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Bence-Jones Protein , Rat Igg, H.R.P , Alkaline Phosphatase, Bovine Serum Albumine used as Antigens in the Immune Response of the Sea Star

Michel Leclerc

556 rue Isabelle Romée, Sandillon, France

***Corresponding Author:** Michel Leclerc, 556 rue Isabelle Romée, Sandillon, France.*

ABSTRACT

Immunocompetent cells were described in the sea star Asterina gibbosa: they recognize specifically various antigens. In the present work, Asterina gibbosa were immunized with Bence-Jones protein , other ones with Rat IGG, at last certain with H.RP (Horse-radish Peroxydase) and the last ones with Alkaline Phosphatase: a crossed immune reaction occurs between Rat IGG and B.J protein, but not between B.J and H.R.P not between Rat IGG and H.R.P and not between Alkaline Phosphatase and B.J, not between Alkaline Phosphatase and Rat IGG, used as antigens.

INTRODUCTION

Recently it was shown that immunized Asterina gibbosa (Asterids Echinodermata) to various proteins (Peroxydase; Alkalin phosphatase, Trypsin) shown specific immunocytochemical reactions in T.E.M (Ref; 1-2-3).

In the present study we attempt to estimate such reactions by the use of more sophisticated proteins: the Rat immunoglobulin IGG and Bence-Jones protein (Light chain of immunoglobulins produced by human myeloma) in comparison to H.R.P (Horse - radish Peroxydase).

MATERIALS AND METHODS

Sea Stars

Asterina gibbosa were kept in aquarium, at 10° C, in running sea water.

Methods

100 µg of HRP (Sigma Products) were injected in 5 Asterina gibbosa each: they were kept in a control aquarium. 7 days after the first injection, they were injected a second time with the same quantity of HRP, always in the coelomic cavity of each animal. They were placed in aquarium I.

A same experiment was done with Alkaline phosphatase (Sigma Products): 100 µg were injected in 5 Asterina gibbosa which were placed after in aquarium II.A second injection was performed as precedently

In the same time, 100 µl of a solution at 1mg/ml of Bence-Jones (BJ) protein were injected to 5 other Asterina gibbosa each, twice, like in the first experiment. They were placed in an aquarium III.

On the other hand, 100 µl of a Rat IGG solution at 1mg/ml were injected to 5 other Asterina gibbosa, placed in an other aquarium IV with running sea water as precedently.

At last 5 Asterina gibbosa were injected with 100µg each of Bovine Serum Albumine (Pentex) in aquarium V.

4 days after the last injection, all axial organs (AO) (Ref.3) were excised and fixed separately in glutaraldehyde (1, 5% in cacodylate buffer) then rinsed in the same buffer.

Incubation in antigenic solution (Rat IGG coupled to peroxydase -Sigma Products) were performed, for each animal of Aquarium I, II, III, IV.V

A new fixation at glutaraldehyde of 10 minutes occur for all AO (1 % in cacodylate buffer).

AO were rinced in cacodylate buffer then treated with diaminobenzidine (Ref.3) at last dehydrated (Alcohol 70 to Alcohol 100°) and finally embedded in Epon.

Incubation in Rat IGG coupled to peroxydase was also performed for each animal of Aquarium I, II, III, IV, V. Following steps have been precedently described.

Incubation in HRP was done for also each animal of Aquarium I, II, III, IV, V. Following steps are now well-known (Ref.3)

At last incubation in antigenic solution of Alkaline Phosphatase was realized fo each animal of Aquarium I, II, III, IV, V. Following steps have already been described. (Ref.3)

Cuts were done with a LKB ultrotome. Observations in TEM were realized with a Hitachi Microscope.

RESULTS

Immuunolabelling was seen in treated animals either with Rat IGG, Bence-Jones protein, HRP, Alkaline phosphatase, BSA antigens.

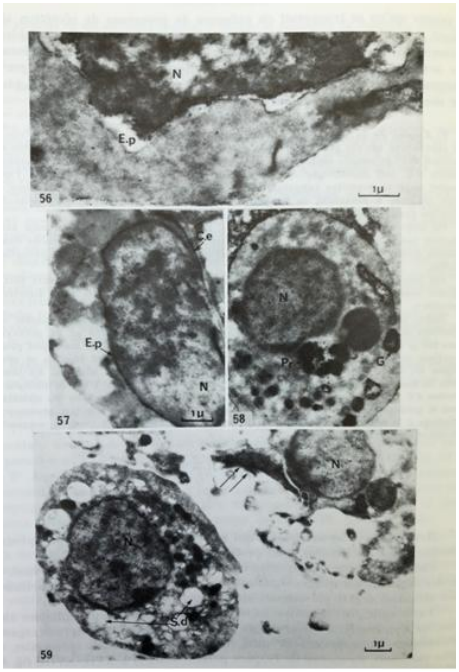

Table1. *Immunoenzymatic Reactions In Asterina Gibbosa (Ref 3) Labelling With Hrp As Antigen.*

These labelling are situated at the level of perinuclear space (EP) next to Nucleus (N) Reticulum endoplasmic (RE) Golgi apparatus (G) and Lysosomes (L) of the sea star plasmolymphocytes in TEM as previously described (Table.1): Labelling with HRP.

DISCUSSION CONCLUSION

- Labelling occurs in treated animals to Rat IGG, Bence-Jones protein, HRP, Alkaline phosphatase Bovine Serum Albumine (BSA) antigens.
- It is noticeable that sea star Immune system makes no differences between Rat IGG and BJ protein antigens: there is a CROSSED reaction. BUT:
- There is no crossed reactions, between HRP and BJ protein, between Alkaline phosphatase and BJ protein
- There is no crossed reactions between Rat IGG and HRP, between Rat IGG and Alkaline phosphatase
- The sea star recognizes specifically HRP from Phosphatase alkaline, from Bovine Serum Albumine (BSA) (Ref.3) and vice versa.
- We retain that Asterina gibbosa is able to recognize many antigens but not all the antigens the man
- Does: it must be claimed.

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