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Research on new immunomodulators from marine animals

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ABSTRACT A review on the mechanism of action of Rapamycine and FK-506 and the discovery of bistramide, an immunosuppressive compound from *Lissoclinum bistratum*, were discussed.

ABSTRAK Satu tinjauan mengenai mekanisma tindakan Rapamisina dan FK-506 dan penemuan bistramida sebatian yang memaparkan aktiviti perencat imun, dibincangkan.

(Immunomodulators, *Lissoclinum bistratum*, therapeutic agent, bistramide A, agelastatin A)

INTRODUCTION

Plants and marine organisms contain immunostimulant as well as immunosuppressive substances, some of them being utilized with great efficiency to cure diseases. In the seventies and the eighties, immunosuppressors, like cyclosporine, have been discovered. Such molecules are able to inhibit the immune response and their studies have led, indirectly, to a better understanding of lymphocyte activation mechanisms which constitute the basis of the immune response.

The immune response is a defensive mechanism unique to vertebrates and it is only during the last decade that its mechanism of action is understood. In brief, it was acknowledged that the leucocytes were critical for the immune response; the lymphocytes, T and B cells, playing the predominant role. B cells excrete specific antibodies while T cells express specific molecular structures on their cell surface that are critical in molecular recognition processes. These distinctions were formerly recognized as reflecting the classical humoral and cellular aspects of immunity, respectively.

Humoral aspects included responses found in disorders involving autoimmunity, immune complex phenomena and enhanced killing of microbial invaders. Cellular aspects included tissues rejection and tumour, viral and microbial supervision and killing [1].

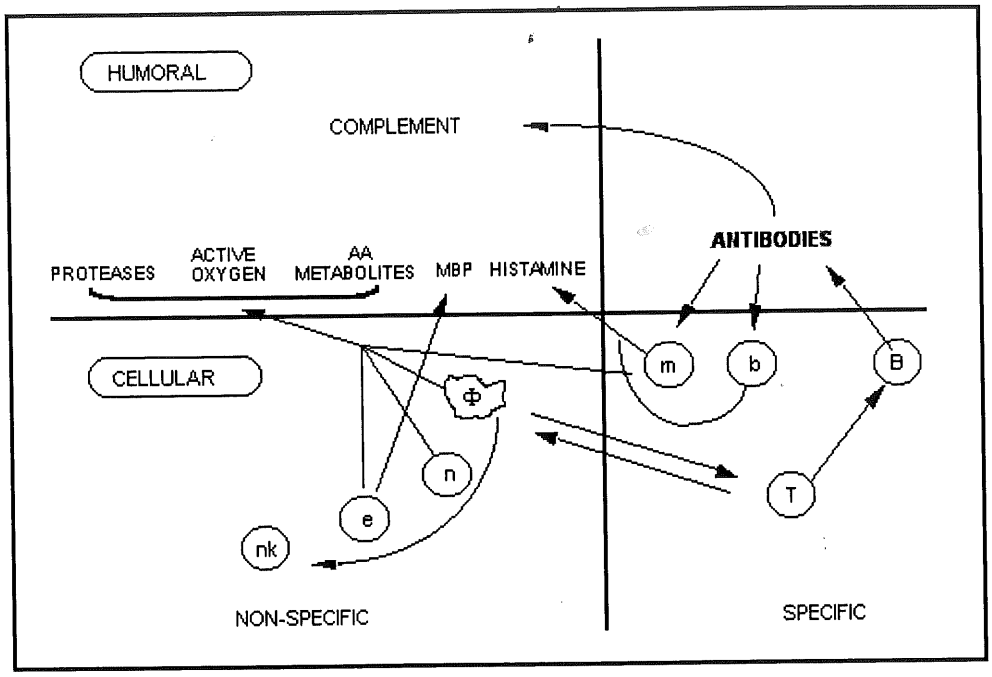
Figure 1 shows an interdependancy between the different cell classes, like lymphocytes T or B, and activation of secondary cell types such as the eosinophil, neutrophil, basophil and macrophage. After triggering the activation of T lymphocyte by the antigen-presenting cell (APC), a series of events occur during the immune response. With the help of many mediators, the different cells of this complex system are acting on each other. For example T lymphocytes enhance the activity of B lymphocytes, which induce the production of antibodies, or macrophages which induce the activity of natural killer cells. For these interactions a lot of intercellular signals are used by the cells, in particular the different interleukines and interferons which are secreted by the different cells which often possess receptors for the same mediators.

Immunomodulators are substances that promote or depress the ability of an animal to mount an immune response or to defend itself against pathogens or tumours [2]. The understanding of the mechanism of immune response offers opportunities for the search of novel and specific immunomodulators.

Immunomodulators may be divided into two types:

a) Immunosuppressors; Cyclosporin A, FK506, Rapamycin, Didemnin B
b) Immunostimulants;

Today many such compounds have been described and their activities are known. For example Table 1 shows some of the immunosuppressive agents with their targets and their mode of action.



Interdependencies between different cell classes in the immune response

Figure 1

Table 1. Immunosuppressive agents

Therapeutic agent/strategy	Target	Result
Cyclophosphamide	DNA Alkylation	Blocks DNA synthesis
Methotrexate	Dihydrofolate reductase	Blocks DNA synthesis
Azathioprine	Purine biosynthesis	Blocks DNA synthesis
Mizoribine, mycophenolic acid	IMP dehydrogenase	Blocks DNA synthesis
Glucocortocoids, Vitamin D_3	Steroid receptor family	Blocks immune cell activation
Brequinar	Dihydroorate dehydrogenase	Blocks DNA synthesis
Anti-CD_4	CD_4	Blocks lymphocyte activation
Anti-IL_2, IL_2 toxins	IL_2 receptor	Blocks lymphocyte activation
Cyclosporine, FK 506	Calcineurin	Blocks lymphocyte activation
Rapamycin	Unknown	Blocks lymphocyte activation
Bone-marrow transplantation	Unknown	Tolerance induction
MHC blockade	Disease-associated MHC	Blocks antigen presentation

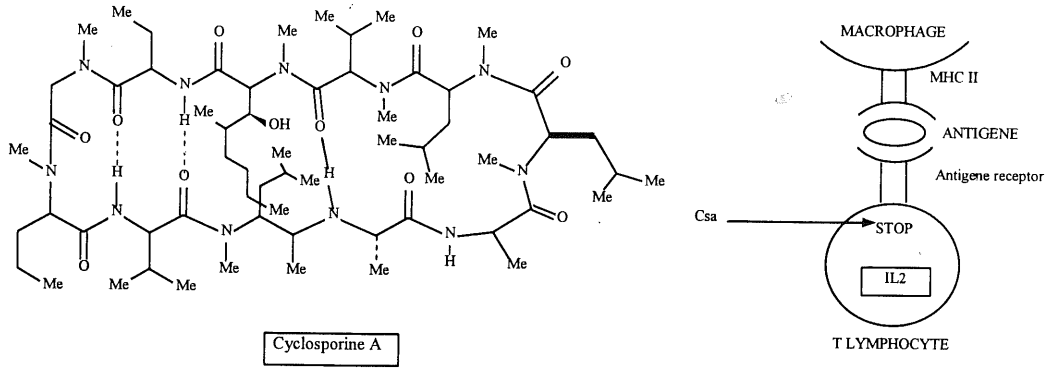


Figure 2. Cyclosporin and his mode of action on T lymphocyte.

FK 506 and Rapamycin were isolated from different *Streptomyces* species. The former, produced by *Streptomyces tsukubaensis* (soil bacterium from the Tsukuba region of northern Japan), is a 23-membered macrocyclic lactone with the unusual hemiketal masked αβ diketopipecolate moiety. The latter, produced by *Streptomyces hygroscopicus* (bacterium isolated from a soil sample collected in Rapanui, Easter island), is a 31 membered macrocyclic lactone with the same unusual substructure.

FK 506 and Rapamycin block respectively T-cell activation and proliferation *in vitro* as they interfere with components of cell-cycle related signal transduction pathway.

Cyclosporin, used against organ rejection, is among the most powerful immunosuppressors [3]. This compound, isolated from a fungus is a cyclic oligopeptide that selectively inhibits T cell activation and blocks the release of IL2 and INF γ by T cells (see Figure 2).

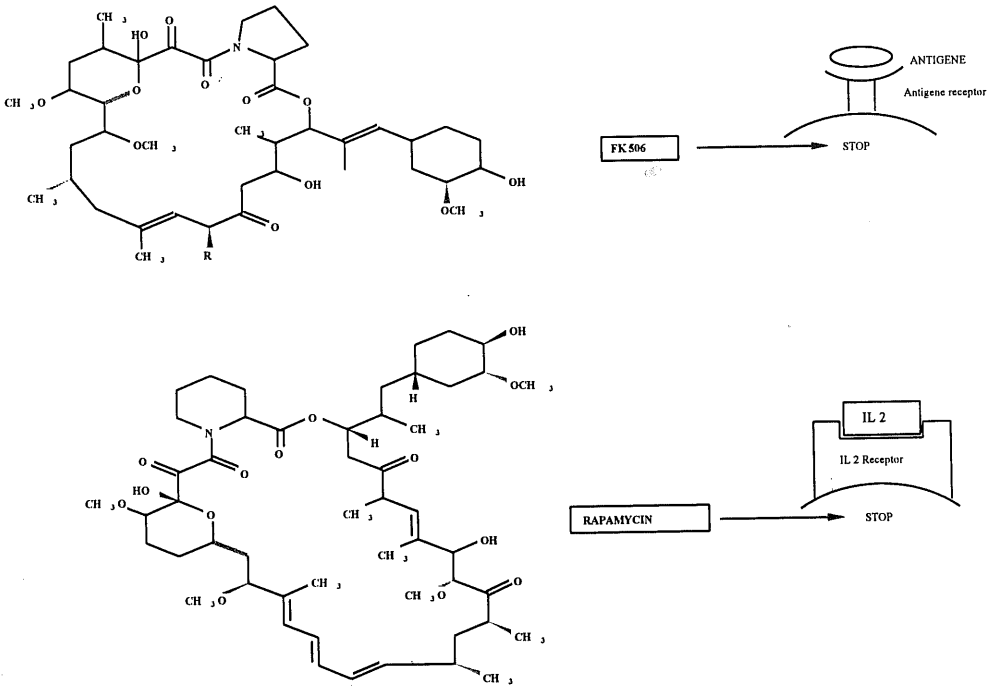


Figure 3. FK 506, Rapamycin and their targets.

FK 506 has an IC_{50} 50 to 100 fold lower than cyclosporine and rapamycine has an IC_{50} intermediate between cycloporine and FK 506. The didemnins are another example of immunosuppressive agents which are a class of cyclic depsipeptides isolated from a Caribbean tunicate *Trididemnum solidum*. Didemnin B is the most powerful of this class of compounds. From another marine organism, the sponge *Discodermia dissoluta*, an immunosuppressive compound discodermolide, has been recently isolated [4].

b) Immunostimulants: fungal glycans, bacterial lipopolysaccharides endotoxins, muramyl dipeptides, microbe derived agents, thymic extracts.

Some immunostimulant agents have also been obtained from natural sources. For example fungal glycans, like bestatin and krestin which enhance the macrophages activity, then IL1 secretion and T lymphocytes activation [5]. Lipopolysaccharide endotoxins show the complexity of the interaction between mediators and cells. We can observe that there is not only one target for these mediators and this lack of specifity could generate some side-effects.

Thymic peptides: an example is thymopentin, which induces T-cell differentiation and promotes proliferation and interleukin production. In this class of stimulating agents, more specific compounds would be of interest in this field.

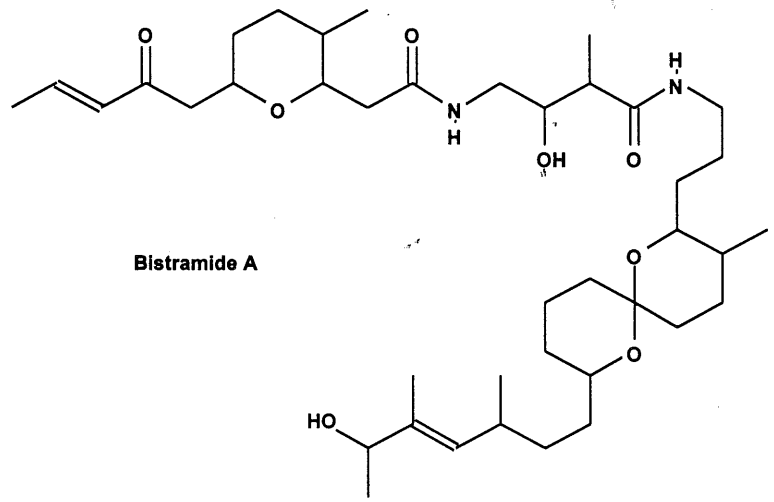
A large program of research on marine animals had been developed in New Caledonia and we had learnt that a large number of marine animals extracts were available for any pharmacological screening. As we were interested by the research of new immunomodulators and able to realise a biological assay for this purpose, we have decided to screen these extracts.

Among 632 extracts from more than 300 animals, 124 extracts have given positive results with the assay on splenocytes proliferation. Table 2 shows the results obtained.

Table 2

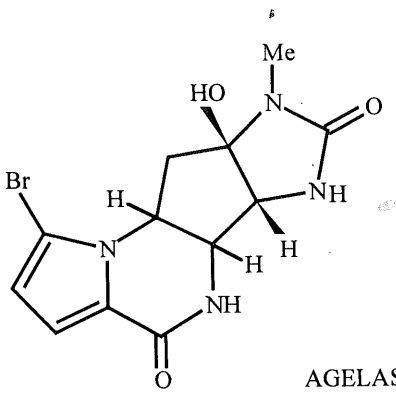
ANIMALS	Extracts tested	Active	Stimulants	Extracts
Sponges	431	89	52	37
Gorgonians	21	5	2	3
Ascidians	77	13	10	3
Algae	9	4	4	-
Bryozoaires	6	2	2	-
Crinoïds	3	1	1	-
Sea Urchins	17	2	2	-
Antipathaires	8	2	2	-
Madrepores	24	2	2	-
Molluscs	8	3	3	-
Holothurians	3	1	-	1
Alcyonaria	24	0	-	-
Ophiuroid	1	0	-	-
TOTAL	**632**	**124**	**80**	**44**

The first positive result have been obtained with the Ascidian *Lissoclinum bistratum* Sluiter.



Bistramide A

From this extract a known compound, bistramide A [6, 7, 8] has shown a suppressive activity. Our screening had also shown that the sponge *Agelas dendromorpha* contained an immunosuppressive agent. The active compound is agelastatin A which belongs to the oroidin family.



AGELASTATIN A

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