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Recombinant Protein (Anti-HRP) issued from the Sea Star Igkappa Cloning by the use of CHO: Its Antibody Specificity Revealed by Elisa

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Abstract

This work was performed in the Laboratory: *UMR-INRA ISP 1282 Tours (France).*

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It allows, by the use of CHO (Chinese Hamster Ovarian) protocol cloning to produce the « Young Protein » or anti-HRP (Horse-Radish Peroxydase) from the sea star IGKappa gene which corresponds to the IPA: (Invertebrate Primitive Antibody). Two elisas confirm the anti-HRP activity.The first Elisa was done with Protein L-HRP antigen. The second one with Streptavidine-HRP at classical concentrations which are used for Mammals.

Introduction

10 years ago, we tried to clone, for the first time, the Asterias rubens sea star IGKappa gene by the use and the help of E.coli as amplificator (1). It allowed, in a second time, to verify that the Young Protein, or anti-HRP Protein recognizes the HRP antigen (1).

But, this verification of the affinity between the IPA (Invertebrate Primitive Antibody) and the antigen ,seemed unclear at that time ,for many of us.

Thus, we decided to operate a new cloning (2) of the IGKappa gene with new parameters and new affinity tests. This second one did not allow to obtain the protein of interest or « Young Protein ».

A third assay was attempted: It used a CHO method, as described in various experiments (3) and Elisa to verify the specificity of our « antibody » against the HRP antigen.

Material and Methods

We use the « Young protein » or anti-HRP protein as primitive antibody.

Secondly, two Elisas are performed in classical microplates coated with BSA to saturate the bottom of each well.

Classical dilutions (half by half) are done.

In the first microplate we add Protein L HRP as antigen, in the second one, Streptavidine-HRP as antigen.

At least, in each well, we place anti-His protein, then substrat TMB to reveal the reaction

Results

As shown in fig.1, by the use of Protein L-HRP antigen, a positive reaction occurs for the first dilutions of the IPA in « sensibility » to the antigen

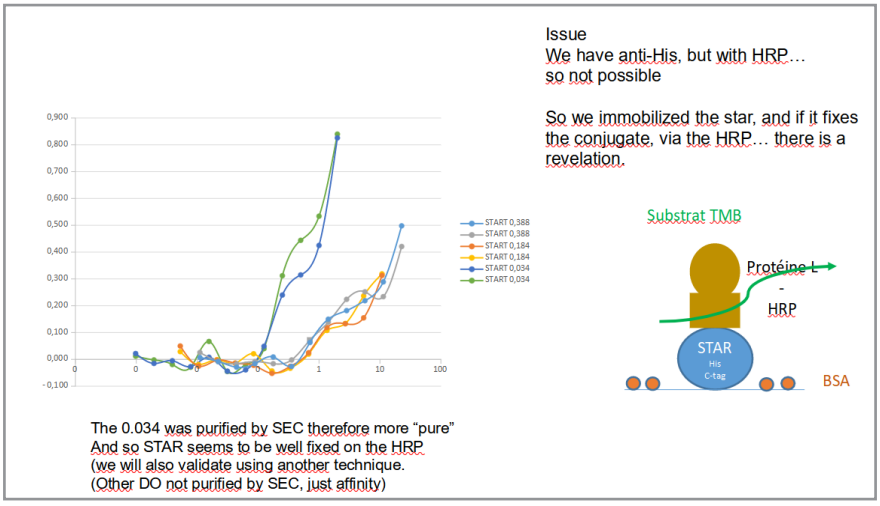

Figure 1: Elisa with STAR or sea star antibody anti-HRP against Protein L-HRP as antigen
Note the affinity specially with the yellow curve for an IPA 0,034 dilution.

A similar positive reaction was obtained with the antigen : Streptavidine -HRP.

It indicates that the « Young Protein » has a ANTIBODY BEHAVIOUR towards the antigen HRP or HRP complex.
Of course it was possible to perform an Elisa test directly against HRP antigen, it is why in a next assay we 'll realize first :
An immobilization of His protein in microplates.

The immobilization of Histidine 'll allow to determine better the specificity of the reaction in our conditions of manipulation.

Discussion, Conclusion

We think now that our primitive antibody **(IPA) anti-HRP** recognizes the antigen HRP either directly (4)

Or : - after cloning with E.coli (1) or with CHO protocol as it is proved to-day with Elisa.

We think now, to immunize other sea stars with anti-tumoral antigens to product specific nanobodies (5) from sea stars, against specific cancer proteins (in a general way) Sequencing and cloning, ''ll allow us to obtain a specific recombinant specific protein we 'll test against cancerous cells.

On the other hand, we 'll try to determine other parameters of the IPA such as CDR 1 and CDR2 with these new invertebrate primitive antibodies

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