" from the SWISSPROT database, had an e-value of 3.97232e-10. On an aligned region of 44 amino acids, 39 positive and 28 identical amino acids were found then.

### 3.5. Control-Complement C1q Subcomponent Subunit C

One contig (NODE\_37343\_length\_278\_cov\_12.356115). On an aligned region of 39 amino acids, 25 positive and 19 identical amino acids were found.

### 3.6. HRP-Complement C1q Subcomponent Subunit C

Three contigs could be annotated via BLASTX to mouse "Complement C1q subcomponent subunit C" from the SWISSPROT database. The best contig (NODE\_29181\_length\_277\_cov\_7.790614) had an e-value of 3.67148e-09. On an aligned region of 45 amino acids, 27 positive and 22 identical amino acids were found.

### 3.7. We Observe Now the C2 Component

Five contigs could be annotated via BLASTX to mouse "Complement C2" from the SWISSPROT

database. The best contig (NODE\_23089\_length\_519\_cov\_15.136802) had an e-value of 7.65302e-07. On an aligned region of 153 amino acids, 59 positive and 38 identical amino acids were found. And now what about the component C4?

### 3.8. Control - Complement C4-B

One contig (NODE\_95392\_length\_215\_cov\_10.818604) could be annotated via BLASTX to mouse "Complement C4-B" from the SWISSPROT database, had an e-value of 3.48532e-06. On an aligned region of 76 amino acids, 40 positive and 21 identical amino acids were found.

### 3.9. HRP-Complement C4-B

One contig (NODE\_58696\_length\_164\_cov\_11.884147) could be annotated via BLASTX to mouse "Complement C4-B" from the SWISSPROT database, had an e-value of 7.51259e-05. On an aligned region of 44 amino acids, 26 positive and 21 identical amino acids were found.

Then, we have a look about the C3 component which is central, in mammals, to both the classical and alternative pathways. We try, at the end of this chapter to attempt a comparison with the SpC3 of the sea urchin.

Seven contigs could be annotated via BLASTX to mouse "Complement C3" from the SWISSPROT database. The best contig (NODE\_25262\_length\_1200\_cov\_21.958334) had an e-value of 3.2395e-49. On an aligned region of 355 amino acids, 128 positive and 173 identical amino acids were found.

### 3.10. HRP-Complement C3

Eleven contigs could be annotated via BLASTX to mouse "Complement C3" from the SWISSPROT database. The best contig (NODE\_15219\_length\_398\_cov\_19.402010) had an e-value of 4.96748e-24. On an aligned region of 146 amino acids, 79 positive and 59 identical amino acids were found. It must be noted that C3 belongs to, also, the ALTERNATE pathway.

Comparisons with the sea urchin (*Strongylocentrotus purpuratus*) **Fig. 1:**

```
NODE_25262_length_1200_cov_21.958334
-> >gi|47551023|ref|NP_999686.1| complement
component C3 precursor [Strongylocentrotus purpuratus]
e-value: 1e-70
Indetique aa: 260
Positive aa: 410
```

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TCACTGTCTGGCTCTGGGATGTGGTAGCTTGACTCAAACCTTCAGCTGGCCAACCTCTGTG
CCTGAAGAGTCAAAAATGAACACGGAATTCCGGCTCATTTCCTGGAATCACAAAGTTTAGA
GTGCTGTCTGACGATTGCGTTGAACGGTTGCAGAACGAACTCATCTTCAAATGACTCTC
AATACAACCTGACCTGGCAATTGATATCAATGTTGCTCGTCTCGGACTTGATAGCATACTCC
GACAACGCCTGCAGAGCTATAACTGTATCCTGAGAAGACACAAATCCACCTTCATAATTC
TCTGTTCCTGTGAGCCAATTAACAATGGCATGACTGTAGGGCAGGTCGTCAGCTGAAGT
AAAGCCAGCAGTGCATAGCTTGTCTCTACATCAATGGCTTTCGGCCGGTTGATGTAC
CAATATGGTTTTCGGACCCGCGCCAAAGGACGAGTCGTCTGCTCCCCAGTGACGATAATTG
GTTGCTCTATCGTACGTAGCGATGTCTTTGAGCATCGTGAGAGCTTCATCAGCTTTGTTGC
TATTGGCCAGAGCGAGGGCGTATGCGGTGATGGCAATGGCGTAAGGTCTCGTCAATTGT
TGCAGTTGACCCTCTAAGAAAGCCGTAGCTGAAGCAACAGAGTCAGTCTTACTTACTGTT
TCACATTCACACTCCAGAAGAGCTATCAGCACGTATGCAGTAAGCGAGGGCGTCACCTTGC
CACCACCAATCATTTCTTGGTGATGCACTTTGTACAATTCCCCGAACGCACCGTTGTCAA
TTCTGTGTCCTCTATCAGCCATCTCATAGCATTACAGGTGATGCTTCCATCAATGTAGG
CAAATTTCTTGGCCTGGCAGAACACCTTGTGACAAAGGCCGTCAACCAGGTACTGCTTG
GATACTGTAAATTATCTCCCATACTGAAAATGAACCGTCCGAACGACGATGCGTCAATT
TCTTGAGTTATACCGCTCCGATGTACTGCTGAGCGGACACCTCCAGCTCAGCCGTGAAC
TGATTAGTGTGCTTACAGATAACCGGTAGACGTAGACGTTGGGTGCTAGGGTGATCATCGTT
TGTTACCCACACCCTCGAGGGATCTGCAAAATGTGCGCCCAACCCACTGATGACCGTGTG
ATGACGTTACCCAAAATATTTCCCATGATGCTGATATGGGTTTGTGGGTACCAGGGATA
CGTCACTATGCAAGGAGTATCGT
```

Fig. 1. DNA sequence of C3 component (comparisons with *S. purpuratus*. Echinid)

>NODE\_25262\_length\_1200\_cov\_21.958334

We give now the obtained results concerning the components of the membrane-attack complex as compared to mammals. So with the C9 component.

### 3.11. Control-Complement Component C9

Three contigs could be annotated via BLASTX to mouse "Complement component C9" from the SWISSPROT database. The best contig (NODE\_46472\_length\_143\_cov\_10.104895) had an e-value of 1.41746e-05. On an aligned region of 31 amino acids, 17 positive and 15 identical amino acids were found.

### 3.12. HRP-Complement Component C9

#### 3.12.1. No Contig Matched, no hit was Found. As for the C5 Component

#### 3.12.2. Control-Complement C5

One contig (NODE\_75605\_length\_236\_cov\_32.677967) could be annotated via BLASTX to mouse "Complement C5" from the SWISSPROT database, with an e-value of 0.000634854. On an aligned region of 55 amino acids, 27 positive and 19 identical amino acids were found.

### 3.13. HRP-Complement C5

Two contigs could be annotated via BLASTX to mouse "Complement C5" from the SWISSPROT database. The best contig (NODE\_7717\_length\_665\_cov\_13.831579) had

an e-value of 6.42528e-17. On an aligned region of 212 amino acids, 99 positive and 57 identical amino acids were found. At last we observe the C8 Component and we find:

### 3.14. Control-Complement Component C8 Alpha Chain

Twelve contigs could be annotated via BLASTX to mouse "Complement component C8 alpha chain" from the SWISSPROT database. The best contig (NODE\_68667\_length\_311\_cov\_10.845659) had an e-value of 6.7995e-15. On an aligned region of 95 amino acids, 33 positive and 42 identical amino acids were found.

### 3.15. HRP-Complement Component C8 Alpha Chain

Six contigs could be annotated via BLASTX to mouse "Complement component C8 alpha chain" from the SWISSPROT database. The best contig (NODE\_43169\_length\_2163\_cov\_11.209893) had an e-value of 6.36046e-14. On an aligned region of 45 amino acids, 42 positive and 33 identical amino acids were found.

## 4. CONCLUSION

The main components of Complement as compared to mammal ones were found in the sea star: How genes are expressed? By which pathway? In the present time we have no answers to these questions.

It could be a working hypothesis which needs to be explored. Nevertheless these observations indicate that

certain mammal structures are present in the immune system of the sea star: and this finding strongly supports the idea that an effective immune system is already present in Echinoderma.

Since the typical immune system is not present in invertebrates, it can be suggested that it was developed at this point in evolution. The main acquisition of Asterids 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 antibody factor (Delmotte *et al.*, 1986) in relation with the Complement to be expressed.

Alternative and direct pathway genes correlated with « mammal » genes were discovered in this study, at the difference of another Echinoderma: the sea-urchin: *Strongylocentrotus purpuratus* (Smith *et al.*, 2001) which shows only two components of the complement such as a homologue of C3, called SpC3, having similarities with sea star C3 component (**Fig. 1**) and factor Bf possessing significant similarity to the vertebrate Bf/C2 family. No humoral immune reaction occur in the sea urchin at our knowledge. At last, It could be expected that the sea-star specific immune reaction needs all the genes (7 components) from C1 to C9 to be expressed. This study reveals a true progress in the comprehension of the immune system in Echinoderma.

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