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INSILICO DESIGNING AND DEVELOPMENT OF VACCINE FOR Rickettsia Rickettssi IN ROCKY MOUNTAIN SPOTTED FEVER

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ABSTRACT:

Rickettsia is a pathogenic gram negative organism which causes Rocky Mountain spotted fever, Scrub typhus and murine typhus. Among this Rocky Mountain spotted fever was found to be the dreadful disease caused by Rickettsia Rickettssi. More than thousands of people are dying yearly due to the infection caused by Rickettsia. To eradicate this Rickettsia, Vaccine was designed by Bioinformatics Approach.

Main focus of this work is on Insilco analysis, find out various genes expressed and proteins developed in diseased human condition. Separate the protein to find out what common domains these proteins share that are functionally active during the disease and then to analyze and are altered and modified in these diseased proteins. Rickettsia Rickettssi consists of 2000 odd protein sequences, these protein sequences are screened and the one with least identity (24.13), and least E value was identified. The least identity sequence is further used to get antigenic determinants from the Immunomedicine group tool. Then the best vaccine candidate was found out by docking process.

Keywords: *Rickettsia, pathogenic, rocky mountain fever, vaccine, immunomedicine, docking*

[I] INTRODUCTION

Rickettsia is a gram negative organism. The tribe rikettsiae has two genera Rickettsia and Orientia. Rickettsia is involved in both spotted fever and typhus groups. Rickettsia is responsible for spotted mountain fever and murine typhus. This is an obligate organisms involving life cycle in both vertebrates and non vertebrates. They divide by binary fission and they metabolize host-derived glutamate via aerobic respiration and the citric acid (TCA) cycle.

The rickettsiae that are pathogens of humans are subdivided into three major;

1. spotted fever group.
2. Typhus group.
3. Scrub typhus group.

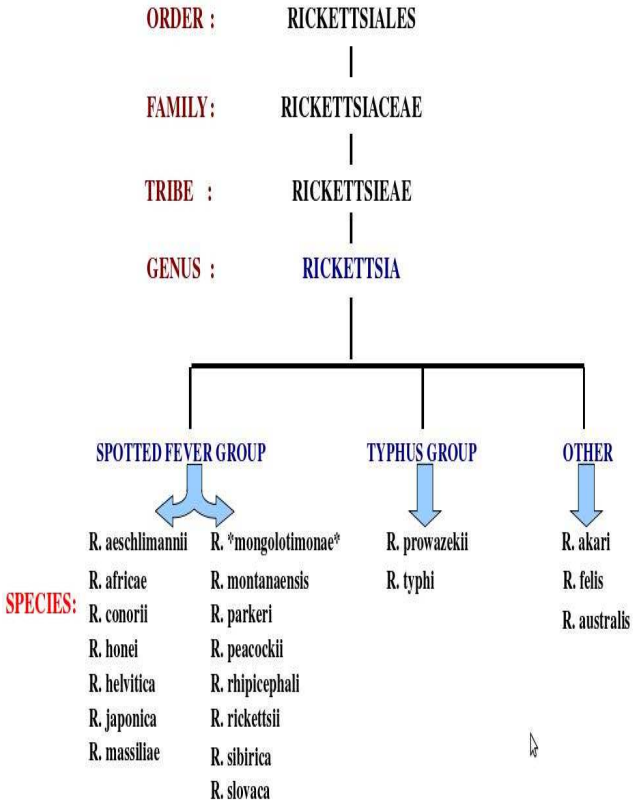

Fig. 1. Rickettsiae Groups

1.1 Rocky Mountain spotted fever

It is the common disease that is caused by rikettsia rickettsii. It is infected in humans due to the bite of infected ticks. The bacterium infects human vascular endothelial cells, producing an inflammatory response.

Upon attaching to the host cell membrane, rickettsiae are phagocytosed by the host cell. The rickettsiae induce host cell phagocytosis because they can enter cells that normally do not phagocytose particles. Once phagocytosed by the host cell, they quickly escape from the phagosome membrane and enter the cytoplasm. This is mediated by a rickettsial enzyme, phospholipase A2.

1.2 Murine typhus

Rickettsial infections have played a significant role in the history of Western civilization. Epidemic typhus has been known since the 16th century and it has long been associated with famine and war. The outcome of several wars was influenced by epidemic typhus. Typhus killed or caused debilitated over 100,000 people in the two World Wars. In spite of its long history, it was not until the early part of the 20th century that the causative agent was determined.

It is caused mainly due to organism Rickettsia typhi. Murine typhus occurs worldwide with approximately 40 - 60 cases being reported in the United States annually. Rats are the primary reservoir for the disease which is transmitted by the rat flea vector. The bacteria are in the flea feces and are inoculated into abraded skin by scratching the area irritated by the bite. The symptoms of fever, chills headache and myalgias appear abruptly 1 - 2 weeks after infection.

2. RESULTS

2.1. Immunomed Group

This tool is used to find out the best antigenic determinant that binds with the MHC molecule .

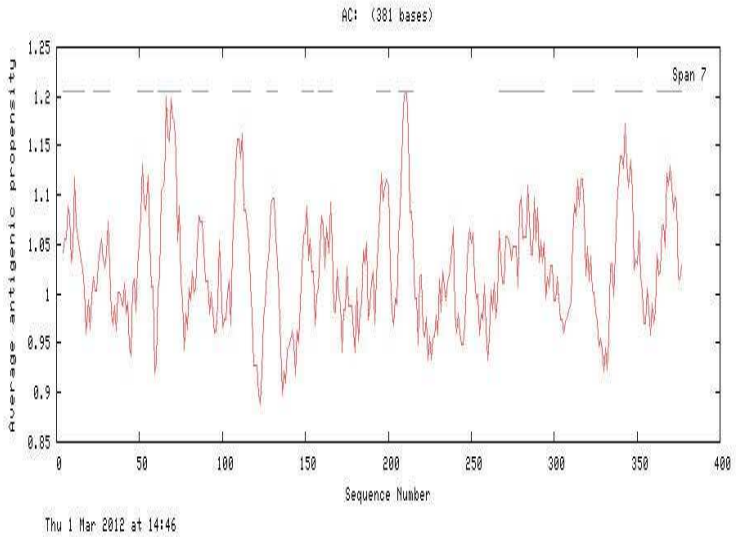

Fig.2 Graph for Immunomed Group

Figure shows the Average antigenic propensity for this protein is 1.0263

There are 15 antigenic determinants in sequence:

Sr. no	Start Position	Sequence	End Position
1	4	NYLKDLISFKSVTP	17
2	22	AIEYINDLLKQ	32
3	49	QVTNLYAVFG	58
4	61	EPNICFVGHVDVVLE	75
5	82	HNASPFKVSQ	91
6	106	GAIACFLAASLD	117
7	127	GSISFLL	133
8	148	MLQYIYDQ	155
9	158	KINFAIVGE	166
10	193	GLSGHVAYP	201
11	206	NPLPCLIIIL	215
12	267	SAETLAKQVEEIIKQHCKEYKVDYKLEY	294
13	311	EFAKVVEHTLKIKP	324
14	337	FVKNYCPLVEFGLLSET	353
15	362	KISDLQKLYDVYYNFL	377

Table.1-15 antigenic determinants

Immunomed group; - From this LCV value is calculated and the highest LCV value is compared with MAPPP results and thereby the best vaccine candidate was designed and docked

MAPPP: From Mappp results the MHC binding probability and MHC cleavage score was found. This tool even helps to determine the type of MHC molecule to be docked with the epitope molecule.

2.2. Discovery studio and Hex6.3

Discovery studio is used to design the molecule and Hex is used to design the ligand molecule and to dock receptor molecule and ligand molecule.

Minimization of the Molecule -PCLIII

3. CONCLUSION AND DISCUSSION

After screening of proteins the best antigenic determinant was found by their least identity, the good vaccine candidate was observed through various bioinformatics tools such as immunomedicine and Mappp results. So from the above results; can conclude that the protein sequence with least identity i.e. 24.13 was further sent to docking analysis. By this analysis it was found that the antigenic determinant PCLIII binds to the MHC molecule successfully. This Docked molecule consists of Docker energy and that energy was found to be -301.98. Hence by this docking analysis the epitope molecule was proved to be the best Vaccine candidate for Rocky mountain fever.

4. MATERIALS AND METHODS

SDSC Workbench: This is the primary bioinformatics tool which is used in this experiment. This is mainly used in screening of proteins and find the protein sequence with the least identity.

Immunomedicine Group: This tool is used to find out the antigenic determinants of the protein sequences with the least identity.

Mappp: This is primarily used to identify the type of MHC molecule to which epitope molecule binds and thereby helps in docking analysis

Discovery Studio: This freeware software is widely used to design the epitope molecule with the help of PDB ids. This is even used in the minimization of the epitope molecule.

Hex 6.3: In this Insilco designing of vaccine Hex software is used to dock the molecules. Docking is done between the epitope molecule and the ligand (MHC molecule). From this the C docker energy is determined. Through this docker energy one can estimate whether it is the best vaccine candidate or not to a particular disease.

4.1. Screening of Proteins

Screening of proteins is the primary step in the Insilco analysis which is done with help of one of the bioinformatics tool called SDSC workbench. From this tool the 2000 odd protein sequences are screened and the best protein sequence is determined based on their least identity and least E value. Once the least identity sequence is obtained then it is sent to immunomedicine group and Mappp to find out the antigenic determinants and MHC cleavage score respectively.

The following protein sequence with least identity was found to have the following features; Least identity is 24.14, Gene ID was found to be -238064817 and the accession number is DAPE_RICRO

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Table-2.- Query results

Query results							
Protein position	**Length**	**Sequence**					
Epitope	**Position**	**MHC type**	**n-mer**	**Overall score**	**Cleavage Probability**	**MHC binding score**	**Group**
0..380	381	MYINYLKDLISFKSVTPKSD..KISDLQKLYDVYYNFLMEIL					
MYINYLKDLI	0	H2_Kd	10	0.8929	1.0000	0.7857	same length
MYINYLKDLI	0	H2_Kd	10	0.8929	1.0000	0.7857	c-term. trimmed
KDLISFKSV	6	H2_Kk	9	0.8328	0.9990	0.6667	c-term. trimmed
SDGAIEYI	18	H2_Kk	8	0.8245	0.9824	0.6667	trimmed twice
VVLEGNHEL	71	HLA_A_0201	9	0.8332	0.9997	0.6667	trimmed twice
AIACFLAASL	106	HLA_A_0201	10	0.8823	1.0000	0.7647	same length
AIACFLAASL	106	HLA_A_0201	10	0.8824	1.0000	0.7647	c-term. trimmed
DFKGSISFLL	123	H2_Kd	10	0.8189	0.9949	0.6429	same length
KEIGDAIKI	170	H2_Kk	9	0.9000	1.0000	0.8000	c-term. trimmed
RRGSVNFKL	180	HLA_B_2705	9	0.8609	0.9650	0.7568	c-term. trimmed
YPHKANNPL	199	HLA_B_0702	9	0.8143	1.0000	0.6286	c-term. trimmed
YPHKANNPL	199	H2_Ld	9	0.8710	1.0000	0.7419	c-term. trimmed
FVKNYCPL	336	H2_Kb	8	0.8226	1.0000	0.6452	trimmed twice
RFVKNYCPLV	335	H2_Kd	10	0.8214	1.0000	0.6429	c-term. trimmed
FVKNYCPL	336	H2_Kb	8	0.8226	1.0000	0.6452	c-term. trimmed
LLSETAHKI	348	HLA_A	9	0.8470	0.9996	0.6944	c-term. trimmed
YDVYYNFL	369	H2_Kb	8	0.8226	1.0000	0.6452	c-term. trimmed
DVYYNFLMEI	370	H2_Db	10	0.8333	1.0000	0.6667	c-term. trimmed
VYYNFLMEIL	371	H2_Kd	10	0.8750	1.0000	0.7500	n-term. trimmed
VYYNFLMEIL	371	H2_Kd	10	0.8750	1.0000	0.7500	same length

Fig.3. Epitope molecule and MHC 1 molecule before docking

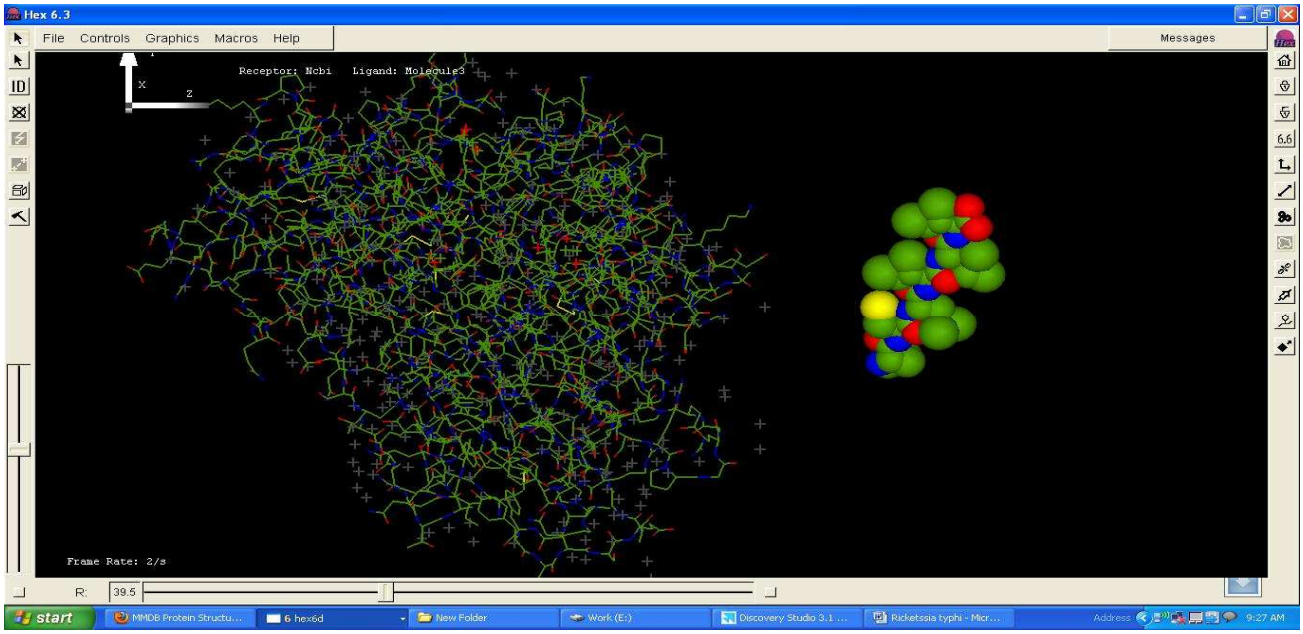

Fig.4 Docked Rickettsia Molecule

Docking: - The Epitope molecule is successfully docked with MHC 1 molecule.