Rosetting and PFC (Plaque-Forming Cell), Anti-SRBC Antibodies and The Sea Star Primitive Antibody

Michel Leclerc*

*556 rue Isabelle Romée, 45640 SANDILLON, Immunology of Invertebrates, France

Abstract

Rosetting test and PFC test (Plaque forming cells) after many injections of SRBC (Sheep red blood cells) were performed in the sea star Asterias rubens (Echinodermata). Positive results, after injection of 5.10^6 red cells/ injection, in the coelomic cavity, occur and were summarized in this work. They indicated the presence of anti-SRBC antibodies in the sea star, according to the method of Biozzi (1966) for rosetting and Cunningham (1965) for PFC.

Keywords: Invertebrate; Sea star antibody; Anti-SRBC.
Introduction

We have recently discovered the IPA (Invertebrate Primitive Antibody)(1). Many antigens, such as various enzymes, generate antibodies in Asterias rubens. In 1965 Cunningham demonstrated the existence of Vertebrate antibodies by the use of the PFC(2). Biozzi in 1966 showed a similar result by the use of Rosetting(3). Then we decided to apply these methods to another model: the sea star Asterias rubens. We attempt to demonstrate the existence of anti-SRBC sea star antibodies.

Materials and Methods

1. Rosetting test

Asterias rubens was injected with 5,000,000 SRBC treated with formol (to avoid osmotic lysis). It corresponds to 0.05 ml of SRBC. 5 days after the injection, the axial organ, a primitive lymphoid organ, was removed and dilacerated(by teasing) in physiologic serum. Fresh SRBC was added to obtain 2 ml of cell suspension which was kept overnight at 4°C. Observations were made in light microscopy. Controls without injection were performed. Specificity was tested with Pigeon red blood cells.

2. PFC test

We used Cunningham test(2). 2 injections (1 per week) were realized in 10 Asterias rubens with the same quantity of SRBC as for Rosetting test. 5 days after the last injection, axial organs were pooled and teased to obtain 1 ml of cell suspension. Fresh SRBC in the presence or not of Guinea pig complement was added to obtain 2 ml of cell suspension. Controls without injection of SRBC were performed. Observations were done in light microscopy.

Results

1. Rosetting appear in treated animals(injected with SRBC) but not in controls (Figure 1). We appreciate 5-6 Rosetting/ml. There are no Rosetting with PRBC (Pigeon red blood cells).

2. PFC also appear in treated animals but not in controls (Figure 2 and 3) It is noticeable that these PFC needs no Guinea pig complement to be shown (sea star complement is already present)(3).

Figure 1: Rosetting around a sea star Plasmolymphocyte
Figure 2 and 3: PFC. Note in 54 the sea star Plasmolymphocyte at higher scale with ghost SRBC
Conclusion

It appears clearly that anti-SRBC antibodies exist in the sea star immune system. It is noticeable this humoral response is specific when the SRBC test is compared to PRBC one (Pigeon red blood cells) : At the opposite of SRBC, PRBC doesn’t agglutinate in presence of sea star plasmolymphocytes. Furthermore, these antibodies may be compared to specific hemagglutinins in the case of Rosetting, to specific hemolysins in the case of PFC.

In this last one, it must be declared that the presence or absence of Guinea-pig complement is not necessary to realize PFC since we discovered the sea star complement in sea star genome (from C1 to C9 complement components)(4). Further studies are necessary to compare anti-SRBC antibodies and IPA (Invertebrate primitive antibody)(1).

In conclusion, these results are of special interest in Comparative Immunology which concerns Invertebrates and Vertebrates.

References