IN SILICO PREDICTION OF EPITOPES OF COMMENSAL VIRUS THAT CROSS-REACT WITH HUMAN AUTOANTIGENS

A DISSERTATION

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CANDIDATE'S DECLARATION

I, hereby certify that the work is which is presented in Major Project –II entitled "In silico prediction of epitopes of commensal virus that cross-react with human autoantigens" in fulfilment of the requirement for the award of the degree of Master of Science (M.Sc.) in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi, is an authentic record of my own, carried out during a period from 7th Jan 2021 to 28th May 2021, under the supervision of Dr. Asmita Das.

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Vanshika Kothari

ABSTRACT

The prevalence and epidemiology of autoimmune diseases in developed, as well as in developing countries have increased over the past decade. The human body consists of trillions of microorganisms and the composition is unique to each individual. It consists of commensal as well as pathogenic viruses. The interactions between host-microbiota helps to regulate immune system. However, there are many factors that can alter the interactions which ultimately leads to dysbiosis. Dysbiosis can lead to development of autoimmune diseases along with other complex diseases. Viruses are obligate intracellular parasites. Commensal viruses is a new concept because there can be some viruses which may not be detrimental to human body. However, sometimes autoimmune reactions are generated as a result of cross-reactivity of epitopes of virus with autoantigens of humans. This study aims, to find various commensal viruses found in human body, to predict potential epitopes in viruses, sequence homology with autoantigens of humans and to check binding energy of viral epitopes with MHC class I and T-cell receptor. This will help us to develop new preventive and therapeutic strategies.

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LIST OF ABBREVIATIONS

SERIAL NO.	ABBREVIATIONS	EXPLAINATION
1	AD	Autoimmune Diseases
2	CNS	Central Nervous System
3	CD	Coeliac Disease
4	MHC	Major Histocompatibility Complex
5	Tregs	Regulatory T cells
6	HV	Herpes Virus
7	PAMPs	Pathogen-Associated Molecular Patterns
8	AIRE	Autoimmune Regulator
9	FEZF2	FEZ Family Zinc Finger 2
10	HIV	Human immunodeficiency viruses
11	NK	Natural Killer
12	VdW	Van der Waals
13	ACE	Atomic Contact Energy
14	HB	Hydrogen Bonds
15	TCR	T Cell Receptor
16	CAV9	Coxsackievirus A9

CHAPTER 1

AUTOIMMUNITY

Autoimmune diseases (AD) arises due to abnormal immune response resulting from recognising self and non-self-antigens. Any deficiency in the ability of the adaptive immune response to recognise and distinguish self and non-self-antigens may increase susceptibility to different types of infection and even cancer in many cases. Although, it was earlier believed that AD are rare. However, with the increase in epidemiological studies it was shown that AD now affects approximately 5% of population. Studies also reflect that two diseases that commonly affect population are type I diabetes and thyroid diseases. AD may affect particular organ or in some cases multiple organs also as seen in systemic lupus erythematosus [1]. More than 80-100 types of AD have been identified till now [2]. Research is still going on to understand etiology of various AD discovered. Age, genetics, environment, bacterial, fungi and viral infections are few factors that have been linked to the autoimmune responses.

1.1 THE IMMUNE SYSTEM AND AUTOIMMUNITY

Autoimmunity is defined as a mechanism which occurs when an organism is not able to recognise its own parts referred to as “self” that leads to an immune response which is generated against its own cells and tissues. This aberrant immune response may result in disease known as AD [3]. Reaction that occurs between the auto reactive T lymphocytes or autoantibodies of immune system against organism’s autoantigen i.e. own antigens.

It can be classified as physiological and pathological autoimmunity. Physiological autoimmunity also referred as natural autoimmunity is commonly transient where no evidence of clinical disease been reported [4]. Autoantibodies that are normally found in healthy people include antinuclear antibodies and rheumatoid factor. It is seen that their prevalence increases with age. Pathological autoimmunity occurs when self-reactive lymphocytes and autoantibodies gets involved in the process of inflammation. This results in tissue damage.

1.2 IMMUNE TOLERANCE

Immune Tolerance and Gut Microbiota Immune tolerance is characterized as a state of unresponsiveness when exposed to substances or tissues and they have the ability to elicit an immune response. It is accomplished by central and peripheral tolerance process [5]. In thymus, T cells that reacts with MHC class I or MHC class II are positively selected for survival which ultimately results in $CD8^+$ OR $CD4^+$ T cell selection. T cell binding to self too stringently die by apoptosis since they are negatively selected for survival. Thymic epithelial cells expresses self-antigens and their expression is regulated by AIRE and FEZF2 transcription factors. B cell selection occurs in bone marrow by the same process. Since, central tolerance is incomplete mature B and T cells after exiting from periphery are subjected to additional tolerance. Various mechanisms makes sure that autoreactive lymphocytes that may have escaped from periphery are removed. Tregs cells help suppress autoreactive B and T cells by silencing via apoptosis, ignorance or unresponsiveness.

1.3 SELF ANTIGENS OR NON SELF ANTIGENS

At the time of birth, mammals are born sterile. Mode of delivery determines type of microbiota infant will be exposed to. Infants born through normal delivery are subjected to vaginal microbiota of mother whereas infants born by caesarean section are not [6]. Many factors that may alter the gut microbiota include mode of delivery, diet intake, geography, and use of antibiotics.

Host environment and its comparison with genetics involved in shaping composition is still under debate. The human gut consists of trillions of microorganisms and the composition is unique to each individual. Although upon analysing the gut microbial communities in case of monozygotic and dizygotic twins it is seen that the degree of variation is somewhat similar in both the pairs. Humans intestine acquire more than 10 times bacterial cells that it actually not present in either of germ or somatic cells. So, where does the concept of self and non-self-starts?

Epigenetic or an environmental factor are two factors which our under debate and what actually defines the microbiome. It is believed that the microbiota composition which is unique and core to an individuals is defined as "self". Our body develops mechanisms that tends to avoid attack on those tissues. Ultimately this helps to maintains balance with the symbionts [7].

1.4 IMPORTANCE OF COMMENSAL MICROBIOTA FOR MAINTAINING HEALTH

There are various communities of microorganisms residing in the various parts of the human system which are non-pathogenic. These are referred to as "commensal microbiota". The interactions between the host and the commensal microbiota have also been studied and is summed up by 3 major aspects. The 1st role of commensal which is studied that they produces essential nutrients which is required by the host. These nutrients are shared by the commensals to host in order to conduct many functions of animal physiology. However, under regular conditions a germ free mice i.e. sterile lacks microbiota is not viable. This indicates the importance and dependence of microbiota to host.

On the same hand, many microbial depends on host as they lack metabolic pathways. These microbial communities are also dependent on other microbial communities residing in the human system [8]. 2nd role emphasises the importance of how commensal microbes makes it difficult for the pathogenic community to inhabit in the human system. This is known as "colonisation resistance" [9].Lastly, it is also studied that development of host is affected by the microbial

community. Abnormalities and underdevelopment of various system have been seen due to lack of microbiota. This is found both in simple and complex organisms. Introducing normal microbiota have resulted in restoring the activities of immune system [10]. However, on the same hand it was also studied that lack of microbes during critical period of growth and development resulted in loss of some functions of the immune system for good [11].

The interactions between host-microbiota may also result in development of food allergies as well as autoimmunity along with other complex diseases. Many factors that may alter the gut microbiota include mode of delivery, diet intake, geography, and use of antibiotics. Commensals microbiota is also responsible for protecting the host against pathogen by process known as "tolerance to pathogens" [12][13]. They help reduce the damage with the help of host immune. Since each individual has a unique structure of commensal microbiota as it is largely shaped by various factors such as diet, genetic makeup as well the kind of environment the individual lives in. These factors largely determines the ability of the host in fighting against pathogens and any dysbiosis may result in development of AD as well as other complex diseases.

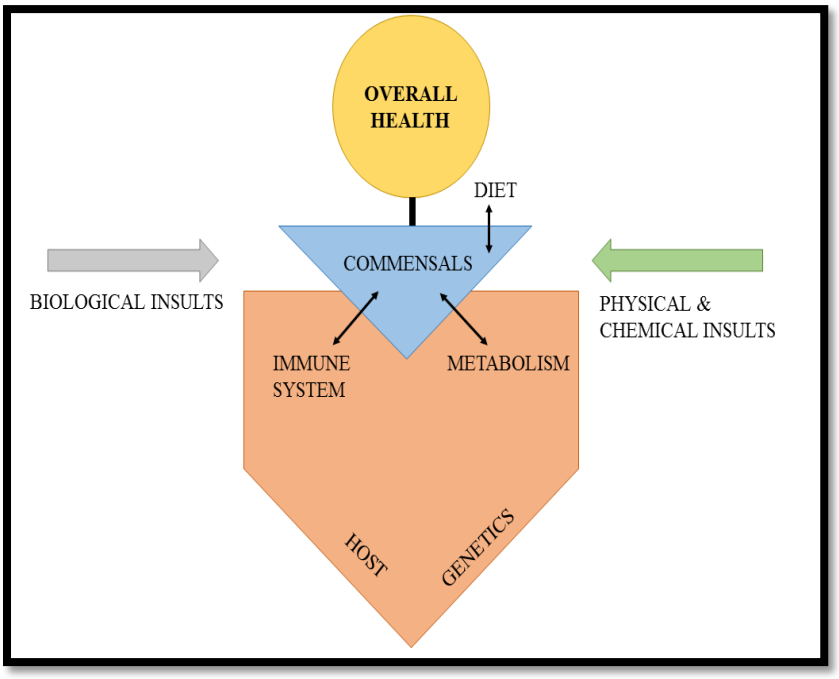

Figure 1.1 Commensals are directly affected by the diet host dietary intake, host metabolism, and immune system

Kothari & Das (Manuscript under preparation)

1.5 ENVIRONMENT AND AUTOIMMUNITY

Biological, physical and chemical insults are the environmental factors that may be linked with autoimmunity. Studies suggest that microbiota is linked with autoimmunity. Autoimmunity is different from inflammatory diseases as inflammatory diseases are not directed against self-antigens [14]. Charles Janeway, gave the theory how microbiota is linked with autoimmunity. His theory formulated the pathway that initiated adaptive immune response. In this theory he talks about the connection between the innate-adaptive system. The ability to distinguish between self and non-self is a key feature of innate system. It can recognise pathogen-associated molecular patterns (PAMPs) which are actually conserved. However, pathogens have different PAMPs. Antigens are either processed into peptide which is then complexed with either MHC class I or class II or in the native form. This recognition is done by adaptive immune system.

Peripheral tolerance and central tolerance helps reduce the chance for development of autoimmunity. However one can also find potential autoreactive cells in humans. The connection between innate and adaptive immune system is used by the microbiota in order to exercise its role. There are 2 groups by which one can classify AD. This is based on whether or not the connection between the innate and adaptive system is involved [14]. In case of group I, this connection is responsible for the onset of disease and on the other hand the connection is not important in group II diseases. AIRE, which is a transcription factors and any mutation in this transcription factor may give rise to group II disease [15]. Ability of T cells to perform negative selection is lost. Group II disease may also occur when mutations occur in regulatory T cells FoxP3 [16]. Commensal microbiota plays a key role in development of group I diseases. This study came from an experiment which studied whether or not outcome of a disease changes when no microbiota is present or a when one is replaced with another microbes. It was carried out in germ-free and gnotobiotic animals.

1.6 MICROBIOTA QUENCHING THE DEVELOPMENT OF AUTOIMMUNITY

Commensal microbes contains individual lineages and this can ultimately play an important role in development of autoimmunity. This is specific lineage hypothesis. According to this hypothesis, an individual first acquire microbiota from the mother which is eventually changed as the infant interacts with various factors discussed before. As an individual interacts with the environment there are certain mutations which are introduced that may affect the composition of the microbiota. This imbalance can eventually result in autoimmunity. Another hypothesis, balanced signal hypothesis has a different view point. According to this hypothesis multiple lineages can also provide the same role that specific lineages provide. In this interaction is defined by the host genetics with microbiota. This can result in autoimmunity if homeostasis is disturbed [8]. Microbiota needs to minimise the impact of host on its community and the host in order to prevent disturbances needs to have a control on microbiota.

The mechanism by which microbiota affects the host and leads to development of autoimmunity may vary with disease to disease. These mechanisms have been less studies in higher organisms so more research is needed to confirm it [14].

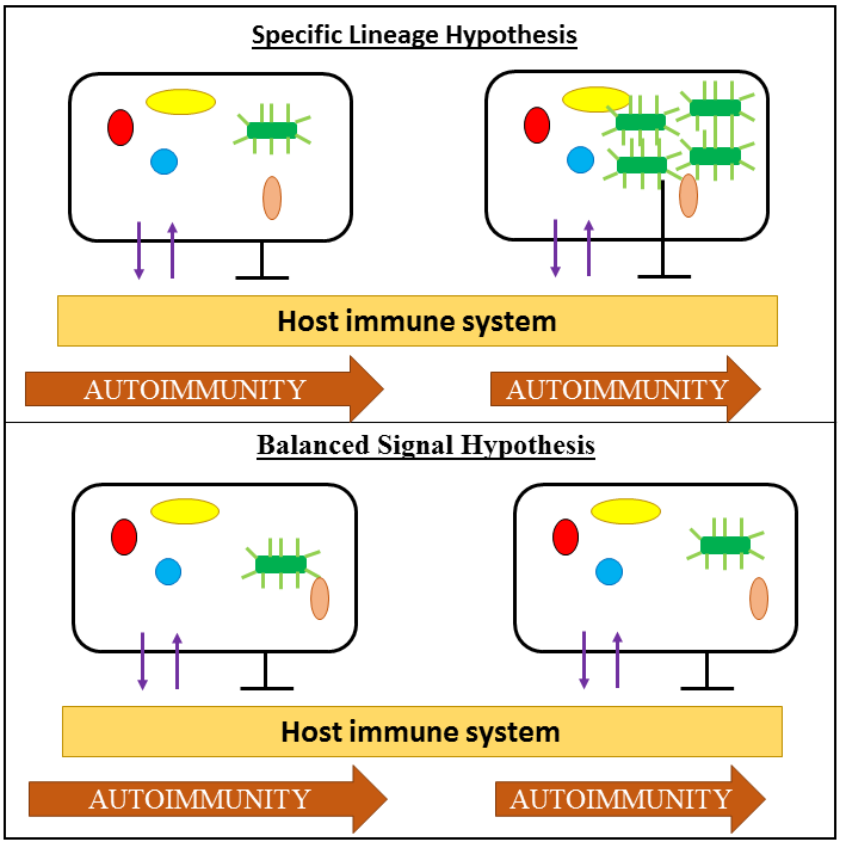

Figure 1.2 Specific lineages hypothesis and balanced lineage hypothesis

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1.7 EPIDEMIOLOGY OF AUTOIMMUNITY

Earlier it was believed that AD are rare and uncommon. But soon effects morbidity and mortality rates became significant. 4-5% of the human population suffers from autoimmunity [17]. Research

has made it possible to diagnose and treat AD. However, the cause and development of AD is still under study. One can find differences in prevalence and incidence amongst different types of AD. When various factors like age, geography, and gender are studied geoepidemiology looks more complex. According to a research, in monozygotic twins prevalence is much higher when compared with other patients [18]. It is also observed that women shows higher frequency of AD than men. However, the reason behind the bias amongst sex is still unclear. One can find differences in incidence and prevalence within different landscapes. The data of multiple sclerosis is a great example of landscapes differences. Europe reports 1-8 cases per 100,000 person and 1-3 cases per 100,000 in Asia [19]. In UK, Coeliac disease (CD) was reported in 20 per 100,000 person [20]. The data obtained from different countries shows that environmental and genetic factors are key factors that have been linked with loss of tolerance [21].

1.8 COMMENSAL VIRUS

It was in 1999, when the concept of commensal virus came into the scientific field. According to P. Griffiths there was a possibility that human body contains commensal virus also and they do exist [22]. Advancement in next generation sequencing made it possible to discover origin of various diseases which earlier didn't had a specific origin. It made it possible to describe etiology of the disease. One example would be infection of the CNS caused by astrovirus [23]. This is made it possible to characterise novel viruses also like Ebola virus [24]. It has also discovered novel viruses that are residing in healthy human body. Last 20 years have been years when researches confirmed that commensal viruses do exist and they comprise human virome. Pegivirus, was seen in blood samples of many donors. Another example is Torque tenovirus [25]. Taking the example of Pegivirus, commensal virus came into existence. It was thought that it causes hepatitis. However, later it was rejected. The concept behind the rejection was that Pegivirus helped reduced activation of NK cells, B cells, T cells and monocytes. As a result of this, HIV progression was reduced. Mortality related to hepatitis was also decreased [26]. According to H.W Virgin, human virome can be more than just a source of pathogens [27]. Virus maintains a state of equilibrium

with other microbiome as well as immune system. As discussed above that each individual has unique microbiota and in the same way there is distinct virotype and immunophenotype. Virus may also provide resistance against some other bacterial infections. Such as resistance to *Listeria monocytogenes* and *Yersinia pestis* is provided by infection with γ HV. But it was also reported that cytomegalovirus promotes *Pneumocystis jiroveci* infection [28]. Thus, virus not only provides resistance but they may also team up with microbial communities against host.

The role of bacteria in microbiota residing in gut has gain interest from the past few decades. On the same hand, how virus living in human body is influenced by factors like age, environment, diet and, antibiotics. One can find wide varieties of RNA and DNA virus in human system. Few examples are anellovirus, adenovirus, rotavirus and many more. Microbiota residing in human body is largely shaped during the early years of life [29]. It has also been studied that interactions takes place between different communities of microbiota, which either promote viral infections or promote viral clearance [30]. Rotavirus infections can be cleared by flagellin. On the other hand, components of bacterial membrane can help enhance polio viral infections. More work is required in the field of transkingdom in order to study more about the interactions between different microbial communities.

1.9 MECHANISMS BY WHICH VIRUS INDUCES AUTOIMMUNITY

There are various mechanisms that have been proposed to explain the process by which viral infections can lead to breakdown of self-tolerance. Viruses carry structurally similar antigens to that of self-antigens. These structurally similar antigens can then activate both B and T cells which ultimately leads to a cross-reactive response against both self-antigens and non-self-antigens. This is “molecular mimicry” [31]. It is seen in many cases[32].

Second mechanism is “bystander activation” in which host and microbe do not share structurally similar antigens. In this mechanism induction of costimulation as well as cytokine production by an antigen presenting cell activated by viral infection, along with this presents self-antigens will ultimately result in activation of autoimmunity. [33]. Third mechanism is known as “epitope spreading”. Viral infection triggers the release of more self-antigens and de novo activation of autoreactive cells. This ultimately is responsible for the spread to target additional self-epitopes [34].

Many cases of molecular mimicry as well as bystander activation are reported [35], [36] and many other autoimmune disorders.

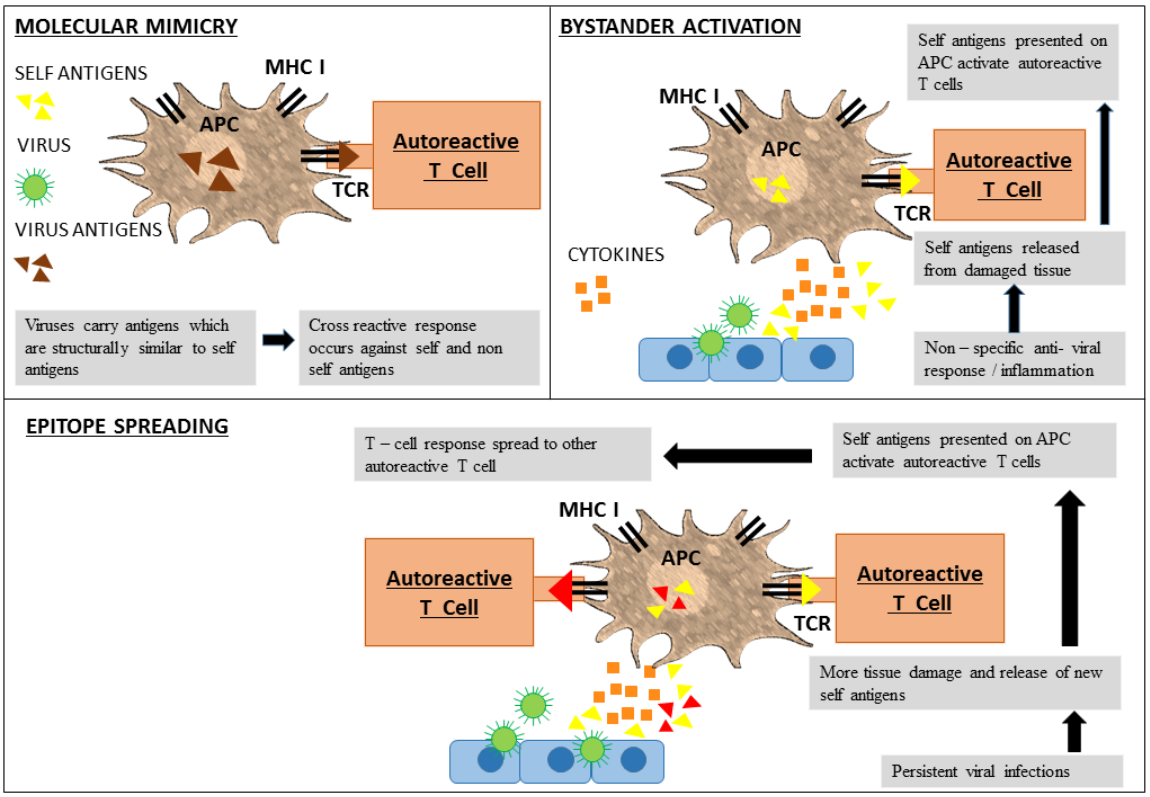

Figure 1.3 Mechanism of virus induced autoimmunity
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1.10 COXSACKIEVIRUS A9 (CAV9)

Coxsackievirus A9 is a single stranded RNA virus and case wide range fatal infections in the CNS. 28 nm in diameter, capsid is nonenveloped and symmetry is icosahedral. Its genome contains 7,452 nucleotides [37]. Capsid contains 4 viral proteins refereed as VP1, VP2, VP3 and VP4. It uses cellular receptors which belong to integrin family $\alpha_{v.}$ With the help of these receptors it binds to host cells before entry and release of genome. Highest affinity studied till now is $\alpha_v\beta_{6.}$ It can cause wide range of diseases such as respiratory infections, CNS infections, aseptic meningitis and Type I Diabetes.

In this study we have checked whether Coxsackievirus A9 can trigger autoimmune reactions and cause autoimmunity. Genome polyprotein is used as an antigen for the study. Sometimes autoimmune reactions are generated as a result of cross-reactivity of epitopes of virus with autoantigens of humans. B and T cell epitopes were predicted to study whether it can trigger both humoral as well as cell mediated immunity. It's similarity with human protein sequence was also studied. The peptide with the highest binding affinity with TCR and MHC class I can be further used for therapeutic purposes in treating Type I diabetes.

CHAPTER 2

MAIN THEME OF THE WORK

2.1 MATERIALS AND METHODS:

Servers used:

i) PEP-FOLD 3
ii) PatchDock
iii) FireDock

Database used:

i) Immune Epitope Database (IEDB)
ii) Protein Data Bank
iii) Uniprot
iv) National Centre for Biotechnology Information (NCBI)
v) Protein BLAST

Software used: PyMOL

2.2 METHODS

Literature review was first carried out to study names of commensal viruses linked with autoimmune diseases. Their pathway and mode of infection was also studied.
UniProt is a database used for retrieving FASTA sequence of the obtained antigen of a particular virus. [UniProt]

IEDB tool is used for B cell and T cell epitope prediction. [IEDB.org: Free epitope database and prediction resource]. IEDB contains list of epitopes from patent applications and peer reviewed papers. FASTA sequence retrieved from UniProt is given as input in B cell epitope prediction of linear peptides from protein sequence. Obtained longest peptide is then given as an input in T cell epitope prediction tool i.e. TepiTool. Output obtained is then saved for further use in protein BLAST.

Protein BLAST compares query sequences to protein database. Predicted peptides are compared to protein sequences of humans to determine the similarity between the 2 sequences.
[Protein BLAST: search protein databases using a protein query (nih.gov)]

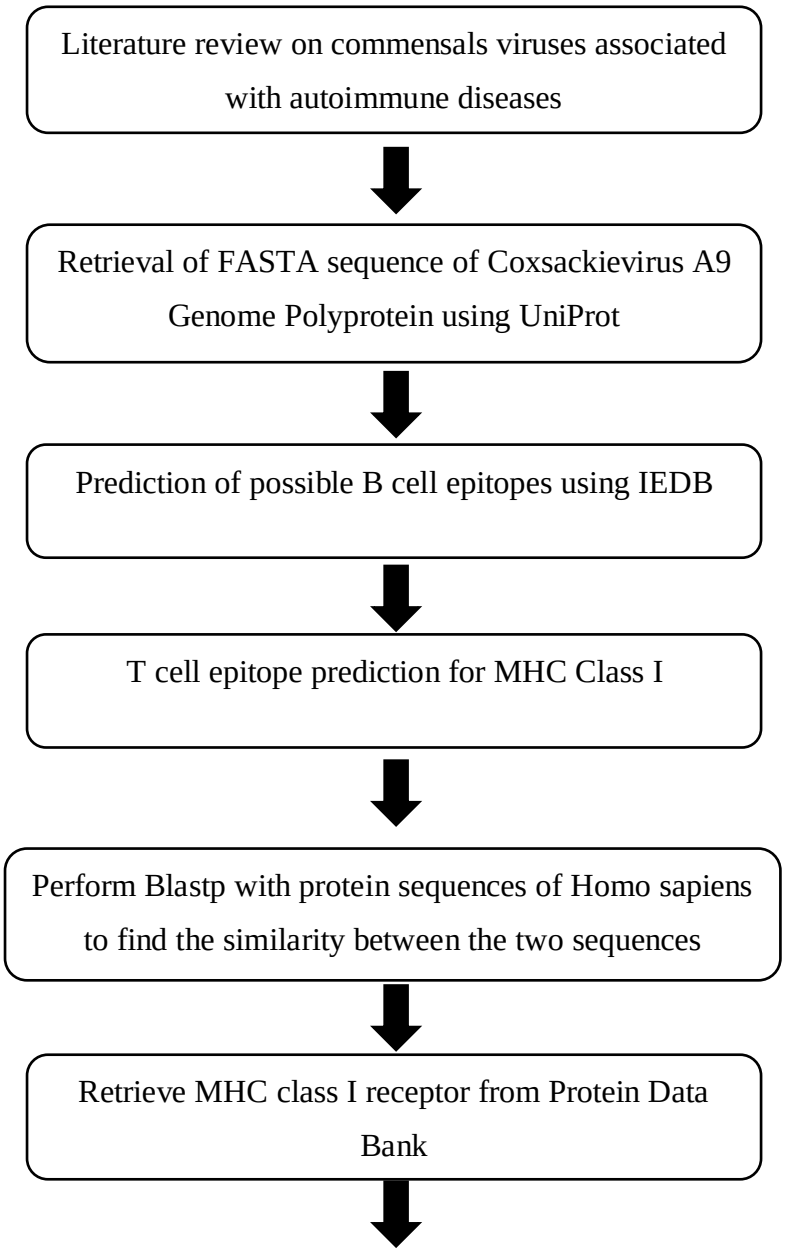
PEP-FOLD3 is used for obtaining peptide structures from the sequence of amino acid given as input. Predicted peptides is used to obtain PDB files. [RPBS Web Portal (univ-paris-diderot.fr)]

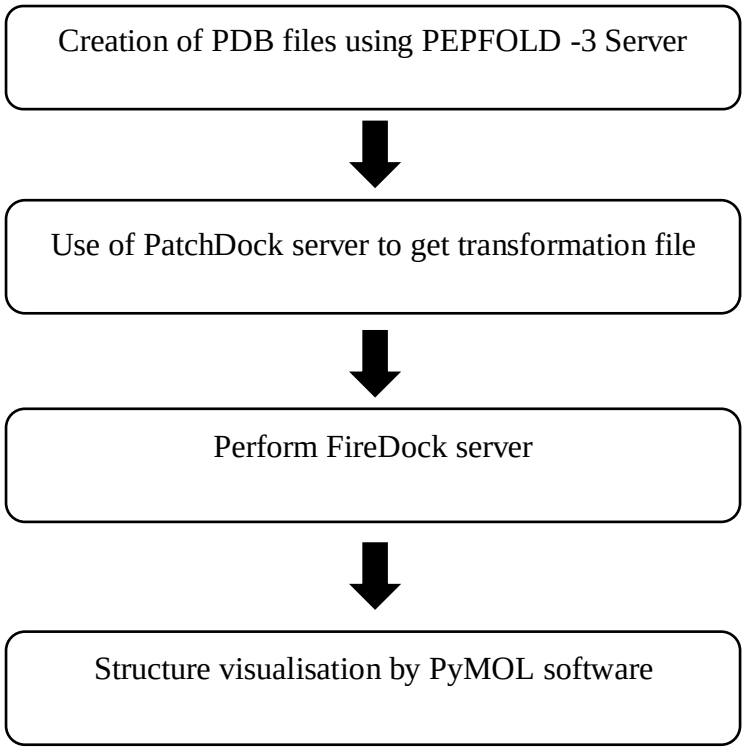
PatchDock server is used for molecular docking. The result contains potential complexes list that are sorted by shape complementarity criteria. PDB files of both receptor and ligand is loaded onto PatchDock to obtain the desired output. [PatchDock Server: An Automatic Server for Molecular Docking (tau.ac.il)]

FireDock server is used to provide scoring of protein-protein docking solutions. Transformation file obtained from PatchDock along with the receptor and ligand PDB files are loaded onto the server. [FireDock Server (tau.ac.il)]

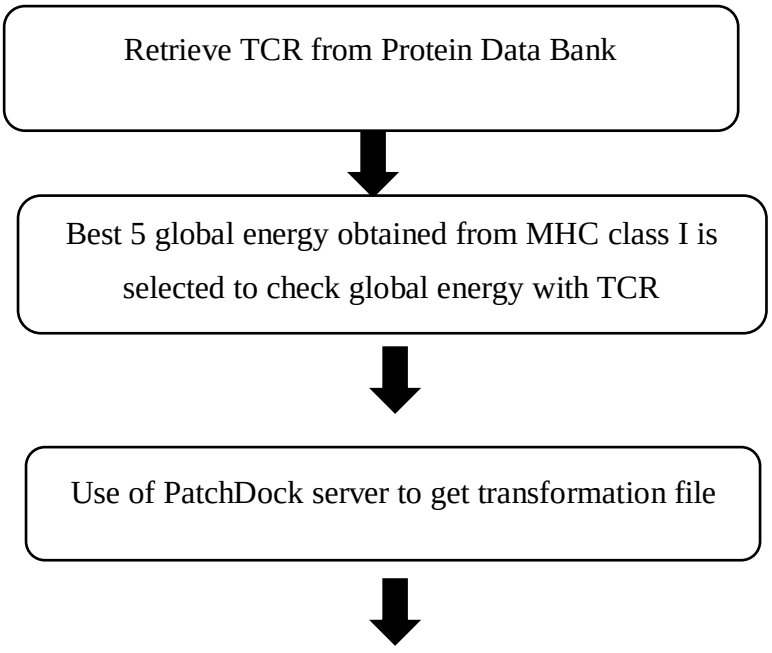
PyMOL is a molecular visualisation software that helps produce high quality of 3D images. Best global energy structure obtained from FireDock is loaded onto PyMOL. The obtained image is then saved in PNG format.

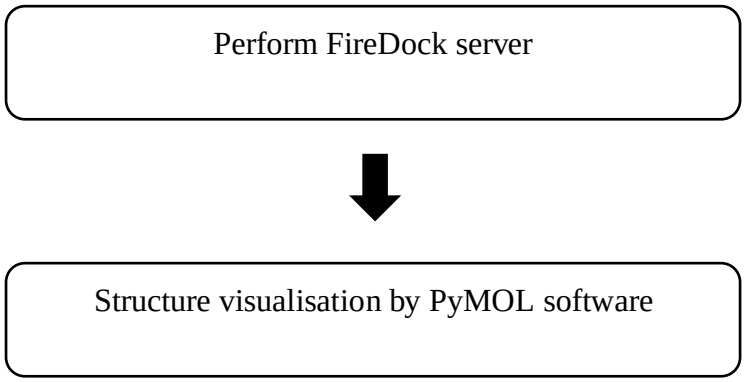
2.3 WORKFLOW TO DETERMINE GLOBAL ENERGY OF PEPTIDES FOR MHC CLASS I





2.4 WORKFLOW TO DETERMINE GLOBAL ENERGY OF PEPTIDES FOR TCR




Perform FireDock server
Structure visualisation by PyMOL software

CHAPTER 3

RESULT, DISCUSSION AND CONCLUSION

3.1 Genome polyprotein of Coxsackievirus A9

Table3.1 Genome Polyprotein cleaved into 17 following chains

1	P1
2	Capsid Protein VP0
3	Capsid Protein VP4
4	Capsid Protein VP2
5	Capsid Protein VP3
6	Capsid Protein VP1
7	P2
8	Protease 2A
9	Protease 2B
10	Protease 2C
11	P3
12	Protease 3AB
13	Protease 3A
14	Viral protein genome linked
15	Protease 3CD
16	Protease 3C
17	RNA directed RNA polymerase

Genome polyprotein of Coxsackievirus A9 is cleaved into 17 chains. The protein is further studied whether or not it can trigger autoimmune reactions.

3.2 B CELL EPITOPE PREDICTION RESULTS

Coxsackievirus A9 which is cross reacting with human autoantigens that may cause autoimmunity by triggering B cell activation. Hence to check whether B cell epitopes of coxsackievirus A9 can activate humoral immunity B cell epitope prediction was carried out.

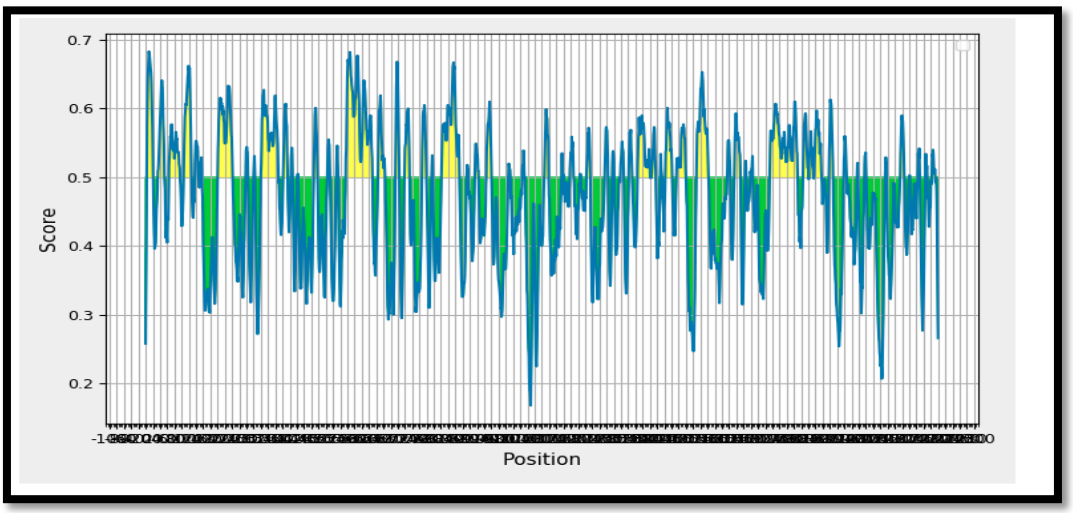
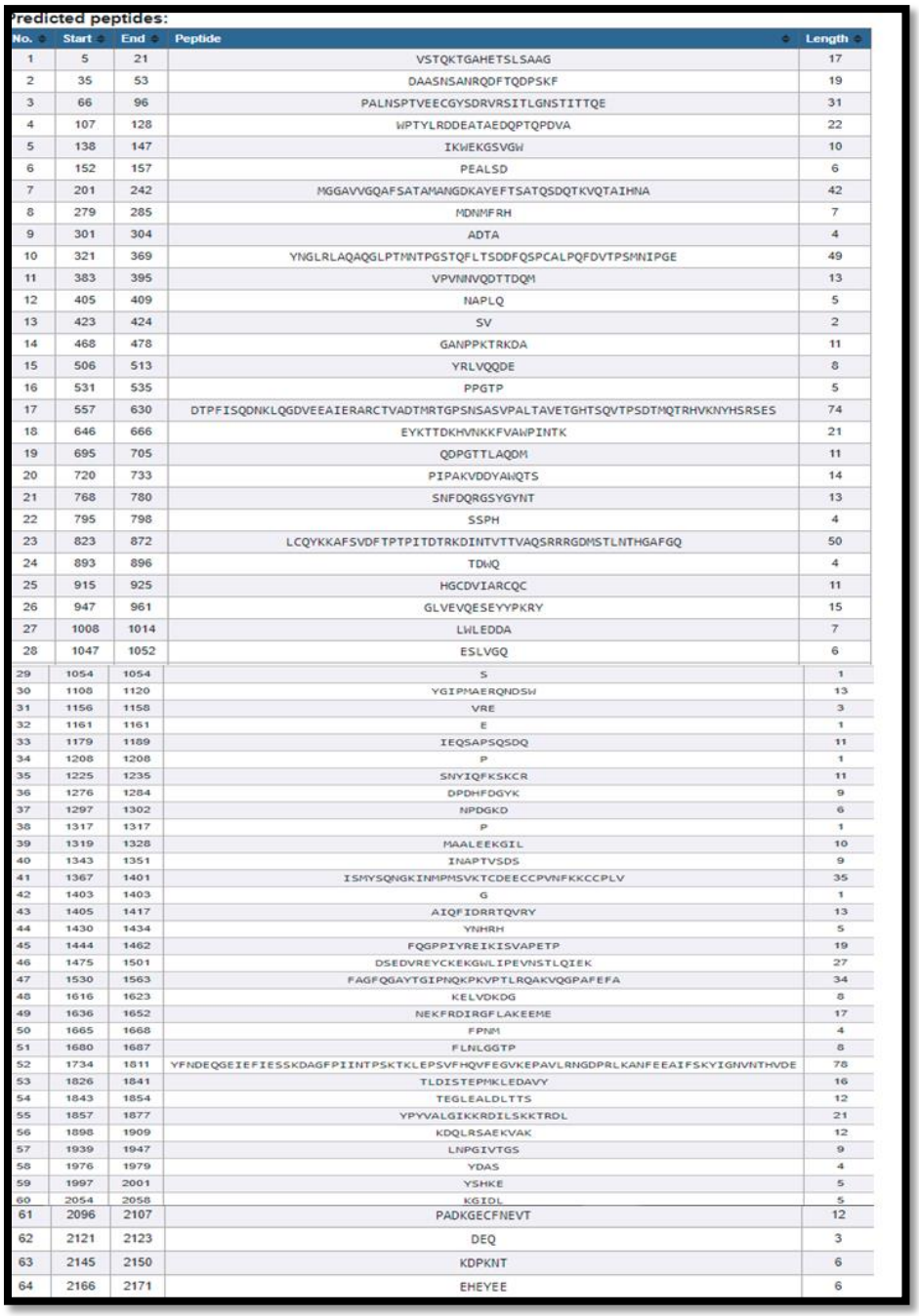


Figure3.1 Bepipred Linear Epitope Prediction of Coxsackie A9 (Genome Polyprotein)



Predicted peptides:

No.	Start	End	Peptide	Length
1	5	21	VSTQKTGAHETSLSAAG	17
2	35	53	DAASNSANRQDFTQDPSKF	19
3	66	96	PALNSPTVEECGYSDRVRSITLGNSTITTQE	31
4	107	128	WPTYLRDDEATAEDQPTQPDVA	22
5	138	147	IKWEKGSVGW	10
6	152	157	PEALSD	6
7	201	242	MGGAVVGQAFSATAMANGDKAYEFTSATQSDQTKVQTAIHNA	42
8	279	285	MDNMFRH	7
9	301	304	ADTA	4
10	321	369	YNGLRLAQAQGLPTMNTPGSTQFLTSDDFQSPCALPQFDVTPSMNIPGE	49
11	383	395	VPVNNVQDTTDQM	13
12	405	409	NAPLQ	5
13	423	424	SV	2
14	468	478	GANPPKTRKDA	11
15	506	513	YRLVQQDE	8
16	531	535	PPGTP	5
17	557	630	DTPFISQDNKLQGDVEEAIERARCTVADTMRTGPSNSASVPALTAVETGHTSQVTPSDTMQTRHVKNYHSRSES	74
18	646	666	EYKTTDKHVNKKFVAWPINTK	21
19	695	705	QDPGTTLAQDM	11
20	720	733	PIPAKVDDYAWQTS	14
21	768	780	SNFDQRGSYGYNT	13
22	795	798	SSPH	4
23	823	872	LCQYKKAFSVDFTPTPITDTRKDINTVTTVAQSRRRGDMSTLNTHGAFGQ	50
24	893	896	TDWQ	4
25	915	925	HGCDVIARCQC	11
26	947	961	GLVEVQESEYYPKRY	15
27	1008	1014	LWLEDDA	7
28	1047	1052	ESLVGQ	6
29	1054	1054	S	1
30	1108	1120	YGIPMAERQNDSW	13
31	1156	1158	VRE	3
32	1161	1161	E	1
33	1179	1189	IEQSAPSQSDQ	11
34	1208	1208	P	1
35	1225	1235	SNYIQFKSKCR	11
36	1276	1284	DPDHFDGYK	9
37	1297	1302	NPDGKD	6
38	1317	1317	P	1
39	1319	1328	MAALEEKGIL	10
40	1343	1351	INAPTVSDS	9
41	1367	1401	ISMYSQNGKINMPMSVKTCDEECCPVNFKKCCPLV	35
42	1403	1403	G	1
43	1405	1417	AIQFIDRRTQVRY	13
44	1430	1434	YNHRH	5
45	1444	1462	FQGPPIYREIKISVAPETP	19
46	1475	1501	DSEDVREYCKEKGWLIPEVNSTLQIEK	27
47	1530	1563	FAGFQGAYTGIPNQKPKVPTLRQAKVQGPAFEFA	34
48	1616	1623	KELVDKDG	8
49	1636	1652	NEKFRDIRGFLAKEEME	17
50	1665	1668	FPNM	4
51	1680	1687	FLNLGGTP	8
52	1734	1811	YFNDEQGEIEFIESSKDAGFPIINTPSKTKLEPSVFHQVFEGVKEPAVLRNGDPRLKANFEEAIFSKYIGNVNTHVDE	78
53	1826	1841	TLDISTEPMKLEDAVY	16
54	1843	1854	TEGLEALDLTTS	12
55	1857	1877	YPYVALGIKKRDILSKKTRDL	21
56	1898	1909	KDQLRSAEKVAK	12
57	1939	1947	LNPGIVTGS	9
58	1976	1979	YDAS	4
59	1997	2001	YSHKE	5
60	2054	2058	KGIDL	5
61	2096	2107	PADKGECFNEVT	12
62	2121	2123	DEQ	3
63	2145	2150	KDPKNT	6
64	2166	2171	EHEYEE	6

Figure3.2 Predicted peptides of Coxsackie A9 (Genome Polyprotein)

Longest peptide was chosen for its ability to trigger humoral immune response. Longer peptides are capable of acting as conformational epitopes and hence are capable of triggering potent B cell response. T cell epitope prediction was carried out within conformational B cell epitope for efficient T cell triggering capacity. Thus such an epitope would be capable of both cell mediated immunity and humoral response.

3.3 PROTEIN BLAST RESULTS

TABLE3.2 Blastp results of Coxsackievirus A9 (Genome polyprotein)

PEPTIDE	HLA CLASS I ALLELE	TARGET (HOMO SAPIENS)	% SIMILARITY
GVKEPAVLR	HLA-A*31:01	Collagen alpha-6(VI) chain isoform X2	88.89%
GVKEPAVLR	HLA-A*33:01	Collagen alpha-6(VI) chain isoform X2	88.89%
GVKEPAVLR	HLA-A*68:01	Collagen alpha-6(VI) chain isoform X2	88.89%
GVKEPAVLR	HLA-A*11:01	Collagen alpha-6(VI) chain isoform X2	88.89%
GVKEPAVLR	HLA-A*03:01	Collagen alpha-6(VI) chain isoform X2	88.89%
GVKEPAVLR	HLA-A*30:01	Collagen alpha-6(VI) chain isoform X2	88.89%
EPSVFHQVF	HLA-B*53:01	A-kinase anchor protein 6 isoform X1	85.71%
EPSVFHQVF	HLA-B*35:01	A-kinase anchor protein 6 isoform X1	85.71%
EPSVFHQVF	HLA-B*08:01	A-kinase anchor protein 6 isoform X1	85.71%
EPSVFHQVF	HLA-B*51:01	A-kinase anchor protein 6 isoform X1	85.71%
EPSVFHQVF	HLA-B*07:02	A-kinase anchor protein 6 isoform X1	85.71%

TABLE 3.2 (Continued)

IINTPSKTK	HLA-A*03:01	Diaphanous homolog 3 (Drosophila), isoform CRA_b	87.50%
IINTPSKTK	HLA-A*30:01	Diaphanous homolog 3 (Drosophila), isoform CRA_b	87.50%
IINTPSKTK	HLA-A*11:01	Diaphanous homolog 3 (Drosophila), isoform CRA_b	87.50%
FEEAIFSKY	HLA-B*44:03	Heterogeneous nuclear ribonucleoproteins C1/C2 variant, partial	100%
FEEAIFSKY	HLA-B*44:02	Heterogeneous nuclear ribonucleoproteins C1/C2 variant, partial	100%
FEEAIFSKY	HLA-A*01:01	Heterogeneous nuclear ribonucleoproteins C1/C2 variant, partial	100%
FEEAIFSKY	HLA-B*40:01	Heterogeneous nuclear ribonucleoproteins C1/C2 variant, partial	100%
FEEAIFSKY	HLA-A*30:02	Heterogeneous nuclear ribonucleoproteins C1/C2 variant, partial	100%
FEEAIFSKY	HLA-B*35:01	Heterogeneous nuclear ribonucleoproteins C1/C2 variant, partial	100%
EEAIFSKYI	HLA-B*44:02	Titin isoform IC	81.82%
EEAIFSKYI	HLA-B*44:03	Titin isoform IC	81.82%
EEAIFSKYI	HLA-B*40:01	Titin isoform IC	81.82%

TABLE 3.2 (Continued)

KTKLEPSVF	HLA-B*57:01	WASH complex subunit 2C isoform 7	88.89%
KTKLEPSVF	HLA-B*57:01	WASH complex subunit 2C isoform 7	88.89%
KTKLEPSVF	HLA-B*58:01	WASH complex subunit 2C isoform 7	88.89%
KTKLEPSVF	HLA-A*30:01	WASH complex subunit 2C isoform 7	88.89%
KTKLEPSVF	HLA-A*30:02	WASH complex subunit 2C isoform 7	88.89%
YIGNVNTHV	HLA-A*02:06	Immunoglobulin light chain junction region	83.33%
YIGNVNTHV	HLA-A*02:03	Immunoglobulin light chain junction region	83.33%
YIGNVNTHV	HLA-A*02:01	Immunoglobulin light chain junction region	83.33%
YIGNVNTHV	HLA-A*68:02	Immunoglobulin light chain junction region	83.33%
RLKANFEEA	HLA-A*02:03	Plectin	88.89%
RLKANFEEA	HLA-A*30:01	Plectin	88.89%
KYIGNVNTH	HLA-A*30:02	Diamine oxidase, copper/topa quinone containing	71.43%
KYIGNVNTH	HLA-A*24:02	Diamine oxidase, copper/topa quinone containing	71.43%
KYIGNVNTH	HLA-A*23:01	Diamine oxidase, copper/topa quinone containing	71.43%
LEPSVFHQV	HLA-B*40:01	Nucleolar complex protein 4 homolog	85.71%

TABLE 3.2 (Continued)

LEPSVFHQV	HLA-B*44:03	Nucleolar complex protein 4 homolog	85.71%
LEPSVFHQV	HLA-B*44:02	Nucleolar complex protein 4 homolog	85.71%
IESSKDAGF	HLA-B*44:02	TBC1 domain family member 5 isoform a	75%
IESSKDAGF	HLA-B*44:03	TBC1 domain family member 5 isoform a	75%
IESSKDAGF	HLA-B*40:01	TBC1 domain family member 5 isoform a	75%
KANFEEAIF	HLA-B*58:01	Mimecan isoform 1	85.71%
KANFEEAIF	HLA-B*57:01	Mimecan isoform 1	85.71%
VLRNGDPRL	HLA-A*02:03	Immunoglobulin heavy chain junction region	85.71%
VLRNGDPRL	HLA-A*02:01	Immunoglobulin heavy chain junction region	85.71%
EIEFIESSK	HLA-A*68:01	hCG1642839, isoform CRA_b	100%
GEIEFIESS	HLA-B*40:01	hCG1642839, isoform CRA_b	100%
GEIEFIESS	HLA-B*44:03	hCG1642839, isoform CRA_b	100%
SSKDAGFPI	HLA-B*30:01	TBC1 domain family member 5 isoform a	77.87%
FEGVKEPAV	HLA-B*40:01	RBAP2	87.50%
DEQGEIEFI	HLA-B*44:03	Pancreas transcription factor 1 subunit alpha	75%
DEQGEIEFI	HLA-B*44:02	Pancreas transcription factor 1 subunit alpha	75%

TABLE 3.2 (Continued)

AVLRNGDPR	HLA-B*11:01	PDZ domain-containing protein 2 isoform X1	100%
NFEEAIFSK	HLA-B*33:01	Mimecan isoform 1	87.50%
FPIINTPSK	HLA-B*35:01	Cadherin EGF LAG seven-pass G-type receptor 1 isoform 1 precursor	100%

To study the similarity of peptides of Coxsackievirus A9 with protein sequence of human's protein BLAST was performed.

3.4 FIREDOCK RESULTS

TABLE 3.3 Firedock Results of Coxsackievirus A9-MHC Class I complex

Peptide	Global Energy	Attractive VdW	Repulsive VdW	ACE	HB
1.GVKEPAVLR	-4.96	-3.18	0.26	-1.01	0.00
2.EPSVFHQVF	-8.26	-3.12	0.99	-2.87	0.00
3. IINTPSKTK	-2.27	-0.82	0.00	1.42	0.00
4. FEEAIFSKY	-8.23	-30.20	16.15	1.47	-2.49
5.EEAIFSKYI	-5.58	-1.93	0.83	-2.41	0.00
6. KTKLEPSVF*	-23.74	-37.85	62.20	-6.83	-2.09
7.YIGNVNTHV*	-27.08	-32.48	34.4	8.01	-3.77
8.RLKANFEEA	13.94	-39.47	45.16	13.17	-4.82
9.KYIGNVNTH*	-14.24	-21.37	19.21	1.93	-3.21
10.LEPSVFHQV*	-15.50	-40.32	69.37	-1.78	-3.15
11. IESSKDAGF	-2.76	-2.54	0.32	3.30	-0.53
12. KANFEEAIF	-0.16	-6.19	0.95	0.87	0.00
13.VLRNGDPRL	-2.97	-25.06	33.20	0.69	-4.89
14. EIEFIESSK	1.33	-5.42	9.98	0.01	0.00
15. GEIEFIESS	7.52	-14.32	8.49	4.44	-0.53

TABLE 3.3 (Continued)

16. SSKDAGFPI	34.58	-29.70	53.24	9.65	0.00
17.FEGVKEPAV*	-26.65	-37.69	21.20	5.41	-2.92
18. DEQGEIEFI	101.98	-32.11	168.79	7.28	-2.46
19.AVLRNGDPR	-12.53	-31.64	22.17	9.89	-4.47
20. NFEEAIFSK	-12.51	-8.01	3.79	-1.99	-0.81
21. FPIINTPSK	-12.38	-34.81	11.03	13.83	-2.03

FireDock server was performed. Out of the 21 peptides results only 5 were selected on the basis of highest binding energy with MHC class I and then FireDock server was performed for those 5 peptides with TCR.

Table 3.4 Firedock Results of Coxsackievirus A9-TCR complex

(BEST 5)

Peptide	Global Energy	Attractive VdW	Repulsive VdW	ACE	HB
6	-56.84	-32.13	13.88	-5.70	-1.99
7	-37.09	-35.30	41.44	-8.33	-3.19
9	-28.68	-28.08	17.55	1.47	-3.11
10	-83.08	-28.39	23.60	-21.15	-0.73
17	-38.52	-34.79	9.68	-2.09	-1.02

FireDock results of the 5 peptides with TCR. Peptide 10 with the highest binding efficiency with TCR.

3.5 PyMOL RESULTS OF COXSACKIEVIRUS A9 PEPTIDES COMPLEX WITH MHC CLASS I

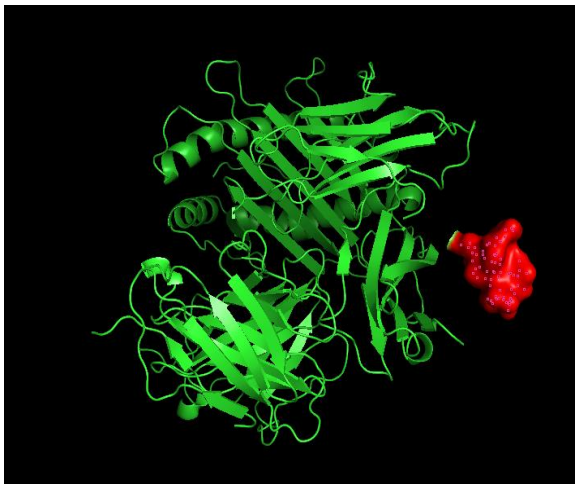


Figure 3.3 MHC class I Peptide 1 complex

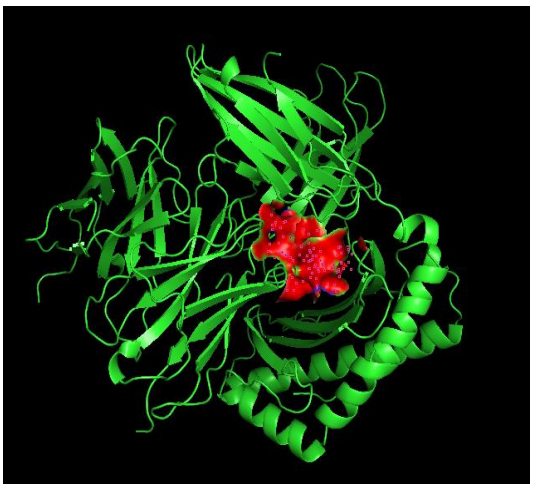


Figure 3.4 MHC class I Peptide 2 complex

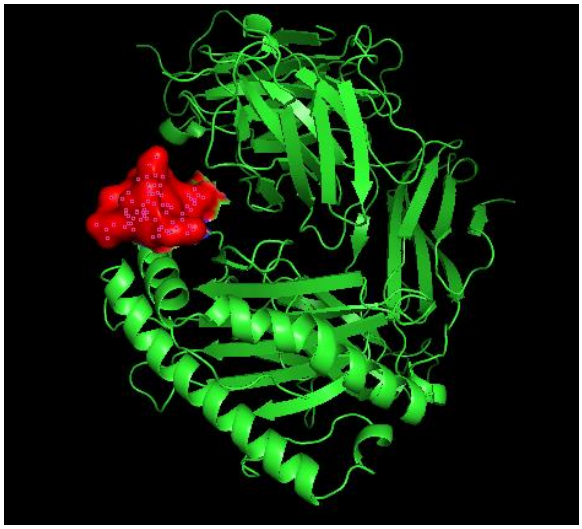


Figure 3.5 MHC class I Peptide 3 complex

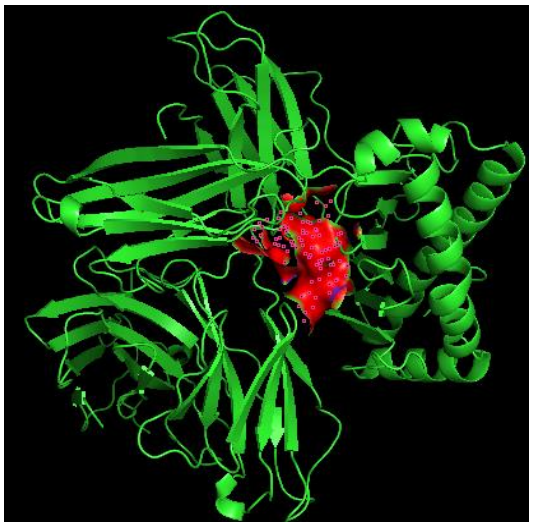


Figure 3.6 MHC class I Peptide 4 complex

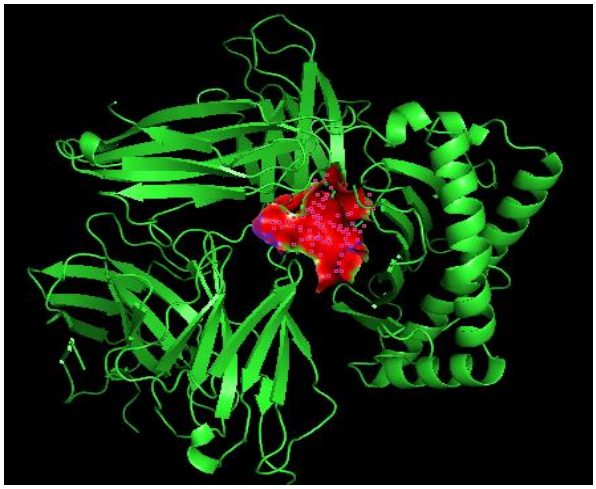


Figure 3.7 MHC class I Peptide 5 complex

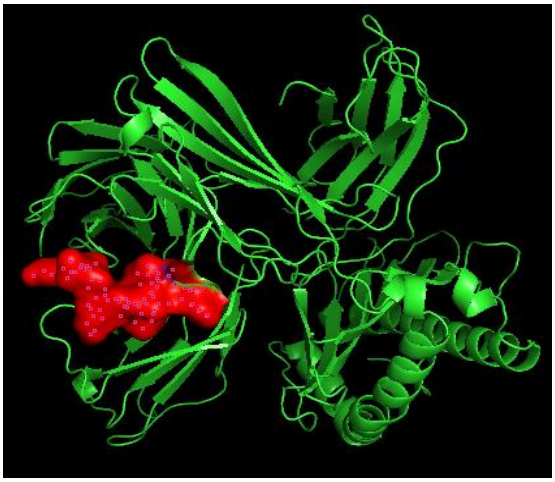


Figure 3.8 MHC class I Peptide 6 complex

Figure 3.9 MHC class I Peptide 7 complex

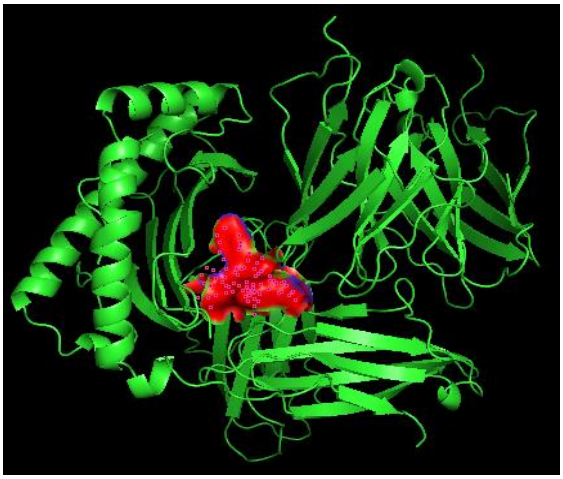


Figure 3.10 MHC class I Peptide 8 complex

Figure 3.11 MHC class I Peptide 9 complex

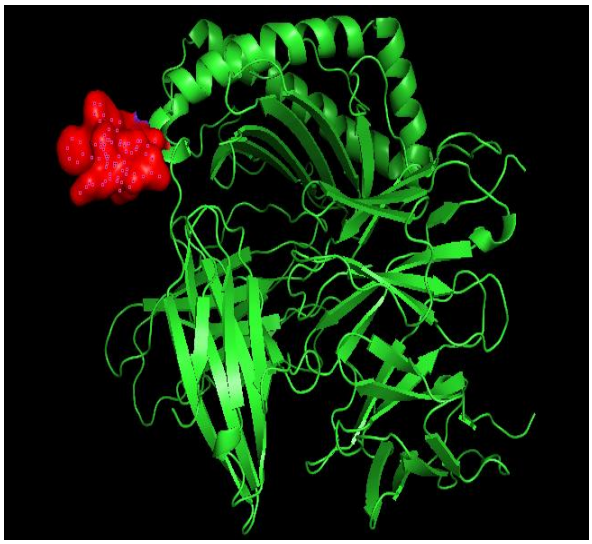


Figure 3.12 MHC class I Peptide 10 complex

Figure 3.13 MHC class I Peptide 11 complex

Figure 3.14 MHC class I Peptide 12 complex

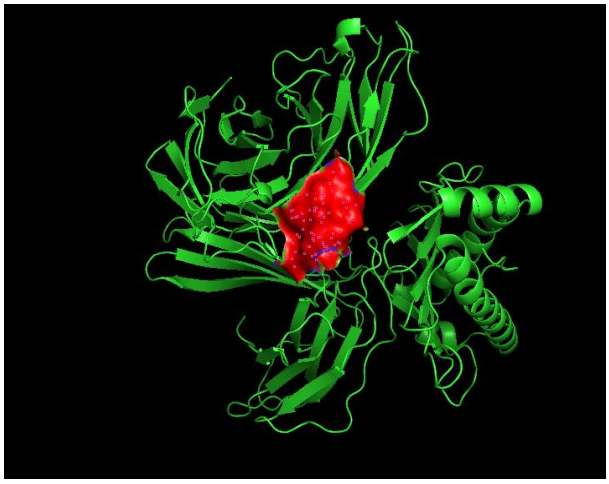


Figure 3.15 MHC class I Peptide 13 complex

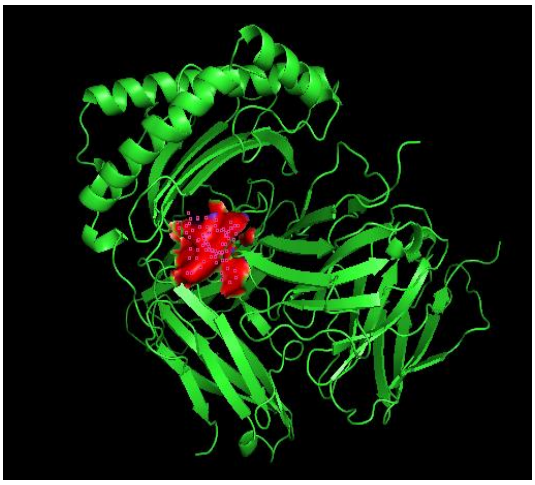


Figure 3.16 MHC class I Peptide 14 complex

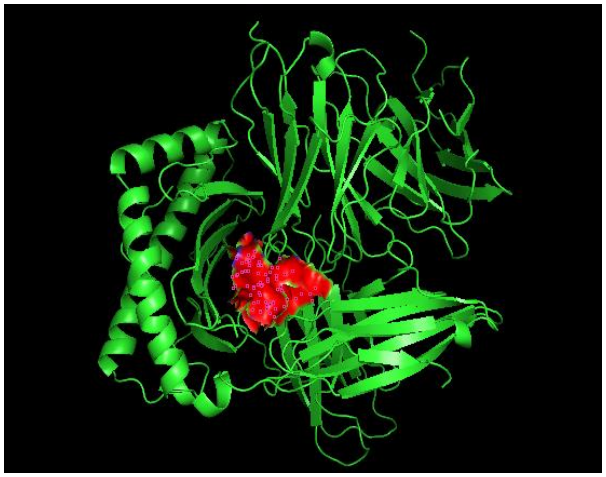


Figure 3.17 MHC class I Peptide 15 complex

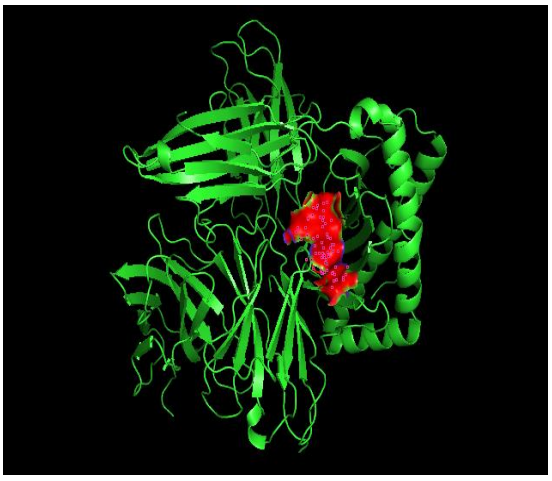


Figure 3.18 MHC class I Peptide 16 complex

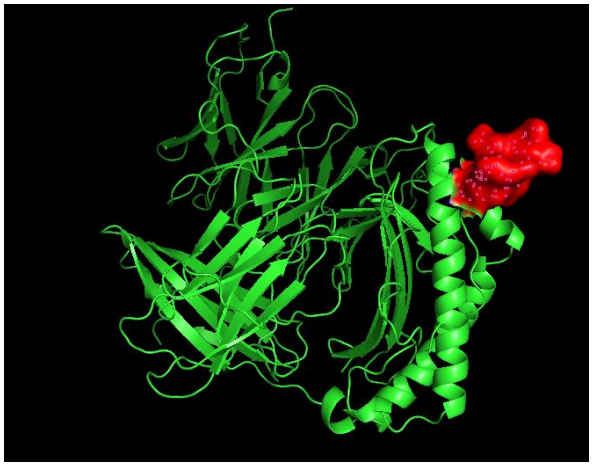


Figure 3.19 MHC class I Peptide 17 complex

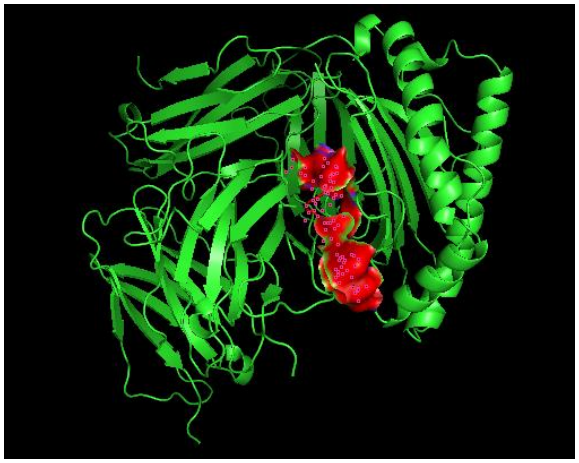


Figure 3.20 MHC class I Peptide 18 complex

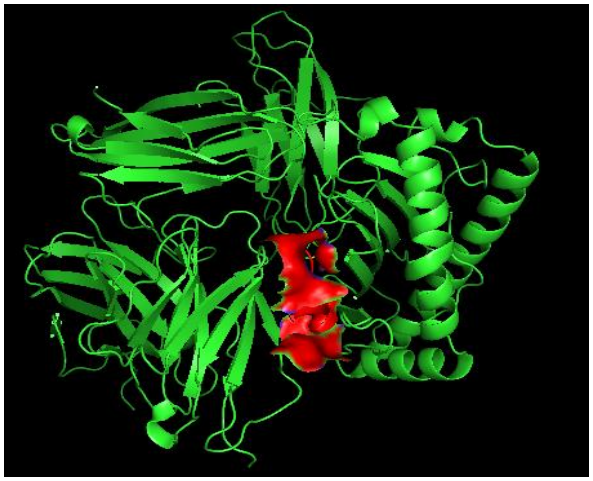


Figure 3.21 MHC class I Peptide 19 complex

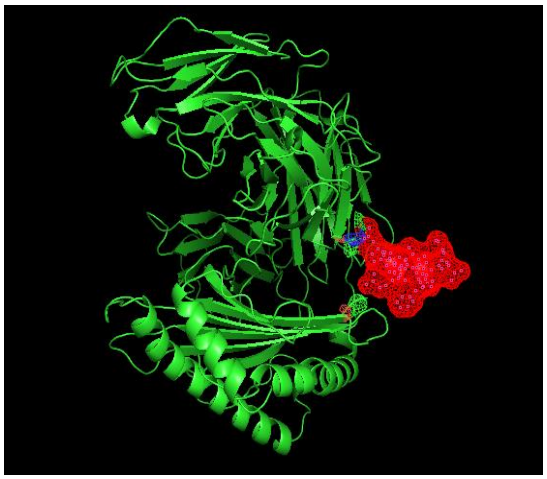


Figure 3.22 MHC class I Peptide 20 complex

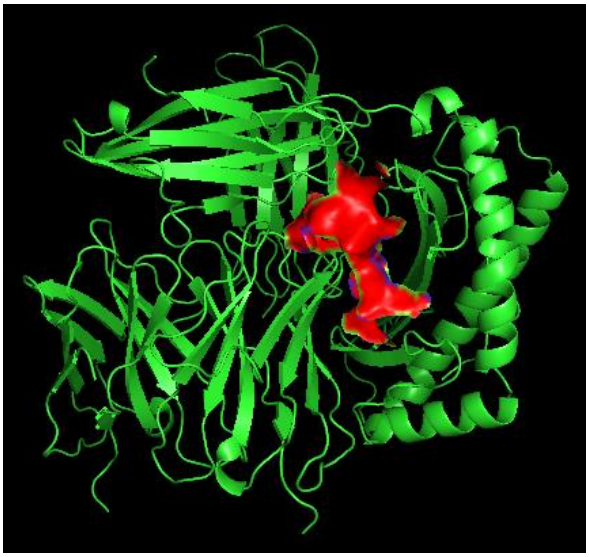


Figure 3.23 MHC class I Peptide 21 complex

Using PyMOL software the 3D structures was visualised for the respective peptides with MHC class I.

3.6 PyMOL RESULTS OF COXSACKIEVIRUS A9 PEPTIDES COMPLEX WITH TCR

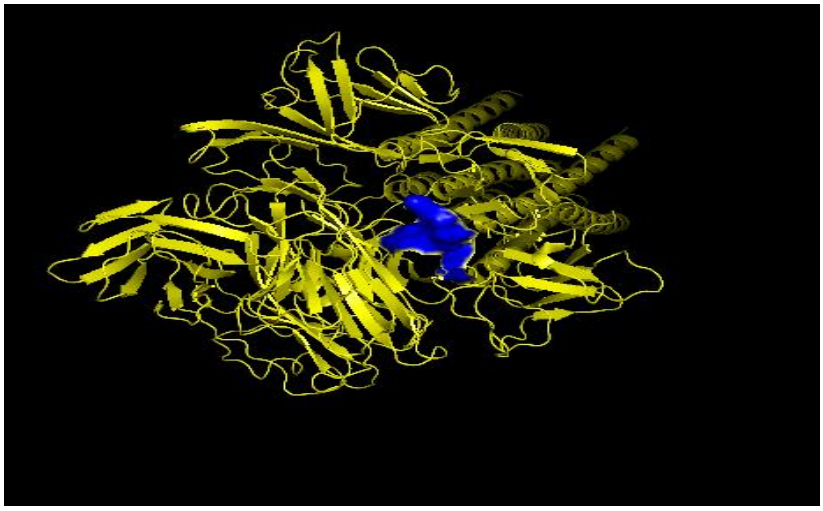


Figure 3.24 TCR- Peptide 6 complex

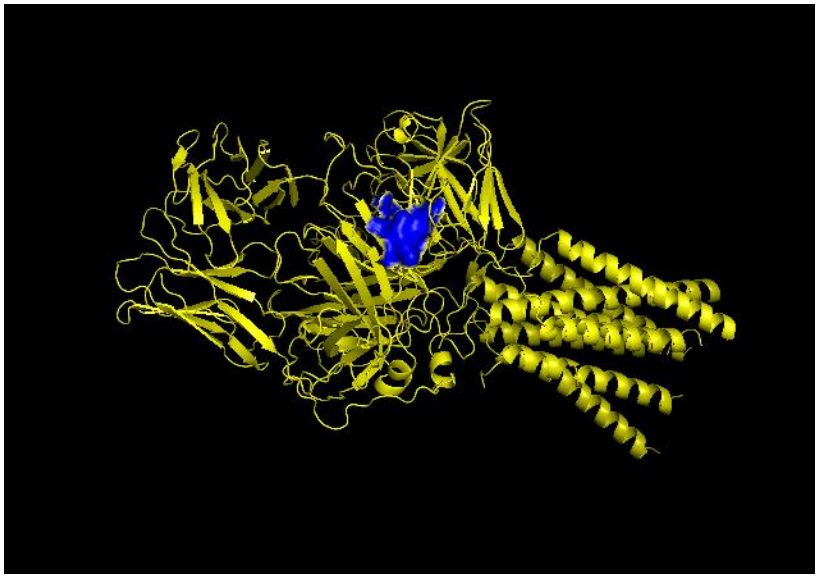


Figure 3.25 TCR- Peptide 7 complex

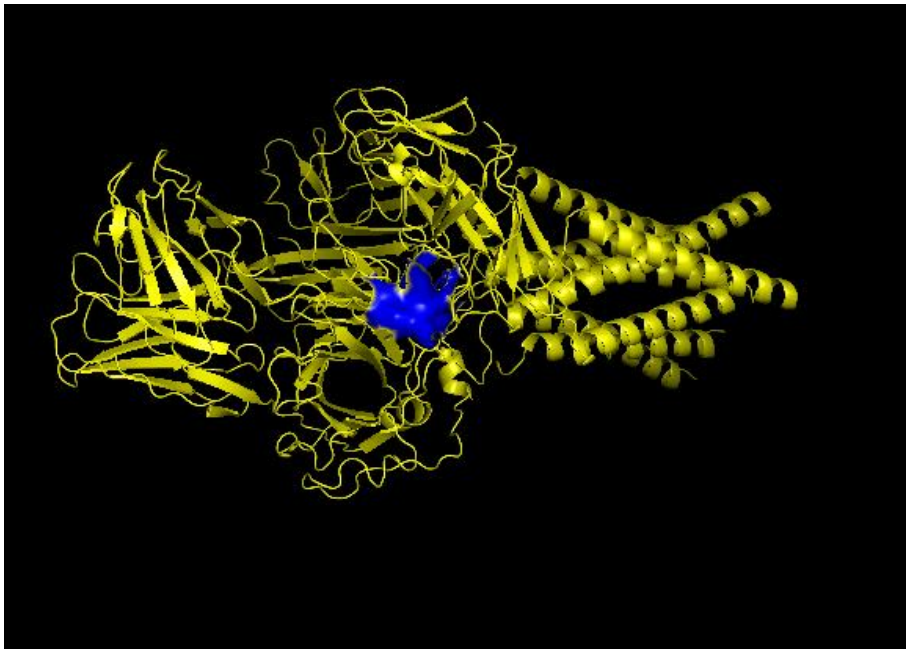


Figure 3.26 TCR- Peptide 9 complex

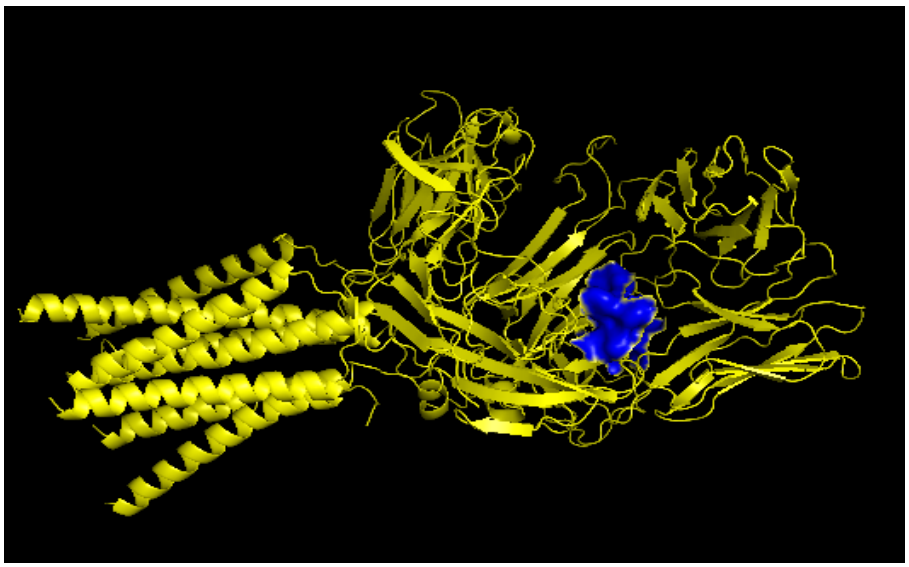


Figure 3.27 TCR- Peptide 10 complex

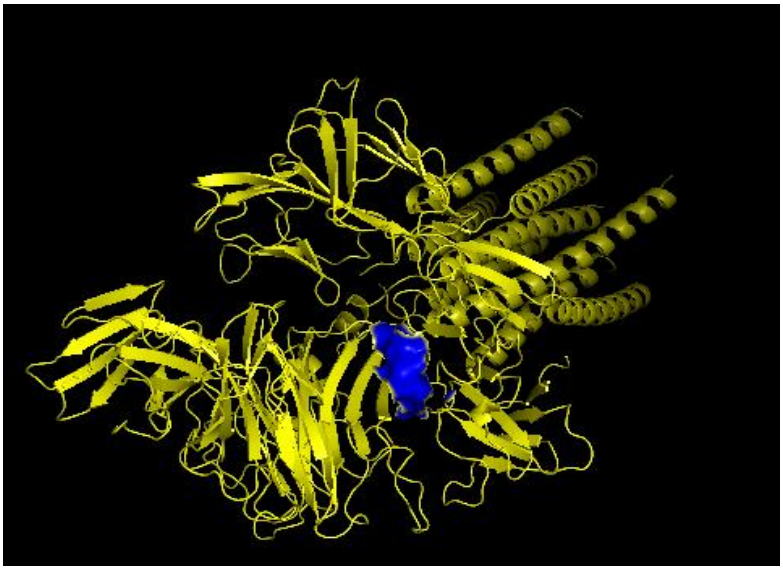


Figure 3.28 TCR- Peptide 17 complex

Using PyMOL software the 3D structures was visualised for the respective peptides with MHC class.

3.7 IINDIAN POPULATION COVERAGE OF PEPTIDE 10:

India - Class I Coverage

Number of epitope hits / HLA combinations recognized	Percent of individuals	Cumulative percent of population coverage
0	98.49	100.0
1	1.51	1.51

Population: India

MHC class	Coverage	Average hit	PC90
I	1.49%	0.01	0.1

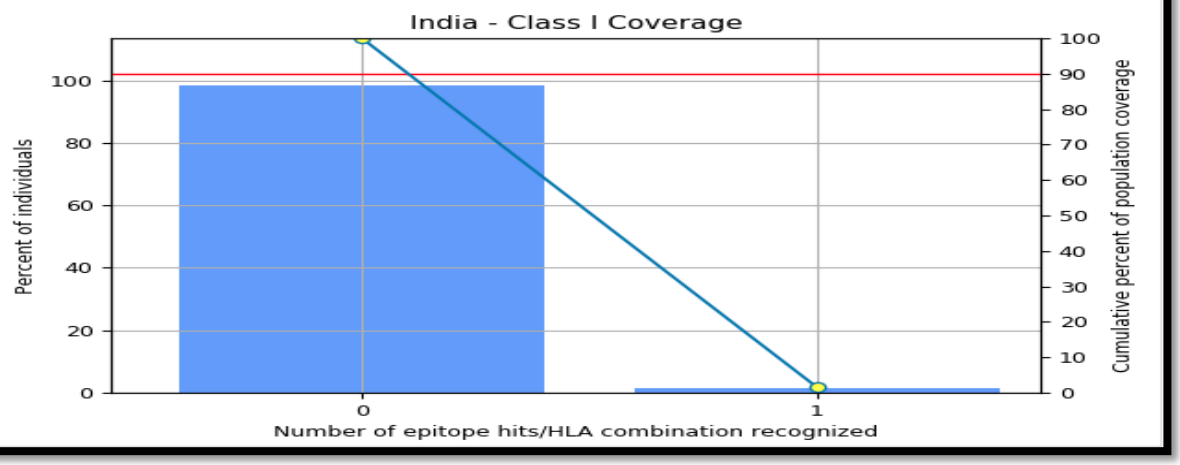


Figure 3.29 HLA-B*40:01

India - Class I Coverage

Number of epitope hits / HLA combinations recognized	Percent of individuals	Cumulative percent of population coverage
0	95.91	100.0
1	4.09	4.09

Population: India

MHC class	Coverage	Average hit	PC90
I	4.09%	0.04	0.1

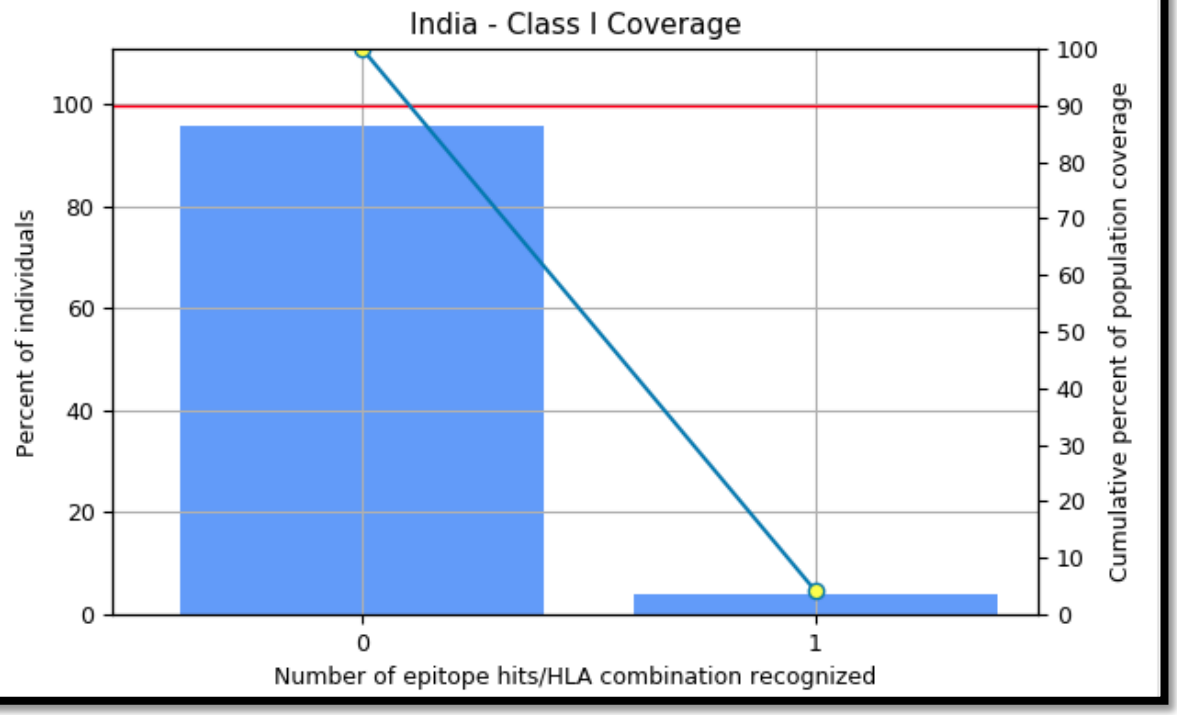


Figure 3.30 HLA-B*44:03

India - Class I Coverage

Number of epitope hits / HLA combinations recognized	Percent of individuals	Cumulative percent of population coverage
0	98.49	100.0
1	1.51	1.51

Population: India

MHC class	Coverage	Average hit	PC90
I	1.51%	0.02	0.1

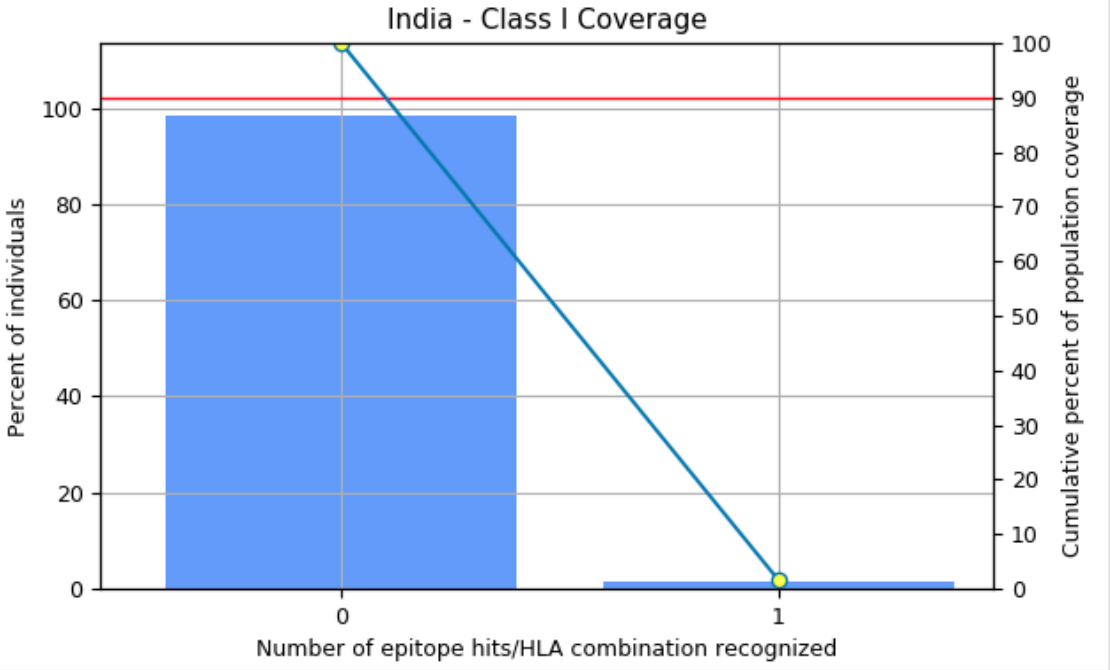


Figure 3.31 HLA-B*44:02

Population coverage of India was studied to check whether the effectiveness of the peptide in Indian population and whether or not it will be well presented by Indian population.

3.8 RESULT AND DISCUSSION

In this study, virus commonly found in our body that are linked with AD are used. This was done to check whether commensal viruses have any kind of epitopes that show similarity with human epitopes, which can trigger autoimmune reactions and cause AD. B cell epitopes prediction was carried out and longest peptide obtained was used for T cell epitopes prediction for a particular virus i.e. Coxsackievirus A9.

Longest peptide was chosen for its ability to trigger humoral immune response. Longer peptides are capable of acting as conformational epitopes and hence are capable of triggering potent B cell response. T cell epitope prediction was carried out within conformational B cell epitope for efficient T cell triggering capacity. Thus such an epitope would be capable of both cell mediated immunity and humoral response.

Protein BLAST was carried out to check similarity with the sequence of Homo sapiens. Molecular docking performed to identify global energies for different peptides. This was done to check binding with both MHC class I and TCR. Since, MHC has a broader stringency, it is important to check binding affinity for TCR also. 5 peptides with the highest binding efficiency with MHC class I are KTKLEPSVF, YIGNVNTHV, KYIGNVNTH, LEPSVFHQV and FEGVKEPAV. These 5 peptides are now used to check their binding affinity with TCR. Peptide with the highest binding efficiency is LEPSVFHQV i.e. -83.08. PyMOL used to visualise and obtain 3D structures of peptide-MHC class I and peptide-TCR complex. The peptide with the best binding efficiency with TCR was then used to check its population coverage. This is done to check the effectiveness of the peptide in Indian population and whether or not it will be well presented by Indian population.

3.9 CONCLUSION

There are many factors that have been linked with dysbiosis such as age, genetics, environmental and genetics. Dysbiosis further leads to autoimmune diseases as well as other complex diseases. Prevalence and incidence of autoimmune diseases in both developing as well as in developing countries have increased over the past few decades. However, there still is still a dynamic gap between commensals and pathogens members. Viruses are obligate intracellular parasites and some viruses may not be detrimental to human body. However, this study will help look for commensal viruses that may turn this relation of commensalism to parasitism and cause autoimmunity. Their similarity with human sequences was studied so that later it could be used as a biomarker for detection of autoimmune diseases. Research in the field considering virus should be carried out more in order to find more such pathways that can lead to AD. Accordingly, more of epidemiological and molecular research in this field is needed. It is important to understand how the interaction between viral infections and host can trigger autoimmune responses. With this information new novel therapeutics strategies could be designed in future.

REFERENCES

[1] C. Yu, M. E. Gershwin, and C. Chang, "Diagnostic criteria for systemic lupus erythematosus: a critical review.," *J. Autoimmun.*, vol. 48–49, pp. 10–13, 2014, doi: 10.1016/j.jaut.2014.01.004.

[2] A. M. Ercolini and S. D. Miller, "The role of infections in autoimmune disease," *Clin. Exp. Immunol.*, vol. 155, no. 1, pp. 1–15, Jan. 2009, doi: https://doi.org/10.1111/j.1365-2249.2008.03834.x.

[3] D. Fairweather, "Autoimmune Disease: Mechanisms," *eLS*. Jul. 16, 2007, doi: https://doi.org/10.1002/9780470015902.a0020193.

[4] S. Panda and J. L. Ding, "Natural antibodies bridge innate and adaptive immunity.," *J. Immunol.*, vol. 194, no. 1, pp. 13–20, Jan. 2015, doi: 10.4049/jimmunol.1400844.

[5] G. Eberl, "Immunity by equilibrium," *Nat. Rev. Immunol.*, vol. 16, no. 8, pp. 524–532, 2016, doi: 10.1038/nri.2016.75.

[6] J. Molloy, K. Allen, F. Collier, M. L. K. Tang, A. C. Ward, and P. Vuillermin, "The potential link between gut microbiota and IgE-mediated food allergy in early life.," *Int. J. Environ. Res. Public Health*, vol. 10, no. 12, pp. 7235–7256, Dec. 2013, doi: 10.3390/ijerph10127235.

[7] D. Mathis and C. Benoist, "Microbiota and Autoimmune Disease: The Hosted Self," *Cell Host Microbe*, vol. 10, no. 4, pp. 297–301, 2011, doi: https://doi.org/10.1016/j.chom.2011.09.007.

[8] A. V Chervonsky, "Microbiota and autoimmunity," *Cold Spring Harb. Perspect. Biol.*, vol. 5, no. 3, pp. a007294–a007294, Mar. 2013, doi: 10.1101/cshperspect.a007294.

[9] B. Stecher and W.-D. Hardt, "The role of microbiota in infectious disease.," *Trends Microbiol.*, vol. 16, no. 3, pp. 107–114, Mar. 2008, doi: 10.1016/j.tim.2007.12.008.

[10] H. Chung *et al.*, "Gut immune maturation depends on colonization with a host-specific microbiota.," *Cell*, vol. 149, no. 7, pp. 1578–1593, Jun. 2012, doi: 10.1016/j.cell.2012.04.037.

[11] T. Olszak *et al.*, "Microbial exposure during early life has persistent effects on natural killer T cell function.," *Science*, vol. 336, no. 6080, pp. 489–493, Apr. 2012, doi: 10.1126/science.1219328.

[12] R. Medzhitov, D. S. Schneider, and M. P. Soares, "Disease tolerance as a defense strategy.," *Science*, vol. 335, no. 6071, pp. 936–941, Feb. 2012, doi: 10.1126/science.1214935.

[13] J. S. Ayres and D. S. Schneider, "Tolerance of infections.," *Annu. Rev. Immunol.*, vol. 30, pp. 271–294, 2012, doi: 10.1146/annurev-immunol-020711-075030.

[14] A. V Chervonsky, "Influence of microbial environment on autoimmunity.," *Nat. Immunol.*, vol. 11, no. 1, pp. 28–35, Jan. 2010, doi: 10.1038/ni.1801.

[15] D. Mathis and C. Benoist, "Aire.," *Annu. Rev. Immunol.*, vol. 27, pp. 287–312, 2009, doi: 10.1146/annurev.immunol.25.022106.141532.

[16] S. Z. Josefowicz, L.-F. Lu, and A. Y. Rudensky, "Regulatory T cells: mechanisms of differentiation and function.," *Annu. Rev. Immunol.*, vol. 30, pp. 531–564, 2012, doi:

10.1146/annurev.immunol.25.022106.141623.
[17] W. W. Eaton, N. R. Rose, A. Kalaydjian, M. G. Pedersen, and P. B. Mortensen, "Epidemiology of autoimmune diseases in Denmark," *J. Autoimmun.*, vol. 29, no. 1, pp. 1–9, 2007, doi: https://doi.org/10.1016/j.jaut.2007.05.002.
[18] M. Wahren-Herlenius and T. Dörner, "Immunopathogenic mechanisms of systemic autoimmune disease.," *Lancet (London, England)*, vol. 382, no. 9894, pp. 819–831, Aug. 2013, doi: 10.1016/S0140-6736(13)60954-X.
[19] R. Milo and E. Kahana, "Multiple sclerosis: geoepidemiology, genetics and the environment.," *Autoimmun. Rev.*, vol. 9, no. 5, pp. A387-94, Mar. 2010, doi: 10.1016/j.autrev.2009.11.010.
[20] K. Fleming, L. Tata, T. Card, and C. Crooks, "Incidence and Prevalence of Celiac Disease and Dermatitis Herpetiformis in the UK Over Two Decades: Population-Based Study," *Am. J. Gastroenterol.*, vol. 109, Mar. 2014, doi: 10.1038/ajg.2014.55.
[21] P. S. C. Leung *et al.*, "Environment and primary biliary cirrhosis: electrophilic drugs and the induction of AMA.," *J. Autoimmun.*, vol. 41, pp. 79–86, Mar. 2013, doi: 10.1016/j.jaut.2012.12.007.
[22] P. Griffiths, "Time to consider the concept of a commensal virus?," *Reviews in medical virology*, vol. 9, no. 2. England, pp. 73–74, 1999, doi: 10.1002/(sici)1099-1654(199904/06)9:2<73::aid-rmv254>3.0.co;2-5.
[23] D.-L. Vu, S. Cordey, F. Brito, and L. Kaiser, "Novel human astroviruses: Novel human diseases?," *J. Clin. Virol. Off. Publ. Pan Am. Soc. Clin. Virol.*, vol. 82, pp. 56–63, Sep. 2016, doi: 10.1016/j.jcv.2016.07.004.
[24] C. B. Matranga *et al.*, "Enhanced methods for unbiased deep sequencing of Lassa and Ebola RNA viruses from clinical and biological samples.," *Genome Biol.*, vol. 15, no. 11, p. 519, 2014, doi: 10.1186/PREACCEPT-1698056557139770.
[25] D. Focosi, G. Antonelli, M. Pistello, and F. Maggi, "Torquetenovirus: the human virome from bench to bedside.," *Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis.*, vol. 22, no. 7, pp. 589–593, Jul. 2016, doi: 10.1016/j.cmi.2016.04.007.
[26] E. T. Chivero and J. T. Stapleton, "Tropism of human pegivirus (formerly known as GB virus C/hepatitis G virus) and host immunomodulation: insights into a highly successful viral infection.," *J. Gen. Virol.*, vol. 96, no. Pt 7, pp. 1521–1532, Jul. 2015, doi: 10.1099/vir.0.000086.
[27] H. W. Virgin, "The virome in mammalian physiology and disease.," *Cell*, vol. 157, no. 1, pp. 142–150, Mar. 2014, doi: 10.1016/j.cell.2014.02.032.
[28] E. S. Barton *et al.*, "Herpesvirus latency confers symbiotic protection from bacterial infection.," *Nature*, vol. 447, no. 7142, pp. 326–329, May 2007, doi: 10.1038/nature05762.
[29] E. S. Lim *et al.*, "Early life dynamics of the human gut virome and bacterial microbiome in infants.," *Nat. Med.*, vol. 21, no. 10, pp. 1228–1234, Oct. 2015, doi: 10.1038/nm.3950.
[30] J. K. Pfeiffer and H. W. Virgin, "Viral immunity. Transkingdom control of viral infection and immunity in the mammalian intestine.," *Science*, vol. 351, no. 6270, Jan. 2016, doi: 10.1126/science.aad5872.
[31] B. Kim, S. D. Kaistha, and B. T. Rouse, "Viruses and autoimmunity.," *Autoimmunity*, vol. 39, no. 1, pp. 71–77, Feb. 2006, doi: 10.1080/08916930500484708.
[32] C. J. Gauntt *et al.*, "Molecular mimicry, anti-coxsackievirus B3 neutralizing monoclonal antibodies, and myocarditis.," *J. Immunol.*, vol. 154, no. 6, pp. 2983–2995, Mar. 1995.

[33] R. S. Fujinami, M. G. von Herrath, U. Christen, and J. L. Whitton, “Molecular Mimicry, Bystander Activation, or Viral Persistence: Infections and Autoimmune Disease,” *Clin. Microbiol. Rev.*, vol. 19, no. 1, pp. 80 LP – 94, Jan. 2006, doi: 10.1128/CMR.19.1.80-94.2006.

[34] D. R. Getts, E. M. L. Chastain, R. L. Terry, and S. D. Miller, “Virus infection, antiviral immunity, and autoimmunity,” *Immunol. Rev.*, vol. 255, no. 1, pp. 197–209, Sep. 2013, doi: https://doi.org/10.1111/imr.12091.

[35] A. A. Leis, G. Szatmary, M. A. Ross, and D. S. Stokic, “West nile virus infection and myasthenia gravis.,” *Muscle Nerve*, vol. 49, no. 1, pp. 26–29, Jan. 2014, doi: 10.1002/mus.23869.

[36] C. S. Constantinescu, N. Farooqi, K. O’Brien, and B. Gran, “Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS).,” *Br. J. Pharmacol.*, vol. 164, no. 4, pp. 1079–1106, Oct. 2011, doi: 10.1111/j.1476-5381.2011.01302.x.

[37] S. Shakeel *et al.*, “Structural and Functional Analysis of Coxsackievirus A9 Integrin α_vβ₆ Binding and Uncoating,” *J. Virol.*, vol. 87, no. 7, pp. 3943 LP – 3951, Apr. 2013, doi: 10.1128/JVI.02989-12.