IN SILICO B-CELL AND T-CELL EPITOPE PREDICTION OF *ASPERGILLUS FUMIGATUS* FOR EPITOPE-BASED PEPTIDE VACCINE AGAINST ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS

A Dissertation

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by

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I, Anjani, 2k23/MSCBIO/09, student of M.Sc. (Biotechnology), hereby declare that the project dissertation titled "***In-silico* B-cell and T-cell epitope prediction of *Aspergillus fumigatus* for Epitope-based peptide vaccine against Allergic Bronchopulmonary aspergillosis** " which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Masters of Science, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

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I hereby certify that the Project Dissertation titled "***In-silico* B-cell and T-cell epitope prediction of *Aspergillus fumigatus* for Epitope-based peptide vaccine against Allergic Bronchopulmonary aspergillosis**" which is submitted by **Anjani**, 2k23/MSCBIO/09, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of **Masters of Science**, is a record of the project work carried out by her under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

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ABSTRACT

Allergic bronchopulmonary aspergillosis (ABPA) is a pulmonary fungal infection caused by an allergic reaction to the common mold, *Aspergillus fumigatus*. Predominantly, immunocompromised persons are more feasible to ABPA however ABPA can progress into a chronic state known as chronic pulmonary aspergillosis which is in ordinary non-immunocompromised persons. The prevalence of ABPA in adults who have severe asthma and cystic fibrosis with fungal sensitization is estimated at 1 million to 2.8 million cases in India. Consideration of vaccine formulation for the treating ABPA has not yet been made. Anti-fungal medication (like itraconazole) and corticosteroids could not emphasize satisfactory results due to high rates of acute morbidity and mortality. . Antifungal drugs, anti-inflammatory drugs like corticosteroids, anti IgE therapy and antibiotics are introduced in treatment of ABPA . There has been growing concern about *Aspergillus* species' resistance to the azole antifungals itraconazole, voriconazole, and posaconazole. The fact that acquired resistance has been reported in azole-naive persons and patients with invasive illness caused by *A. fumigatus* who had exposure to these drugs are particularly concerning. Invasive aspergillosis still has a startlingly high death rate despite improvements in antifungal treatments because of delayed diagnosis and the development of antifungal resistance Epitope-based peptide vaccine (EBPV), a type of subunit vaccine utilizes the epitopic regions of antigenic proteins. In ABPA, allergenic proteins are considered for EBPV. In this study, immunoinformatics are employed to investigate putative B-cell and T-cell epitopes against ABPA to the *Aspergillus fumigatus*. Primarily, allergenic proteins of *A. fumigatus* are extracted from the Allergen Nomenclature server. Obtains the protein sequences of allergens from the NCBI and UniPort databases. Eliminate those allergen proteins that possess cross-reactivity claimed by performing Blastp, restrict by *Homo sapiens* and *mus musculus*. B-cell and T-cell epitopes are identified by the Immune epitope database analysis resource (IEDB-AR) server. Epitope prediction and its characterization are performed by B-cell prediction tools. Correspondingly, T-cell epitope, ^{118}KPLYYVVQANVEYSAD^{133} is interacting with the highest number of MHC-II alleles and population coverage is also significant. Protein-protein docking is performed between epitopes and their receptor. The lowest energy weighted score models are selected to visualize non-covalent interaction between docked clusters. Upon subsequent exploratory study, the recommended B cell and T cell epitopes could potentially be leveraged in peptide-based immunizations in the treatment of ABPA.

Keywords- Allergic bronchopulmonary aspergillosis (ABPA), Epitope-based peptide vaccine, Molecular docking, B-cell and T-cell epitopes.

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LISTS OF ABBREVIATION

Abbreviation	Full Form
ABPA	Allergic Bronchopulmonary Aspergillosis
APCs	Antigen Presenting Cells
ANN	Artificial Neural Network
CF	Cystic Fibrosis
CPA	Chronic Pulmonary Aspergillosis
EBPV	Epitope-Based Peptide Vaccine
ECM	Extracellular Matrix
GRAVY	Grand Average of Hydropathicity
HLA	Human Leukocyte Antigen
HRCT	High-Resolution Computed Tomography
IEDB-AR	Immune Epitope Database Analysis Resource
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IL	Interleukin
ISHAM	International Society for Human and Animal Mycology
MHC	Major Histocompatibility Complex
MW	Molecular Weight
PDB	Protein Data Bank
PARs	Protease Activated Receptors
SMM	Stabilized Matrix Method
TCR	T-Cell Receptor
Th2	T-helper Type 2 Cells
TLR	Toll-like Receptor
Tregs	T-regulatory Cells
WHO/IUIS	World Health Organization / International Union of Immunological Societies

INTRODUCTION

Allergic bronchopulmonary aspergillosis, it is a type of aspergillosis fungal infection which can cause by an allergic reaction to *Aspergillus fumigatus* antigen following colonsiation of the airways. It is characterised by pulmonary infiltrates, eosinophilia, bronchospasm, and immunologic signs of an allergy to *Aspergillus* species antigens.Patients with bronchial asthma and those with cystic fibrosis and immunocompromised are the three groups most commonly affected. In most cases, allergic bronchopulmonary aspergillosis manifests between the ages of three and five. Children are also suspectible to it(1). Although ABPA is not very common however 50% of patients with cystic fibrosis and nearly 25% of asthmatics have an allergy to *Aspergillus,* as indicated by a positive skin prick test to *Aspergillus* antigen. Asthma and cystic fibrosis have ABPA prevalence of roughly 13% and 9%, respectively(2). The ABPA affects about 4 million people globally. In India, where there are approximately 27.7 million adults with asthma, the estimated burden of ABPA is 1.38 million(3). The organism that causes ABPA most frequently is *Aspergillus fumigatus*, and the infection is most common during the winter months worldwide (due to fallen leaves).The airway epithelium is directly damaged by severe acute respiratory syndrome coronavirus 2, which makes it possible for *Aspergillus* to infiltrate(4). There are worries that reports of pulmonary aspergillosis linked to COVID-19 could exacerbate the disease's progression and increase mortality. Furthermore, azole-resistant *aspergillus*-induced pulmonary aspergillosis linked to COVID-19 has been documented for the first time(5) .

Treatment for allergic bronchopulmonary aspergillosis emphasises on reducing progressive lung damage and controlling acute inflammatory episodes. Antifungal drugs, anti-inflammatory drugs like corticosteroids, anti IgE therapy and antibiotics are introduced in treatment of ABPA . There has been growing concern about Aspergillus species' resistance to the azole antifungals itraconazole, voriconazole, and posaconazole. The fact that acquired resistance has been reported in azole-naive persons and patients with invasive illness caused by *A. fumigatus* who had exposure to these drugs are particularly concerning. Invasive aspergillosis still has a startlingly high death rate despite improvements in antifungal treatments because of delayed diagnosis and the development of antifungal resistance(6).The developing of effective vaccines is one of the novel preventive measures that are desperately needed. The need for innovative therapeutic approaches to improve or restore antifungal immunity in people with weakened immune systems is growing. Promising immunotherapeutic approaches in this context include vaccination and the modification of particular innate immune processes(7).

A vaccination is defined as a biological preparation that strengthens immunity, educates the immune system, and protect against a potentially fatal microbial infection. Conventional vaccination for fungal infection has been not observed successful because the requirement for the host to identify and combat the many fungal structures, including hyphae, germ tubes, and conidia possess different constitutes component(8).Consequently, the host experiences dynamic changes in the distribution and quantity of fungal wall components that accompany morphological changes during infection, which exacerbates the difficulties caused by interspecies variations in the fungal cell wall structure. The cell wall can be affected by external stressors (including nutritional limitation, pH, and ROS), the changeover between yeast and hyphae growth, and the process of cellular division, which can reduce host recognition, hinder inflammatory responses, and enhance fungal virulence(9). The development of recombinant DNA technologies gave rise to a novel idea in vaccination: using individual antigens rather than whole disease causing microbes. This category of vaccine is called subunit vaccine. More research has been done on immunology and vaccination, and from that a novel type of vaccine has emerged in which potential epitopes are used moreover these are in the form of peptide to immunized the body, known as Epitope- based peptide vaccine (EBPV)(10). Where all the epitopes on the antigen are unable to generate a sufficient amount of immune response, some potential epitopes can produce a good amount of antibodies. In this way, they enhance our immune response significantly. Additionally, Epitope-based vaccines are being developed to boost the specific activity of neutralising antibodies by decreasing the quantity of ineffective antibodies (by release of undesired epitopes from antigen) present in the diseased person. When producers must cultivate vast amounts of pathogens, vaccine production can become both technically challenging and biohazardous. The manufacture of an epitope-based vaccination is thought to be less complex, safer, and undoubtedly less expensive in the long term. Considering these advantages of Epitope- based peptide vaccine offers a promising treatment for ABPA(11).

Epitopes which recognized by B-cell and T-cell are known as B-cell epitopes and T-cell epitopes, respectively. Allergenic proteins of A. fumigatus are considered for epitope characterisation due to its antigenic function (hypersensitivity reactions). *Aspergillus fumigatus* utilized their allergenic protein to trigger the pulmonary allergic inflammatory reaction i.e. fungal proteases is strong allergens that can cause allergic reactions in the lungs. Proteases released by *A. fumigatus* growing on the airway epithelium may cause IgG and IgE-mediated allergic reactions by interacting with PARs and TLR-mediated signalling pathways, which could result in pulmonary problems like ABPA(12).

The aim of this study is to investigate the putative B-cell and T-cell epitope of *Aspergillus fumigatus* using the systematic vaccine-informatics approach which includes IDEB-AR, molecular docking and

other bioinformatics tools. According to the “Allergen Nomenclature- WHO/IUIS Allergen Nomenclature Sub-Committee” have recorded totally 30 allergens of *Aspergillus fumigatus* (common mold)(13). New allergens have been submitted in this database since 2021, those are Asp f 35, Asp f 36, Asp f 37, Asp f 38 and Asp f 39 moreover have not yet been studied. Raman Thakur and his colleagues have conducted a study named was “*In-silico* predication of potential peptides against A. fumigatus” in which they have studied total 25 allergenic proteins upto Asp f 35(14). Therefore, In spite of 25 researched allergenic proteins new allergens, Asp f 35, Asp f 36, Asp f 37, Asp f 38 and Asp f 39 are subjected to this study. Retrieval of *A. fumigatus* allergens, locating similarities between the host and the protein sequence, allergen antigenicity predication, predicting the primary and secondary structures, determination of B-cell and T-cell potential epitopes and molecular docking , these are the major steps in this study which utilized the application of computational methods offers an innovative strategy to combine the vaccine-informatics approach to epitope based-peptide vaccine development(15). In order to identify possible peptides or allergen shot candidates against infections or allergies caused by A. fumigatus, we employed the reverse vaccinology approach in this investigation. In order to create vaccinations against infectious diseases, a field known as "vaccinemics" combines immunogenetics and immunogenomics with information on system biology and immunological profiling. Here, personalised medicine and next-generation vaccinations are developed using bioinformatics techniques(16).

LITERATURE REVIEW

According to the World Health Organisation, respiratory illnesses brought on by bacteria, viruses, and fungus rank fourth among all causes of death worldwide. However, despite the fact that fungal respiratory infections kill as many people each year as influenza or tuberculosis, they are still often ignored. Although more than 600 fungal species are known to infect people, *Aspergillus* species account for 70% of fungal-associated fatalities. Only a small number of the more than 200 *Aspergillus* species that have been found are thought to be harmful to people.

2.1 *Aspergillus fumigatus* and Allergic Bronchopulmonary Aspergillosis (ABPA): An overview

2.1.1 *Aspergillus fumigatus's* biology and pathogenicity

Aspergillus species are saprotrophic and filamentous fungus that is commonly found in soil. *Aspergillus fumigatus* can cause severe invasive disease in immunocompromised persons; however it usually fails to trigger infection in immunocompetent hosts. By feeding on decomposing vegetation, they actively contribute to the recycling of carbon and nitrogen(17).

- **Taxonomy and Morphology**

Domain - Eukaryota

Kingdom- Fungi

Division- Ascomycota

Class- Eurotiomycetes

Order- Eurotiales

Family- Aspergillaceae

Genus- *Aspergillus*

Species- *Aspergillus fumigatus*

Synonyms- *Neosartorya fumigata*

The characteristic of *A. fumigatus* is its blue-green, tiny (2–3 μm) echinulate conidia, which grow in long chains from conidiophores that extend from the vegetative mycelium. The fungus can grow at 37 °C or 99 °F, which is the typical temperature of the human body. It can also develop at temperatures as high as 50 °C or 122 °F, and its conidia may survive at 70 °C or 158 °F, which are temperatures it frequently faces in compost heaps that heat themselves. *A. fumigatus* can be found on walls or ceilings that have sustained water damage, in indoor (and outdoor) air, and in fungal cultures from basements. It is now acknowledged that A. fumigatus is capable of sexual reproduction, despite the long-held belief that it is solely an asexual organism. However, most natural reproduction is asexual, with *A. fumigatus* sporulating widely to produce tiny, hydrophobic conidia that can be spread by air over great distances(18).

➢ Asexual reproduction

The germination of spores signifies the initiation of *A. fumigatus'* life cycle. The spores are usually found in significant numbers on the fungal colony's surface, these spores are generated in particular structures known as conidiophores. In order to thrive in a variety of environments, *Aspergillus* species generate large amounts of asexual spores called conidia, which are tiny unicellular structures that are extremely resistant to environmental stressors. This species is among the most common fungi with airborne conidia, although not being the most common in the globe. After being discharged into the environment, the conidia can spread by a variety of channels, including water, air currents, and animal carriers. As a result, *A. fumigatus* can establish colonies in new habitats and substrates. The conidia can germinate and start the development of new colonies when the appropriate conditions are there. The conidium swells during germination, giving rise to the germ tube, a specialised hyphal structure. In order to create a network of hyphae that penetrates the substrate, the germ tube lengthens and branches. The hyphae allow the fungus to develop and spread its mycelium by secreting enzymes and absorbing nutrients from the surroundings. As the mycelium grows, it creates an intricate web of hyphae that pierce the substrate and aid in the organic matter's breakdown and nutritional uptake(19).

➢ Sexual reproduction

A. fumigatus is capable of sexual reproduction under specific circumstances, such as the presence of particular mating types or stressors. This process entails the union of two compatible mating types, which results in the formation of sexual spores known as ascospores that are protected in protective structures called ascus4. Like asexual conidia, the ascospores can spread after being expelled from the ascus. Sexual reproduction is a crucial part of the life cycle of *A. fumigatus* because these sexual spores have the capacity to promote genetic variation and adaptation. Notably, many *Aspergillus* species are primarily asexual and depend on the creation and dissemination of conidia for their survival and propagation, but some species engage in sexual reproduction(19).

➢ Pathogenesis

A wide range of pH and temperature conditions can be tolerated and survived by *Aspergillus fumigatus*, and the species can be effectively spread by minimal air currents due to of its hydrophobic cell wall. Likewise, multiple features enable this species one of the most common mould species that infects people.

Melanin in the cell wall allows for resistance to phagocytosis and reactive oxygen species, the abundance of negatively charged sialic acid on the surface allows *A. fumigatus* to bind to the basal lamina proteins once inside the host lung, and the small conidia size facilitates entry to the lower pulmonary system and escape clearance by mucociliary forces.

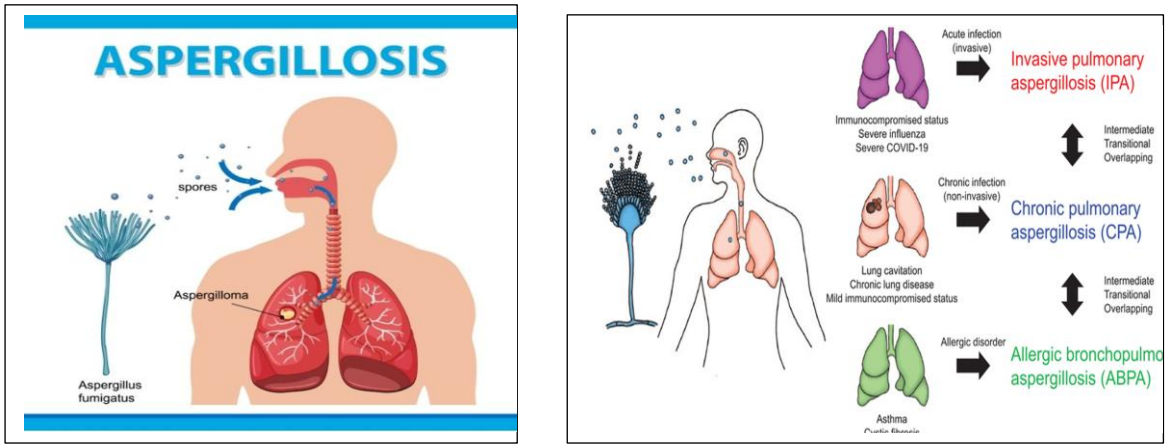
In a healthy host, resting conidia are eliminated through innate immune defences such as phagocytosis and mucociliary clearance before they can cause infection. Conidia may, however, experience a variety of morphological alterations led by isotropic and later polarised growth stages if defence system is unsuccessful. These modifications may result in the formation of hyphae that can infiltrate host tissues and cause illness(20).

Although asthma is a diverse illness, *Aspergillus fumigatus*, or *A. fumigatus*, is most closely linked to asthma and mostly affects the lungs. Patients with compromised lung defences are at a significantly higher risk of developing pulmonary *Aspergillus*-related disease. Compromised lung defences can be caused by immunosuppressive treatments like chemotherapy or by long-term respiratory disorders like asthma, chronic obstructive lung disease, or cystic fibrosis, where the immune system may also be affected. However, only a small percentage of the population will get Aspergillus-related infections despite humans being exposed to *A. fumigatus* on a regular basis(1).

➢ Diagnosing respiratory aspergillosis and its clinical manifestations

The respiratory tract serves as the primary entry point and location of infection for *A. fumigatus* in the majority of patients. However, various infection sites, including the skin, peritoneum, kidneys, bones, eyes, and gastrointestinal tract, have been reported in both healthy and immunocompromised hosts. The state of the host and its immune system has a significant impact on how infections start, progress, and finish.

Invasive pulmonary aspergillosis (IPA), **chronic pulmonary aspergillosis** (CPA), **allergic bronchopulmonary aspergillosis** (ABPA), and (21) **bronchitis** (AB) are the four different forms of **aspergillosis** that are currently recognised as shown in fig2.1. Each of these types affects different patient cohorts and causes varying degrees of disease severity.



(A) (B)

Figure 2.1 (A) Aspergillosis (B)Disease caused *Aspergillus fumigatus*

2.1.2 Allergic Bronchopulmonary Aspergillosis (ABPA)

A fungal infection of the lung caused by an allergic reaction to *Aspergillus fumigatus* antigens following colonisation of the airways is known as allergic bronchopulmonary aspergillosis (ABPA).

- **Epidemiology**

Asthma is a diverse illness characterised by fluctuating expiratory airflow limitation and respiratory symptoms that change over time and in intensity, including wheezing, shortness of breath, chest tightness, and coughing. It can even be lethal at circumstances.

Aspergillus fumigatus is significantly linked to asthma and can impact the lungs in several manners, including Allergic Bronchopulmonary Aspergillosis (ABPA), aspergilloma (fungal ball), and invasive aspergillosis. ABPA is an allergy condition categorised into Serological ABPA (ABPA-S) as well as ABPA with Bronchiectasis (ABPA-B) based on High-Resolution Computed Tomography (HRCT) chest findings.

According to multiple researches, between 17% and 58% of ABPA cases in India are misdiagnosed as pulmonary tuberculosis. Fungal colonisation and sensitisation are more likely to occur in severe asthmatic patients who experience frequent attacks, persistent asthma symptoms, and irreversible lung abnormalities. Over 4 million people have been affected by ABPA globally(22).

- **Etiology**

The clinical manifestations of ABPA, an allergic lung disease brought on by **hypersensitivity to *Aspergillus fumigatus*, include bronchiectasis, recurrent pulmonary infiltrates, and chronic asthma**. In ABPA, there is no invasive illness but the formation *of A. fumigatus* hyphae in bronchial mucus following inhalation of environmental spores. In addition to alveolar macrophages, polymorphonuclear leukocytes are defences against *A. fumigatus* spores.

According to the updated Cell and Coombs categorisation of immunologic hypersensitivity, the disorder has immunologic traits such as eosinophil-rich inflammatory cell responses (type IVb), antigen-antibody complexes (type III), and

acute hypersensitivity (type I). It is estimated that between 1 and 2% of people with asthma and 2 to 15% of people with cystic fibrosis (CF) have ABPA.

There are two categories under the International Society for Human and Animal Mycology's (ISHAM) standards: "obligatory" criteria and "other" criteria . The two characteristics of the mandatory criterion are as follows:

(1) high total IgE levels more than 1000 IU/Ml.

(2) elevated IgE levels against *Aspergillus fumigatus (A. fumigatus)* or positive acute (type I) cutaneous hypersensitivity to *Aspergillus* antigen.

A patient with asthma must satisfy at least five prerequisites in order to be diagnosed with ABPA:

(1) Asthma

(2) Proximal bronchiectasis (dilated bronchi in the inner two thirds of the chest field on a computed tomograph [CT]

(3) Immediate cutaneous reactivity to *Aspergillus* species or *Aspergillus fumigatus*;

(4) An elevated total serum IgE (>417 kU/L or 1000 ng/mL)

(5) Elevated serum IgE– *A. fumigatus* and/or serum IgG-*A. fumigatus* compared to sera from asthma patients with ABPA who do not have ABPA.

Genetic association: HLA-DQ2 contributes to resistance, while HLA-DR molecules DR2, DR5, and potentially DR4 or DR7 contribute to susceptibility(23).

➢ Pathogenesis

It is still unclear how allergic bronchopulmonary aspergillosis develops. If sufficient *A. fumigatus* spores are inhaled, they act as allergens. A low level of IgG against fungal antigens in the bloodstream and a low amount of antifungal secretory IgA in bronchoalveolar fluid often indicate that healthy people are capable of successfully removing fungus spores. In contrast, IgE and IgG antibodies are produced when atopic individuals are exposed to fungal spores or mycelial pieces. Recent research has revealed a strong genetic basis for *Aspergillus* colonisation, linked to decreased transcriptional factor ZNF77 expression in bronchial epithelia. This results in

compromised epithelial cell integrity and increased expression of extracellular matrix (ECM) proteins that promote conidial adhesion.

Helper T cells, or Th2 cells, are crucial to the hypersensitive reaction triggered on by the *A. fumigatus* antigen. IgE production, eosinophilia, mast cell degranulation, and bronchiectasis are some of its symptoms.

The hypersensitive reaction is brought on by the release of proinflammatory cytokines by *A. fumigatus* proteases, such as IL-8, which damages epithelial cells and breaks down protective barriers. The cytokines interleukin (IL)-4, IL-5, and IL-13 are also released, increasing IgE and blood and airway eosinophils(4).

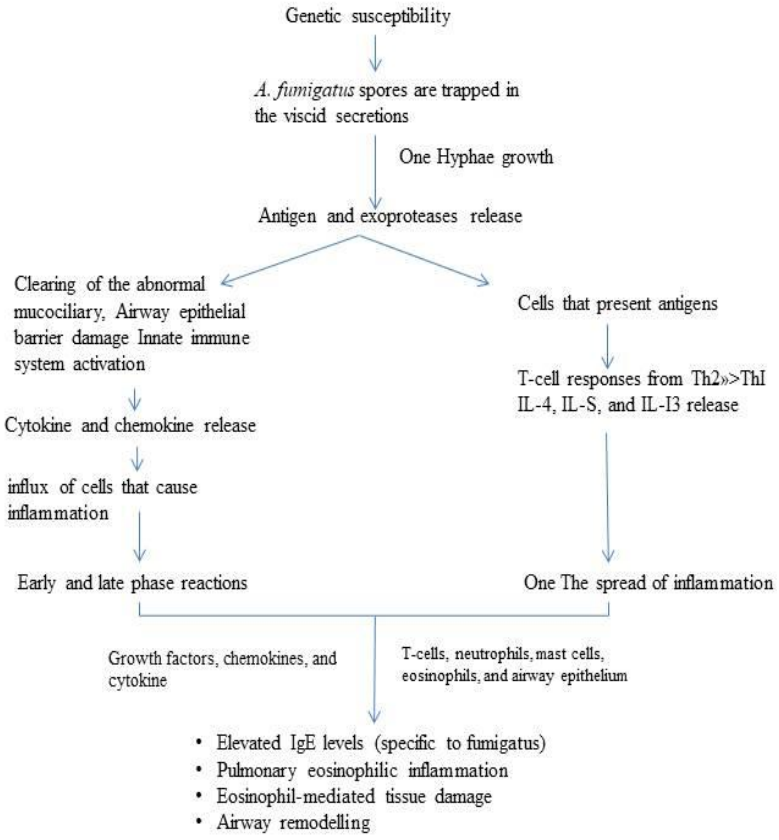


Figure 2.2. An illustration of the pathophysiology of allergic bronchopulmonary disease and aspergillosis. Th is the T-helper(21).

- ❖ Innate immune system processes allow **immunocompetent** people to easily remove *Aspergillus* conidia from the airway; as a result, pulmonary fungal infections do not appear. The isolation in respiratory secretions like as sputum or bronchoalveolar lavage does not indicate an infection; rather, it only indicates colonisation.
- ❖ Due to an imbalance in the host immune system**, immunocompromised** individuals are unable to eradicate *Aspergillus* conidia; as a result, the bacteria colonise airways and develop into somatic hyphae, which trigger a chronic allergic inflammatory response that damages tissue and eventually results in the clinical manifestations of ABPA.
- ❖ Inhaling *Aspergillus fumigatus* spores causes an IgE-mediated hypersensitivity response within the respiratory tract, which results in respiratory symptoms like coughing with expectoration and dyspnoea in **atopic people** (asthmatics), patients with cystic fibrosis, and patients with cavitary lung diseases(8,21).

➢ Evalution

The diagnosis of ABPA cannot be made by a single test. Classic clinical signs, radiographic results, and immunological results are used to make the diagnosis.

❖ **Skin test for *Aspergillus***:

- Sensitisation to A. fumigatus is most usually diagnosed by the *Aspergillus* skin test (AST).
- The cutaneous hypersensitivity to *A. fumigatus* is evident right away.
- When ABPA is present, a positive Type I Hypersensitivity reaction indicates the existence of IgE antibodies specific to *A. fumigatus.*
- Sensitisation to *Aspergillus* can be diagnosed more accurately using intradermal skin testing than with skin prick tests.

❖ **Abnormalities of blood:**

- Greater than 1000 IU/mL of total serum IgE.
- Serum IgE levels for *A. fumigatus (Af)* were elevated.
- Serum precipitins (by gel diffusion) or elevated IgG levels in particular serum samples for *A. fumigatus.*
- Often missing, peripheral blood eosinophilia is particularly noticeable in patients taking oral or inhaled corticosteroids.

❖ **ABPA's radiological manifestations:**

- The sensitivity of a chest X-ray to diagnose ABPA is 50%. All lobes may be affected, but the higher lobes are most likely to demonstrate bronchiectasis alterations and parenchymal infiltration.
- The preferred test for identifying the distribution of bronchiectasis and other anomalies that are not visible on a chest X-ray, like centrilobular nodules and a tree-in-bud appearance is HRCT Chest.
- Serologic ABPA (ABPA-S) is the term used to describe ABPA patients who show no abnormalities on HRCT chest.
- ABPA Central Bronchiectasis (ABPA-CB) is the designation given to patients with central bronchiectasis on HRCT(24).

➢ TREATMENT FOR ABPA

Two crucial components of ABPA management are the use of glucocorticoids to regulate immunologic activity and careful observation to identify relapses. The use of antifungal medications to reduce the fungal burden brought on by fungal colonisation in the airways is another potential target. Moreover, Anti IgE therapy and antibiotics are used for the treatment of ABPA.

Systemic Glucocorticoid Therapy: The preferred course of treatment for ABPA is oral corticosteroids. They have anti-inflammatory properties in addition to suppressing immunological hyper function. Different glucocorticoid regimens have been utilised, and there is no data to recommend the dosage and duration of glucocorticoids (Table 8). We used a higher dosage of glucocorticoids for a longer period of time and saw higher remission rates and a lower rate of glucocorticoid-dependent ABPA (13.5%). The use of lesser doses of glucocorticoids was linked to frequent relapses or corticosteroid dependence (45%).

The choice is based on personal preference, though, and there are no direct comparisons between the two regimens. Along with improvements in symptoms and radiography, the therapeutic efficacy of steroid therapy is demonstrated by significant drops in the patient's overall serum IgE levels (there does not appear to be a relationship between serum antibody levels of *A. fumigatus*-specific IgE levels and disease activity). Instead

of trying to normalise IgE levels, the therapy aims to reduce them by 35 to 50%, which improves radiography and clinical outcomes. A steady blood level of total IgE should also be established in order to guide future relapse identification.

Inhaled Corticosteroids: We only use inhaled corticosteroids to treat asthma after the oral prescribed medication dosage is lowered to less than 10 mg per day.

Oral Itraconazole: The less toxic medication itraconazole has taken the place of ketoconazole(have hepatotoxicity), which was used in the previous decade. The function of itraconazole in ABPA has only been assessed in two randomised controlled trials, involving 84 patients. A pooled research revealed that itraconazole did not significantly enhance lung function, but it could significantly lower IgE levels by about 25% when compared to a placebo. The fact that neither study included long-term results in ABPA was a significant drawback. Therefore, longer-term studies are necessary before a definitive recommendation regarding the administration of itraconazole in ABPA can be made. At this time, itraconazole is only used in individuals with glucocorticoid-dependent ABPA or following the first relapse of ABPA in spite of glucocorticoid therapy(25).

The most common and significant class of antifungal medications are **azoles**. For all types of aspergillosis, ABPA, oral azole antifungal treatment is frequently used. On the other hand, prolonged treatment might make resistance more likely.

Negative impacts of drugs

Itraconazole disrupts the hepatic metabolism of a number of drugs, such as oral hypogl ycemics, cyclosporine, tacrolimus, terfenadine, cisapride, and midazolam; the most com mon side effects include rash, headache, oedema, nausea, vomiting, and diarrhoea; the most significant impairment is with proton pump inhibitors.

Weight gain, osteopenia, acne, skin atrophy, diabetes mellitus, glaucoma, cataracts, avascular bone necrosis, infection, hypertension, and childhood growth retardation are all adverse consequences of **glucocorticoids**.

The most frequent side effect of **omalizumab** is injection site oedema and redness; asthmatics may have anaphylaxis(26).

2.2 Allergens of *Aspergillus fumigatus*

Fungi have long been known to be linked to reactive airway disorders, including asthma. *Aspergillus fumigatus* is the most common fungus that causes severe pulmonary allergic disease, such as allergic bronchopulmonary aspergillosis (ABPA), which is linked to long-term lung damage and a decline in pulmonary function in pateints with cystic fibrosis(CF) and chronic asthma.

A wide range of type I-IV hypersensitivity illnesses are caused by fungi, which are common(12).

Fungal Proteases

Strong allergens that cause pulmonary allergic reactions are fungus proteases. Large quantities of proteases produced by *aspergillus* species are known to cause airway epithelial cells to produce IL-6, IL-8 and MCP-1; these enzymes also cause cellular desquamation and damage epithelial tight junctions. Proteases from A. niger have been shown to cause severe allergic lung illness in mice, according to a recent study by Porter et al.

Proteases have been observed to activate receptors (PARs) in recent times. Airways cells and other cells, including mast cells, eosinophil, neutrophils, macrophages, and lymphocytes, have G-protein coupled receptors called PARs. There are currently four PARs known to exist; in asthmatic patients' airways the expression of PARs are found high when they get infected by allergic airway disease. It is interesting to note that damaged airway epithelial cells also release trypsin,a PAR-2 agonist that exacerbates allergic inflammatory reactions. In mouse models, PAR-2 has been shown airway hyperreactivity and pulmonary eosinophilic infilration, indicating a function in aggravating Th2- mediated responses. In allergic disorders, PAR-2 also encourages fibrosis and elevated IgE production(27).

According to research by Moretti et al., *A. fumigatus* proteases increase host pulmonary inflammatory responses by suppressing PAR-2 expression via a mechanism that is dependent on TLR-4(28). Therefore, through interaction between PARs and TLR-mediated signalling pathways, it is plausible that proteases released by *A. fumigatus* growing on the airway epithelium cause IgG and IgE-mediated allergic responses, which can result in pulmonary problems like ABPA.

For asthmatic individuals to have Th2-mediated allergy reactions, the tolerogenic processes must also be avoided. According to research by Kheradmand et al., fungal proteases can reverse airway tolerance and, when administered to the airways, can trigger Th2-mediated allergic reactions without the need for adjuvant priming(29). The production of IL-10, an immunoregulatory cytokine that stimulates the formation of TGF-β-expressing CD4+ T-regulatory cells (Tregs), is another way whereby DCs contribute significantly to the maintenance of tolerance in the lungs. According to a recent study by Kriendler et al., compared to CF-ABPA patients, CD4+ T-cells from a cohort of *A. fumigatus* colonised non-ABPA CF patients exhibited a higher proportion of TGF-β-expressing Tregs(30).

Allergens of *Aspergillus fumigatus* are observed from a database ,known as ALLERGEN NOMENCLATURE(13). Total 30 allergens are listed in table no. 2.1.

Table 2.1 Allergens of *Aspergillus fumigatus* are listed form ALLERGEN NOMENCLATURE server.

S.No.	Allergen	Biochemical name	MW(SDS-PAGE)	Route of allergen exposure	Date created
1.	Asp f 1	Mitogillin family	18 kDa	Airway	2003-08-21
2.	Asp f 2	Pra1-related protein containing a HRXXH motif-containing metalloprotease domain	37 kDa	Airway	2003-08-21
3.	Asp f 3	Peroxysomal protein	19 kDa	Airway	2003-08-21
4.	Asp f 4	Protein of unknown function	30 kDa	Airway	2003-08-26
5.	Asp f 5	Metalloprotease	40 kDa	Airway	2003-08-26
6.	Asp f 6	Mn superoxide dismutase	26.5 kDa	Airway	2003-08-26
7.	Asp f 7	Expansin-like protein	12 kDa	Airway	2003-08-26
8.	Asp f 8	Ribosomal protein P2	11 kDa	Airway	2003-08-26
9.	Asp f 9	Cell wall associated glycosidase Crf1	34 kDa	Airway	2003-08-26
10.	Asp f 10	Aspartate protease	34 kDa	Airway	2003-08-26
11.	Asp f 11	Peptidyl-prolyl isomerase	24 kDa	Airway	2003-08-26
12.	Asp f 12	Heat shock protein P90	90 kDa	Airway	2003-08-26
13.	Asp f 13	Alkaline serine protease	34 kDa	Airway	2003-08-26
14.	Asp f 15	Cerato-platanin	16 kDa	Airway	2003-08-26
15.	Asp f 16	Cell wall associated glycosidase Crf1	43 kDa	Airway	2003-08-26

16.	Asp f 17	Hydrophobic surface binding protein HsbA	27 kDa	Airway	2003-08-26
17.	Asp f 18	Vacuolar serine protease	34 kDa	Airway	2003-08-26
18.	Asp f 19	Heat shock protein 70 kDa (Hsp70)	69.4 kDa	Airway	2022-05-15
19.	Asp f 22	Enolase	46 kDa	Airway	2003-08-26
20.	Asp f 23	L3 ribosomal protein	44 kDa	Airway	2006-02-26
21.	Asp f 24	putative eukaryotic translation elongation factor 1 subunit EF1-beta	34 kDa	Airway	2022-05-15
22.	Asp f 27	Cyclophilin	18 kDa	Airway	2006-02-26
23.	Asp f 28	Thioredoxin	13 kDa	Airway	2006-02-26
24.	Asp f 29	Thioredoxin	13 kDa	Airway	2006-02-26
25.	Asp f 34	PhiA cell wall protein	20 kDa	Airway	2007-06-05
26.	Asp f 35	Cu-Zn Superoxide dismutase	21 kDa	Airway	2022-05-15
27.	Asp f 36	Fructose-bisphosphate aldolase	42 kDa	Airway	2022-05-15
28.	Asp f 37	Malate dehydrogenase NAD-Dependent	35 kDa	Airway	2022-05-15
29.	Asp f 38	Protein of unknown function	25 kDa	Airway	2022-05-15
30.	Asp f 39	FG-GAP repeat protein	35 kDa	Airway	2022-05-15

2.3 Peptide vaccine: Epitope-based peptide vaccine

Fungal infections are found all across the world, and their prevalence and distribution differ depending on regional factors like topography, climate, and socioeconomic development. The World Health Organisation (WHO) ranked a number of fungal pathogens based on their epidemiology, including annual incidence, lethality, treatment effectiveness, and worldwide distribution, in their first-ever Fungal Priority Pathogens List. They identified *Candida albicans*, ***Aspergillus fumigatus***, *Cryptococcus neoformans*, and *Candida auris* as the critical group.

India ranks seventh in terms of land area and has the second-highest population in the world. Estimates of the prevalence of fungal diseases in India, such as **allergic bronchopulmonary aspergillosis (ABPA)** and chronic pulmonary aspergillosis (CPA), have been published(8).

According to estimates, there were approximately 40 million adult cases of asthma in India in 2021 (2.9% prevalence in 2016). The ~5% prevalence in asthma patients was used to calculate the burden of ABPA. It was hypothesised that the fungal sensitisation rate was 33% and that 10% of people had severe asthma. Although they do occur, we were unable to determine the prevalence of ABPA or severe asthma with fungal sensitisation in children(31).

Aspergillus is common saprophytic fungi found in the environment that can lead to a variety of health issues, from allergic reactions to potentially fatal infections. Less than 20 of the genus's approximately 200 members are considered pathogenic(8).

Subunit vaccines, which contain purified components of *Aspergillus*, have also been tested in preclinical models. Vaccination with individual recombinant proteins from Aspergillus, including peroxiredoxin (Asp f3), 1,3-betaglucanosyltransferase (Gel1), extracellular cell wall glucanase Crf1 (Asp f9) and aspartic protease (Pep1), induce protection against aspergillosis, and purified cell wall glycans and cell wall mannans have also been used as immunogens.

When combined with lipidated Tucaresol, vaccination with *A. fumigatus* surface antigens Asp f3 and Asp f9 (VesiVax Af3/9R) shields mice suffering from steroid-induced immune suppression or neutropenia from infections brought on by various strains of the bacteria, including azole-resistant strains. Three days after the last vaccination boost, higher quantities of IgG1 antibodies against *Aspergillus* antigens and enhanced splenic IL-4 release are connected to the vaccine-induced protection(9).

- **Epitope – based peptide vaccine**

The original focus of immunoinformatics, or bioinformatics applied to immunology, was B-cell epitope prediction (BCEP). In order to facilitate the previously suggested production of synthetic peptide-based vaccines for eliciting protective antibody-mediated immunity, protein sequence analysis was used to find hydrophilic peptide fragments bound by antibodies that recognise entire proteins(10).

An efficient line of defence against infectious diseases is the presence of antibodies. Strong neutralising abstract antibodies have the ability to stop a virus before it can infect its target cell, rendering it inactive. The essential components of antigen molecules that define their specificity, known as **epitopes**, directly trigger the production of particular humoral and/or cellular immune responses(11). There are two types of epitopes are as follows:

1. B-cell epitope

A peptide sequence or protein structure of an antigen which recognized by receptor(IgM and Ig D) of B-cells or antibodies consequently induce humoral immunity as shown in fig 2.3.

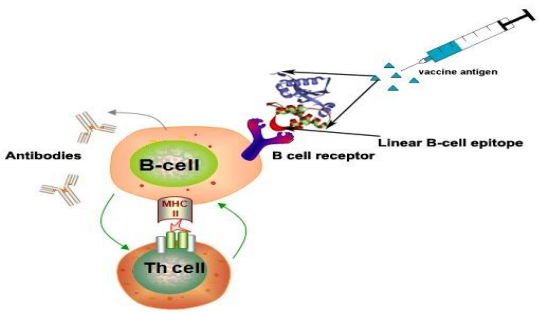


Figure 2.3. B-cell epitope

2. T-cell epitope

An immunogenic protein's epitope is a region that the immune system can identify. T-cell epitopes are peptides that are detectable by T-cell receptors following intracellular processing of a specific antigen, binding to at least one MHC molecule, and expression on the antigen-presenting cell surface as an MHC-peptide complex as shown in fig 2.4. People are predicted to be genetically predisposed to allergic reactions against an allergen if they possess at least one MHC molecule that can most avidly bind to allergenic amino acid sequences from that allergen and the right T-cell clone that can recognise this MHC-peptide complex(32).

(Konstantinou)

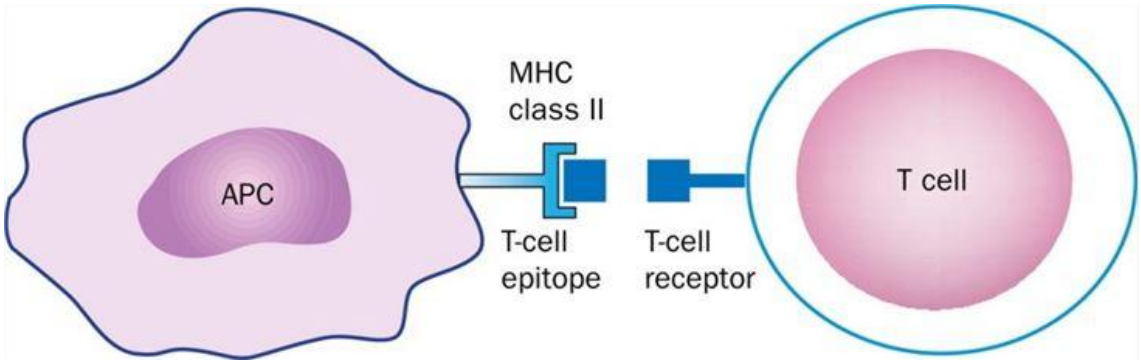


Fig 2.4 T-cell epitope

The particular recognition of epitopes—the locations of the antigen that antibodies bind to—by the antibodies is the basis for this capability. Therefore, a foundation for the logical development of preventive vaccinations is provided by an understanding of the antibody/epitope interaction. It is believed that the vaccine would produce antibodies of comparable potency if they were immunised with the exact epitope, which corresponds to an efficient neutralising antibody(33). The ability to reverse a desired antibody to its matching epitope is necessary for the implementation of a **"B-cell epitope-based vaccine."**

Epitope-based vaccines, which can be made as DNA, mRNA, or protein vaccines, have a number of advantages over traditional vaccines, including fewer side effects, increased effectiveness, and better protection, especially for susceptible groups like the elderly and people with compromised immune systems as mentioned table no 2.2.

Epitope-based vaccines, which are novel immunisation strategies based on the logical design of B- and T-cell epitopes, are progressing through clinical trials**. The next-generation method known as the epitope-focused recombinant protein-based malaria vaccine (RTS,S) has made it to phase-III trials and has the potential to be the first commercial vaccination to protect against a parasite disease in humans**. These new ideas are being developed as a result of advancements in procedures like recombinant DNA technology, sophisticated cell-culture methods, immunoinformatics, and the logical design of immunogens. Because the design assumptions of these methods differ, it is reasonable to infer that their predictions will also differ. Potential allergen T-cell epitopes can be predicted using the software programs that have been built based on this foundation. These algorithms are able to incorporate the growing quantity and complexity of accessible experimental data that have been arranged in immunoinformatics databases(34). Online, all relevant T-cell epitope prediction techniques are available as freeware.

Table 2.2 Comparative analysis between traditional vaccines and Epitope- based peptide vaccines.

S.No.	Feature	Traditional Vaccines	Epitope-Based Peptide Vaccines
1.	**Antigen Source**	Whole pathogens (live-attenuated, inactivated, or subunit)	Selected short peptide sequences (epitopes) from antigens
2.	**Production Complexity**	Often requires culturing large volumes of pathogens; can be biohazardous	Simpler and safer to produce; avoids pathogen culture
3.	**Specificity**	May include irrelevant or non-protective components	Highly specific; targets conserved and immunodominant epitopes
4.	**Risk of Side Effects**	Higher chance due to broader immune activation, including non-neutralizing or auto-reactive responses	Lower risk; avoids undesired epitopes that may lead to autoimmunity or ineffective responses
5.	**Effectiveness**	May produce a broad but less focused immune response	Designed for focused and functional immune response (e.g., neutralizing antibodies)
6.	**Autoimmunity Risk**	Higher if pathogen epitopes mimic host proteins, leading to cross-reactivity	Reduced by excluding epitopes structurally similar to host proteins
7.	**Neutralizing Antibody Production**	May generate antibodies that are not strongly neutralizing	Designed to enhance the specific activity of neutralizing antibodies
8.	**Adaptability**	Less flexible to strain variation or rapid mutation	Highly adaptable; epitopes can be updated quickly to match new variants
9.	**Cost and Scalability**	More expensive due to complex manufacturing and biohazard handling	Potentially cheaper and easier to scale in the long run

CHAPTER 3

MATERIALS AND METHODS

3.1 Retrieval of A. fumigatus allergens

Allergens are identified currently for *A. fumigatus* obtained from http://www.allergen.org which offered the allergen data sets categorized by the WHO/IUIS/allergen nomenclature subcommittee, a global organization assigned in maintaining and developing an exclusive, obvious, and systematic nomenclature for allergenic proteins(13).

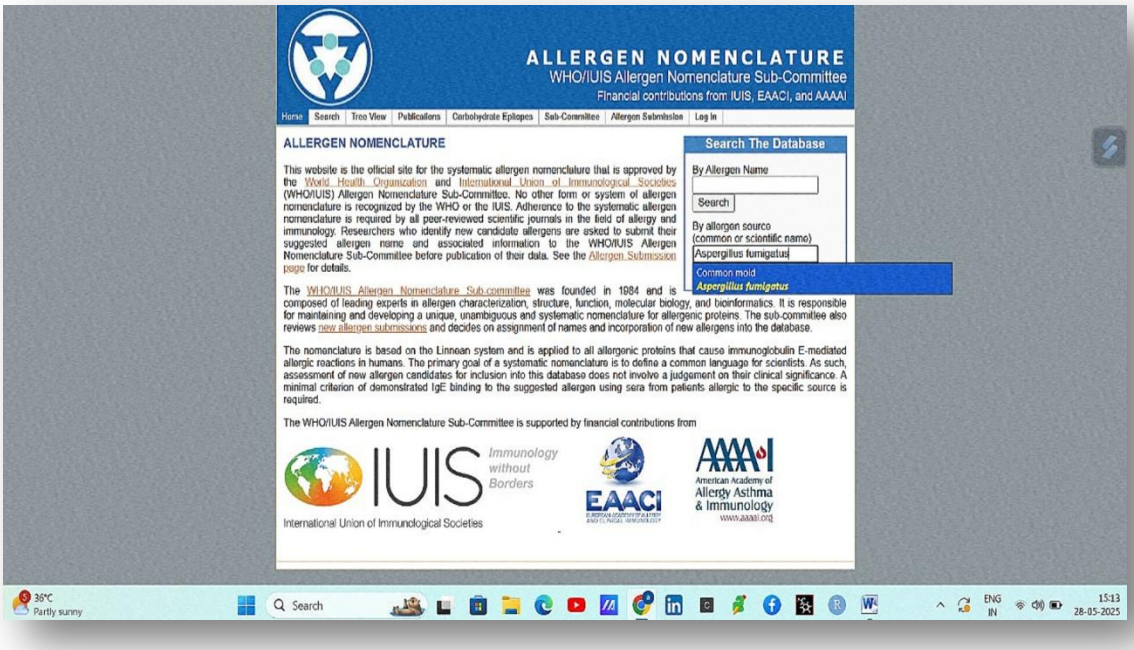


Figure 3.1. Window observed Allergen nomenclature.

3.2 Locating similarities between the host and the allergenic protein sequence

The proteins sequences of the allergen are queried against two taxids: 10090 and 9606, *Mus musculus* and *Homo sapiens* respectively to uniprotkb_refprotswissprot database using BLASTp. Default parameters are used with an E-value cut-off of 1e-4 and the

BLOSUM62 scoring matrix. The analysis rejected the hit with an expectation value (E-value) less than10^{-4}, and it was expected that these protein sequences exhibited a high degree of sequence similarity with the proteomes of the host and model organisms(35).

3.3 Allergen antigenicity prediction

Antigenicity is determined by the online prediction server VaxiJen v 2.0, providing the antigenic profile of proteins from bacteria, viruses, parasites, and fungi. To improve accurate antigenicity and prevent false-positive results, we set the threshold value at 0.4(36).

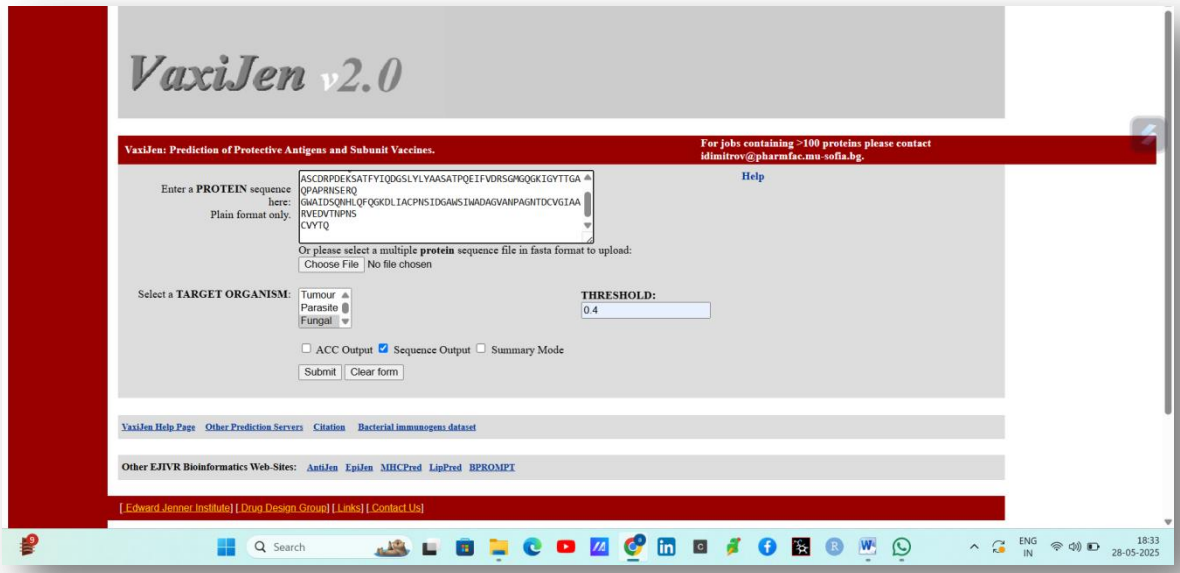


Figure 3.2 Window observed VaxiJen v 2.0.

3.4 Hypothetical Protein sequence retrieval

The Allergen Nomenclature server www.allergen.org and National Centre for Biotechnology Information database (NCBI)(37) (www.ncbi.nlm.nih.gov) provides the FASTA format of the protein sequence of UYL70861.1, a 227 amino hypothetical protein allergen Asp F-38-like/hypothetical protein (AFUA_5G14680) from the fungus *Aspergillus fumigatus.*

3.5 Predicting the primary and secondary structures of the hypothesized hypothetical protein (AFUA_5G14680) from the *Aspergillus fumigatus* organism

ExPASy Prot Param (www.expasy.org, accessed on March 4, 2018) is a proteomics server that has been used to evaluate the linear sequence of the interesting protein. The molecular weight, theoretical pI, protein composition, the overall amount of negatively and positively charged residual amino acids, extinction coefficients, atomic composition, estimated half-life, aliphatic index, instability index, and GRAVY (grand average of hydropathicity) are among the physicochemical properties that are examined (38). The amount and placement of the mentioned features of that protein's secondary structure are thus anticipated, and ultimately, this prediction will assist us in determining which epitopes are best suited for vaccine creation.

3.6 Determination of B cell potential epitopes

Many physicochemical characteristics, including peptide chain flexibility, hydrophilicity, accessibility, exposed surface area, turns, polarity, and antigenic a tendency, are assessed in order to categorize the locations of the target protein's linear epitopes. Therefore, linear or continuous B cell epitopes for *Aspergillus fumigatus* hypothetical protein are estimated using a variety of Immune epitope database analysis resource (IEDB-AR) methods, including classical propensity scale methods like the Kolaskar and Tongaonkar(39) antigenicity scale ,the Parker hydrophilicity prediction, the Emini surface accessibility prediction , the Bepipred linear epitope prediction , the Karplus and Schulz flexibility prediction , the Chou and Fashman prediction tool for beta-turn. The protein's most likely B cell epitope has been determined, along with prognosis ratings and graphical conclusions(40).

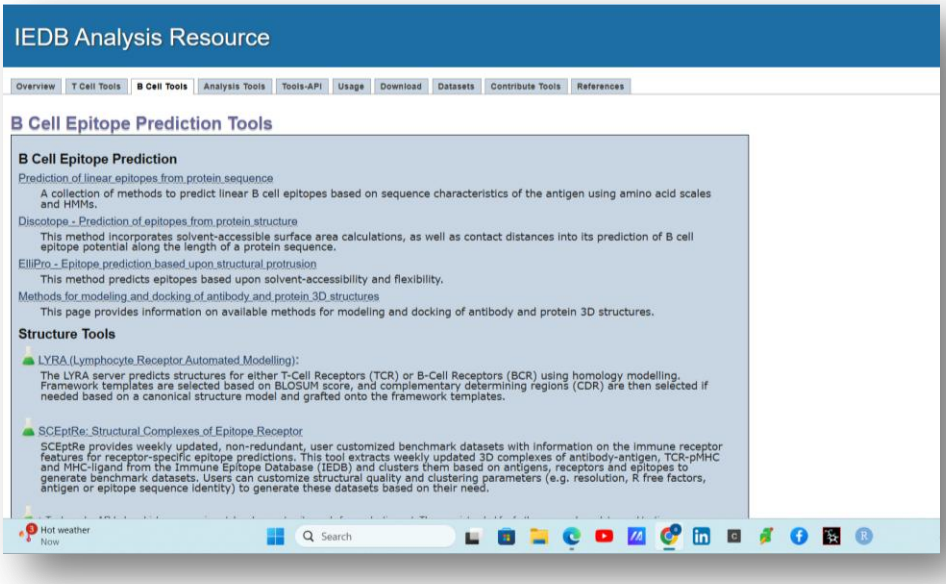


Figure 3.3 Window observed IEDB –AR.

3.7 Determination of T-cell potential epitopes

Antigen presenting cells (APCs) contains the Major Histocompatibility Complex (MHC) class II molecules which assist in the processing of fungal antigens in the human body and present to T-helper cells(41). The outcomes are based on MHC-II binding methods for anticipating using IEDB-AR tools. Two consensus algorithms have been implemented to document the chosen epitopes: the stabilized matrix method (SMM) and an artificial neural network (ANN)(42). This web server-based algorithm analyses binding scores to several MHC supertypes as a percentile rank to forecast the MHC-class II binding epitopes.

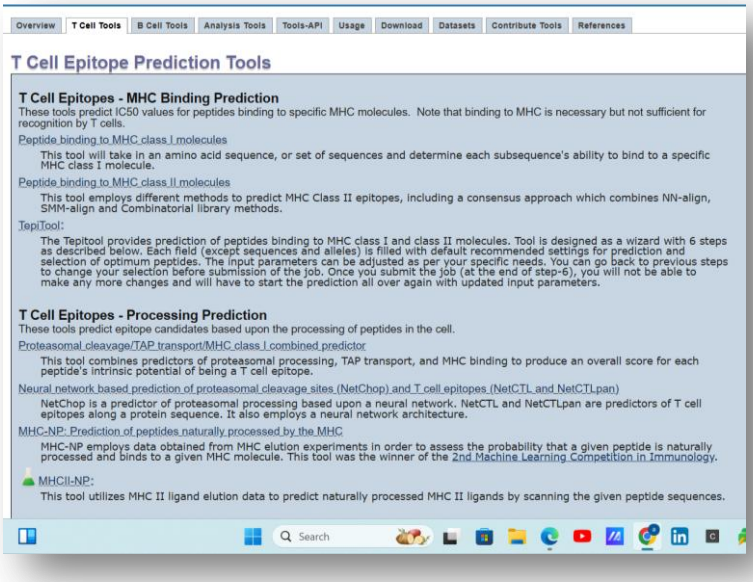


Figure 3.4. Window observed T-cell epitope prediction tools in IEDB-AR.

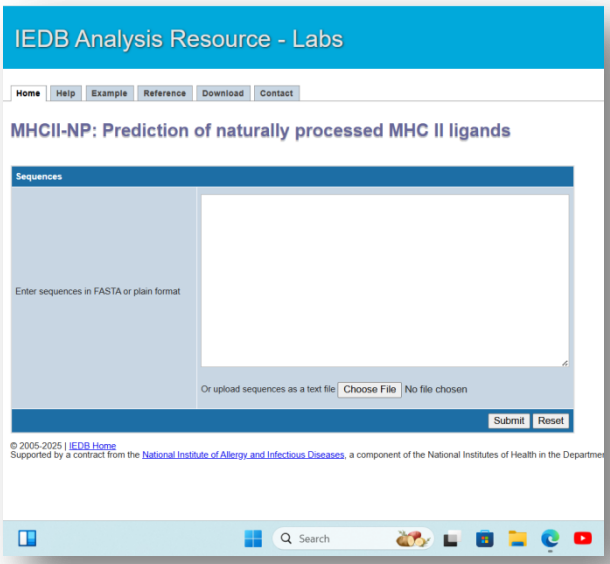


Figure 3.5. Window observed MHC II-NP tool for T- cell epitopes.

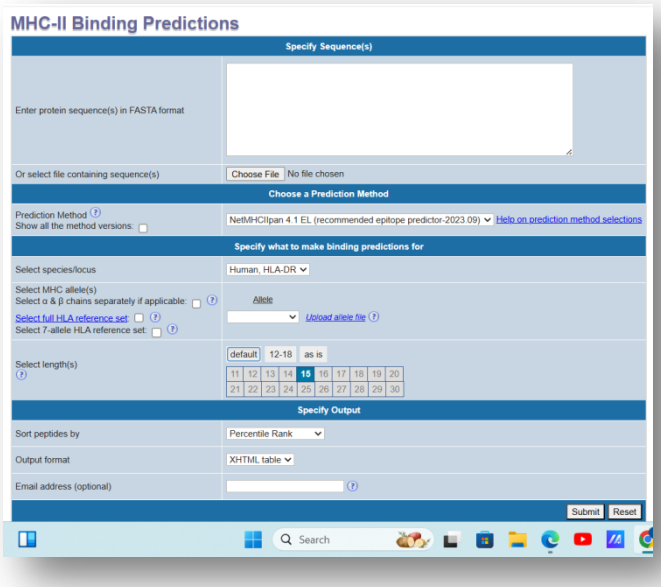


Figure 3.6. Window observed MHC-II binding predictions for further T-cell epitopes.

3.8 Population coverage estimation

T-cell epitopes are analysed through MHCII NP tool and peptide binding to MHC class II molecules tool. Naturally processed peptides are predicted through MHCII NP tool, these peptides are further queried for peptide binding to MHC class II molecules tool to determine their binding affinities towards different HLA- alleles of MHC class II molecules. Predicted T-cell epitopes are subjected to population coverage tool of IEDB-AR(43).

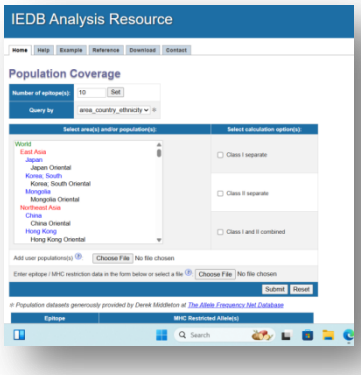


Figure 3.7. Window observed Population coverage in IEDB-AR.

3.9 T-cell and B-cell epitopic peptide formation

PEP-FOLD server is used to generate 3-D structure of depicted peptides for protein-protein docking. PEP-FOLD server use protein sequence of queried peptide(44).

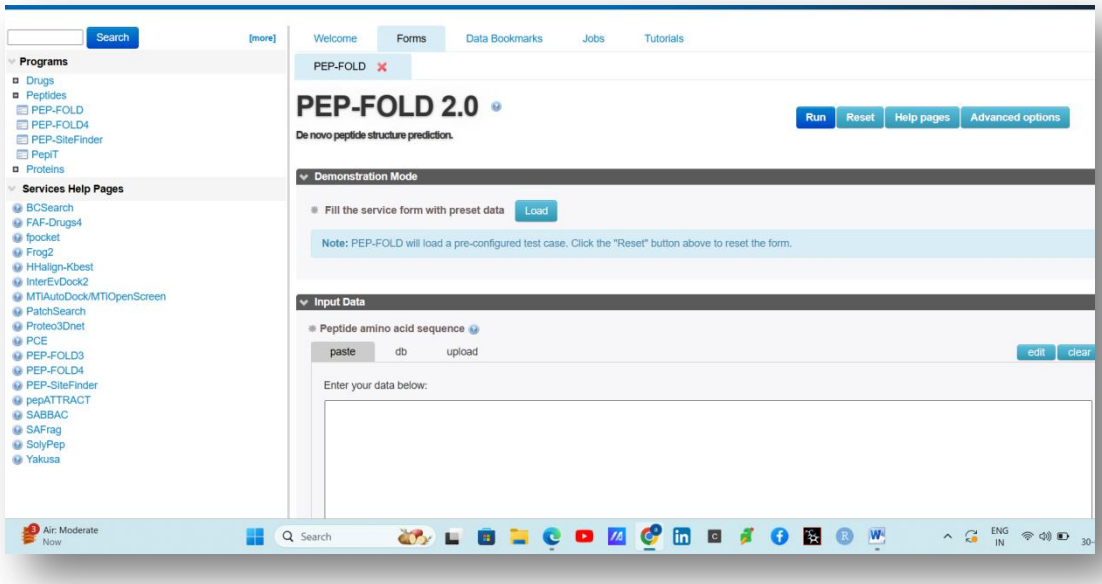


Figure 3.8. Window observed PEP-FOLD 2.0 for peptide formation.

3.10 Molecular Docking study of T and B cell epitopes

For protein–protein docking, the ClusPro server (https://cluspro.org) is a notable tool. Ten models defined by the centers of densely packed clusters of low-energy docked structures are created by docking with each set of energy parameters.

B-cell epitope and allergic antibody, IgE (PDB ID 4J4P) both are subjected for dock(45). B-cell epitopes and T-cell epitopes are generated in 3-D structure through the PEP-FOLD server and that after these epitopes are function as ligand in the docking server.

Antigenic fungal proteins are processed and presented by MHC-class II through APCs for T- cell recognition by their receptor. When T lymphocytes recognize an epitope (processed antigenic peptide) via T cell receptor by the help of MHC molecule connect to one another to form a ternary complex. Stable ternary complex formation is now a

vital need for a chosen T cell epitope in vaccine design. A ternary complex (PDB ID 3T0E) containing T cell receptor, a peptide with MHC molecule and CD4 receptor and the T cell epitope are subjected to docking(46).

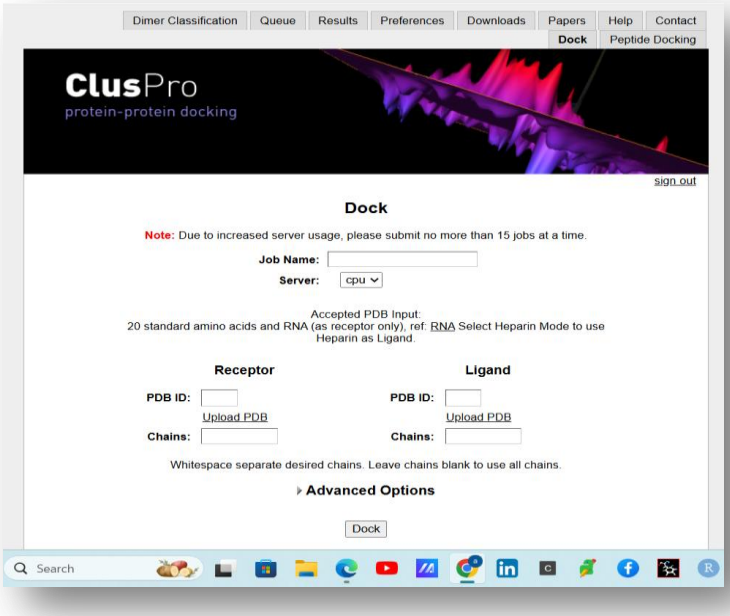


Figure 3.9. Window observed ClusPro server for molecular docking.

3.10.1 Protein –protein docking interaction analysis

Docked structures are analysed through Discovery Studio 2025. The lowest affinity docked structures are selected for analysing them. Bond type and their distance are going to be determined.

CHAPTER 4

RESULT & DISCUSSION

4.1 Identification of *A. fumigatus* Allergen sequences

Newly discovered allergens are Asp f35, Asp f36, Asp f37, Asp f38 and Asp f39 from *Aspergillus fumigatus*, these allergens were retrieved from Allergen Nomenclature Database, displays in table 4.1.

Table 4.1 Allergen retrieved from Allergen Nomenclature Database.

Aspergillus fumigatus						
S.No.	**Allergen**	**Biochemical name**	**Accession ID**	**Molecular weight (KDa)**	**Route of allergen exposure**	**Date created**
1.	Asp f35	Cu-Zn Superoxide dismutase	MZ731825	21	Airway	15-05-2022
2.	Asp f36	Fructose-bisphosphate aldolase	MZ731820	42	Airway	15-05-2022
3.	Asp f37	Malate dehydrogenase NAD-Dependent	MZ731822	35	Airway	15-05-2022
4.	Asp f38	Hypothetical protein	MZ731827	25	Airway	15-05-2022
5.	Asp f39	FG-GAP repeat protein	MZ73181	35	Airway	15-05-2022

Protein sequences of retrieved allergens are mentioned below for performing BLASTp.

1. Asp f 35 : Cu-Zn superox hhide dismutase

Molecular Weight (MW)(SDS-PAGE): 21kDA ,according to allergen database

Length = 154 amino acids

Protein sequence: >UYL70859.1 Cu-Zn superoxide dismutase [Aspergillus fumigatus]

MVKAVAVLRGDSKITGTVTFEQADENSPTTVSWNIKGNDPNAKRGFHVHQFG

DNTNGCTSAGPHFNPYGK

THGAPEDSERHVGDLGNFETDAEGNAVGSKQDKLIKLIGAESVLGRTLVVHAG

TDDLGRGGNEESKKTGN AGARPACGVIGIAA

2. Asp f 36 :Fructose - bisphosphate aldolase

MW(SDS- PAGE)=42kDa

Length=360 amino acids

Protein sequence :

>UYL70854.1 fructose-bisphosphate aldolase, class II [Aspergillus fumigatus]
MGILDKLSRKSGVIVGDDVLRLFEYAQEKNFAIPAVNVTSSSTVVACLEAARDQ
NCPIILQVSQGGAAYF
AGKGVSNDGQKASIAGSIAAAHYIRSIAPSYGIPVVLHTDHCAKKLLPWLDGML
DEDERYFKQHGEPLFS
SHMIDLSEEPVDYNIETTAKYLKRAAPMKQWLEMEIGITGGEEDGVNNEDVDN
NSLYTQPEDILAIYNAL
APISPYFSIAAGFGNVHGVYKPGNVRLHPELLSKHQAYVKEKIGSNKDKPVYFV
FHGGSGSTKEEYKQAI
SYGVVKVNLDTDMQYAYMSGVRDYILNKKDYLMSAVGNPDGEDKPNKKYFD
PRVWVREGEKTMSKRVQVA
LEDFNTAGQL

3. Asp f37: Malate dehydrogenase NAD-Dependent

MW= 35kDa

Length=331 amino acids

Protein sequence: >UYL70856.1 malate dehydrogenase, NAD-dependent [Aspergillus fumigatus]
MVKAAVLGASGGIGQPLSLLLKACPLVDELALYDVVNTPGVAADLSHISSVAK
VSGYLPKDDGLKNALTG
TDIVVIPAGIPRKPGMTRDDLFKVNAGIVRDLVTGIAQYCPKAFVLIISNPVNSTV
PIAAEVLKKQGVFD
PKRLFGVTTLDIVRAETFTQEYSGQKDPSKVQIPVVGGHSGETIVPLFSKASPAL
DIPADKYDALVNRVQ
FGGDEVVKAKDGAGSATLSMAYAGFRFAEKVIRASQGQSGIVEPTYIYLRGVT
GGEEIANETGVEFFSTL
VELGRNGAEKAINILQGVTEQEKKLLEACTKGLKGNIEKGIEFVKNPPPKA

4. Asp f 38: Protein of unkown function

MW = 25kDa

Length=227 amino acids

Protein sequence :

>UYL70861.1 hypothetical protein [Aspergillus fumigatus]
MGSNDTTEKLFHILLTTSHLQKNPNNVVEKVRIPGTYTTVQAAKAAAYRCLFD
AGYEKEWFTTYETKPEI
FENKDLPERSGLAVFAVAPDGTEFRVHIITTPKDDKLLDEVIEGHVTKPLYYVV
QANVEYSADEGSRVRD
IDIEGVFLTYQEARDLASRVLLSKEDAVTKESFAEYTEAAATEKDCGYGENVV
VRAVSEYGTNYLISVIK
NQELEAVHLAEAAMRIL

5. Asp f 39: FG-GAP repeat protein

MW= 35kDa

Length = 283 amino acids

Protein sequence :

>UYL70852.1 putative FG-GAP repeat protein [Aspergillus fumigatus]
MAPYQTRILATGTTFNAPDPYGPWSLTPSANEPGPDLAYIKTSNTGTGKVEVHL
ASRASNYQTRTLEVGT
TFFPEDNGVWQLIDADGDGRDDLVYIKTRNTGTGRVEVHIATAASNFQTRIKD
VGTTFLPEDNGTWQMAD
FDGDGILDLIYIKTRNTGTGRVEVHVASGASNYQTRIQEVGTTFYPEDNGVWQ
MIDFNRDGKLDLVYIKT
RNTGTGRVEVHVASGASTYKTRVQEVGTTFYSEDNGFWQLIDFNKDGVLDLA
YVKTQNTGTGRIEVHVAN
GRN

4.2 Homology and Sequence Analysis

These discovered *A. fumigatus* allergenic proteins were examined for optimal epitopic areas and utilized to forecast a vaccine or allergic shot candidate. These twenty-three allergenic proteins were first compared to the proteomes of the host and the model organism, the mouse. If chosen for vaccination or allergy injections against A. fumigatus, a similar epitopic region could cause autoimmune disorders or a catastrophic host cross-reaction. Therefore, it's critical to identify the finest allergenic protein for experimental research that might be used as a vaccine or allergic shot candidate. As per the result, two allergen Asp f35 and Asp f37 were found less than 1e-4 E-value .Both proteins were excluded from five researchable allergen due to showed high sequence similarity and identity against humans and mouse's genome. High E-value means low similarities. Asp f 36, Asp f 38 and Asp f 39 were showed high E-value 2.6, 1.9 and 5.8 respectively as given in table 4.2.

Table 4.2 Resultant sequence similarities from UniProt-BLASTp.

S.No.	Query ID	Subject ID	Organism	Identity (%)	E-value	Bit Score	Description
1.	UYL70859 (Asp f 35)	P004441& P08228	*Mus musculus* and *Homo sapiens*	59%(90/152)	1e-60	186	Superoxide dismutase Protein
2.	UYL70854 (Asp f 36)	P33261	*Homo sapiens*	25%(27/108)	2.6	32.3	Cytochrome P450 Protein
3.	UYL70856 (Asp f 37)	P08249& P40926	*Mus musculus* and *Homo sapeins*	55%(179/324)	1e-114	337	Malate dehydrogenase Protein
4.	UYL70861 (Asp f 38)	H7C1D9	*Homo sapeins*	23%(17/73)	1.9	32	Centrosome and basal body associated protein
5.	UYL70852 (Asp f 39)	Q5T4F6	*Homo sapeins*	26%(35/135)	5.8	30.8	Cartilage acidic protein

4.3 Antigenicity prediction

The web prediction server VaxiJen v 2.0 determines protein's antigenicity. Asp f36, Asp f38 and Asp f39 were subjected to check their antigenicity .As per the result, Asp f 38 has found high antigenicity score 0.6022 among three allergen proteins as shown in fig 4.1 and mentioned in table 4.3. Therefore, *in-silico* epitope based peptide vaccine determined utilizing Asp f 38 allergen proteins against *Aspergillus fumigatus.*

Table 4.3 Allergen's antigenicity score from VaxiJen server.

Allergen	**GI number**	**Protein name**	**Antigenicity score (Threshold>0.4)**
Asp f 36	2320084074	Fructose-bisphosphate aldolase	0.5204(proably Antigen)
Asp f 38	2320084088	Hypothetical protein	0.6022(Antigen)
Asp f 39	2320084070	FG-GAP repeat protein	0.3872(Non-Antigen)

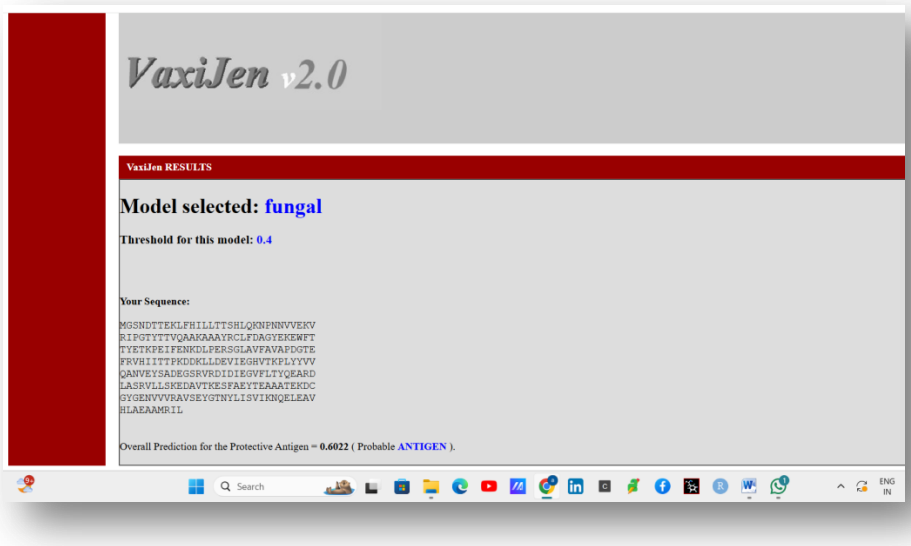


Figure 4.1. Resultant window of VaxiJen v2.0 .

4.4 Aspergillus fumigatus's hypothetical protein, Asp f 38 (Afu5g08030): primary structure prediction

The primary structure of this hypothetical protein is as follows:

>UYL70861.1 hypothetical protein [*Aspergillus fumigatus*]

MGSNDTTEKLFHILLTTSHLQKNPNNVVEKVRIPGTYTTVQAAKAAAYRCLF
DAGYEKEWFTTYETKPEIFENKDLPERSGLAVFAVAPDGTEFRVHIITTPKDD
KLLDEVIEGHVTKPLYYVVQANVEYSADEGSRVRDIDIEGVFLTYQEARDLA
SRVLLSKEDAVTKESFAEYTEAAATEKDCGYGENVVVRAVSEYGTNYLISVI
KNQELEAVHLAEAAMRIL

This fungal hypothetical (Asp f38) AFUA_5G14680 protein contains 227 amino acid residues and a calculated molecular weight is 25 kDa, according to the main structure analysis as mentioned in fig 4.2. It is estimated to have an isoelectric pH of 4.83. There are a total of 38 negatively charged amino acid residues (Asp + Glu) and 24 positively charged amino acid residues (Arg + Lys). In theory, a protein's isoelectric point value and the quantity of charged amino acid residues reveal that the protein is negatively charged. It is clear that this protein is a stable substance because its estimated half-life in rodent reticulocytes and in-vitro is 30 hours, and its calculated instability index (II) is 27.16. The N-terminal amino acid of this antigenic protein is M (Met), and the aliphatic index is determined to be 88.06. The protein is hydrophilic based on its GRAVY (grand average of hydropathicity) value -3.000. The peptide hydrophobicity score is represented by the Grand Average Index of Hydropathicity (GRAVY), which calculates the ratio of the length of the sequence to the sum of all amino acid hydropathy values. The amino acids Glu (E), Ala (A) and Val (V) are observed in large quantity like 11.0%,10.1% and 9.7%, as opposed to the other protein amino acids. On other hands Met(M), Cys(C), His(H), and Gln(Q) are present in low proportion,i.e.,0.9%, 0.9%, 2.2% and 2.2% in the protein.

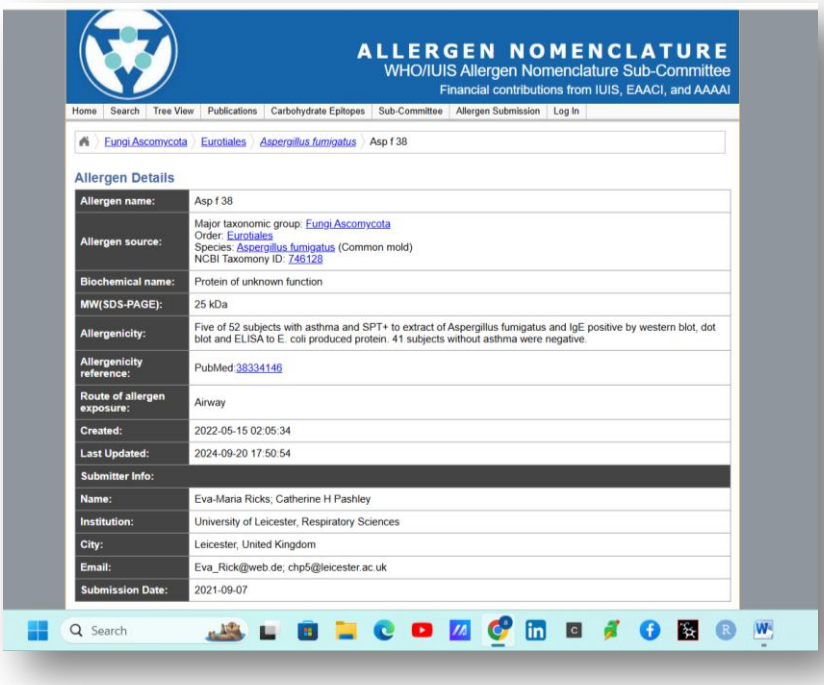


Figure 4.2. Asp f38, allergen details from ALLERGEN NOMENCLATURE server.

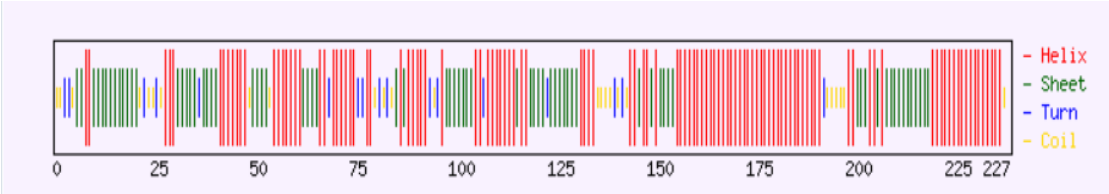


Figure 4.3. Secondary structure of Asp f 38 from ExPASY. Red, yellow, green and blue colour indicates helix, coil, sheet and beta-turn structure.

4.5 Potential B-cell epitope analysis

Selecting favourable epitopic areas from immunodominant proteins using *in silico* methods can reduce the time or cost of screening synthetic peptides. For providing humoral immunity, B- Lymphocytes recognized epitope of an antigen using their receptor (Ig M and Ig D). Epitopic regions of Asp f 38, an allergic antigen was analysed by IEDB–AR. The protein sequence of this allergen was submitted to the IEDB –AR database and analysed by using BepiPred method as shown in fig 4.4.

Total nine epitopic peptide were found with different peptide lengths as showed in table 4.4. ^{55}GYEKEWFTTYETKPEIFENKDLPER^{79} , ^{101}TPKDDKLLDEVIEGHV^{116} and ^{160}VLLSKEDAVTKESFAEYTEAAATEKDCG^{187} three longer peptide were selected to evaluated based on their epitopic's characteristic features like flexible regions, hydrophilic (surface – exposed) regions, surface- accessible regions and beta-turn regions (39). The resultant residue scores of putative B-cell epitope are displayed in table 4.5. Epitopic peptides were subjected to Karplus and Schulz Flexibility prediction, Parker Hydrophilicity prediction, Emini Surfcae Accessibiliy prediction and Chou- Fasman Surface Beta - turn Prediction. Epitopic's characteristic features scores for putative B-cell epitope are mentioned in table 4.6. These suspected B-cell epitope are further subjected to perform protein-protein docking.

Table 4.4 B-cell Epitopes from IEDB-AR server.

S.No.	Start	End	Peptide	Length
1.	4	4	D	1
2.	20	28	QKNPNNVVE	9
3.	34	37	GTYT	4
4.	54	78	GYEKEWFTTYETKPEIFENKDLPER	25

5.	100	115	TPKDDKLLDEVIEGHV	16
6.	128	139	EYSADEGSRVRD	12
7.	147	151	LTYQE	5
8.	159	186	VLLSKEDAVTKESFAEYTEAAATEKDCG	28
9.	210	218	NQELEAVHL	9

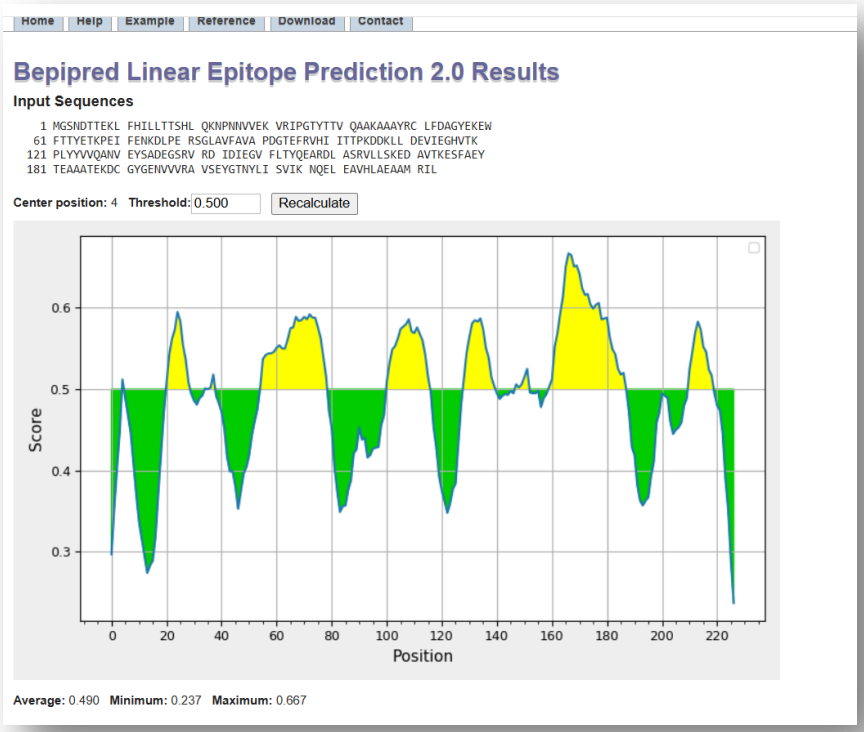


Figure 4.4. Bepipred linear epitope prediction graph representation for allergen Asp f 38(X-axis represents residue position in the sequence, Y-axis represents corresponding score).

Table 4.5. Predicted residue scores for B-cell epitopes from BepiPred method.

Position in sequence	Residue	Score	Assignment
4	**D**	0.512	E
20	**Q**	0.508	E
21	**K**	0.543	E
22	**N**	0.562	E
23	**P**	0.573	E
24	**N**	0.595	E
25	**N**	0.584	E
26	**V**	0.554	E
27	**V**	0.536	E
28	**E**	0.508	E
34	**G**	0.501	E
35	**T**	0.500	E
36	**Y**	0.502	E
37	**T**	0.518	E
54	**G**	0.501	E
55	**Y**	0.537	E
56	**E**	0.542	E
57	**K**	0.544	E
58	**E**	0.544	E
59	**W**	0.546	E
60	**F**	0.551	E
61	**T**	0.554	E
62	**T**	0.550	E
63	**Y**	0.550	E
64	**E**	0.561	E
65	**T**	0.575	E
66	**K**	0.576	E
67	**P**	0.589	E

Position in sequence	Residue	Score	Assignment
68	**E**	0.584	E
69	**I**	0.585	E
70	**F**	0.589	E
71	**E**	0.586	E
72	**N**	0.592	E
73	**K**	0.588	E
74	**D**	0.588	E
75	**L**	0.576	E
76	**P**	0.563	E
77	**E**	0.539	E
78	**R**	0.515	E
100	**T**	0.509	E
101	**P**	0.532	E
102	**K**	0.549	E
103	**D**	0.553	E
104	**D**	0.562	E
105	**K**	0.574	E
106	**L**	0.577	E
107	**L**	0.580	E
108	**D**	0.586	E
109	**E**	0.571	E
110	**V**	0.569	E
111	**I**	0.576	E
112	**E**	0.568	E
113	**G**	0.560	E
114	**H**	0.542	E
115	**V**	0.515	E
128	**E**	0.513	E
129	**Y**	0.545	E
130	**S**	0.564	E

Position in sequence	Residue	Score	Assignment
131	**A**	0.581	E
132	**D**	0.585	E
133	**E**	0.583	E
134	**G**	0.587	E
135	**S**	0.574	E
136	**R**	0.551	E
137	**V**	0.539	E
138	**R**	0.515	E
139	**D**	0.504	E
147	**L**	0.506	E
148	**T**	0.502	E
149	**Y**	0.506	E
150	**Q**	0.515	E
151	**E**	0.525	E
159	**V**	0.503	E
160	**L**	0.512	E
161	**L**	0.552	E
162	**S**	0.569	E
163	**K**	0.593	E
164	**E**	0.614	E
165	**D**	0.651	E
166	**A**	0.667	E
167	**V**	0.665	E
168	**T**	0.651	E
169	**K**	0.652	E
170	**E**	0.642	E
171	**S**	0.624	E
172	**F**	0.616	E
173	**A**	0.617	E
174	**E**	0.604	E

Position in sequence	Residue	Score	Assignment
175	**Y**	0.599	E
176	**T**	0.604	E
177	**E**	0.606	E
178	**A**	0.586	E
179	**A**	0.587	E
180	**A**	0.588	E
181	**T**	0.564	E
182	**E**	0.549	E
183	**K**	0.543	E
184	**D**	0.525	E
185	**C**	0.518	E
186	**G**	0.520	E
210	**N**	0.525	E
211	**Q**	0.546	E
212	**E**	0.569	E
213	**L**	0.583	E
214	**E**	0.574	E
215	**A**	0.552	E
216	**V**	0.546	E
217	**H**	0.524	E
218	**L**	0.517	E

Table 4.6. Epitopic characteristic prediction score for different B-cell epitopes.

S.NO.	Peptide	Karplus and Schulz Flexibility predication Threshold:1.018	Parker Hydrophilicity Predication Threshold:2.505	Emini Surfcae Accessibiliy Predication Threshold :1 000	Chou- Fasman Surface Beta - turn Predication Threshold : 1.025 (>1000)
1.	^{55}GYEKEWFTTYETKPEIFENKDLPER^{79}	Average - 1.021 Max-1.067 Min-0.986	Average - 2.098 Min-0.329 Max-4.557	Average - 1.000 Min-0.488 Max-2.262	Average - 0.949 Min-0.853 Max-1.119

2.	^{101}TPKDDKLLDEVIEGHV^{116}	Average - 1.021 Min-0.978 Max-1.051	Average - 1.901 Min--0.943 Max-4.214	Average - 1.000 Min-0.139 Max-3.841	Average - 0.933 Min-0.727 Max-1.144
3.	^{160}VLLSKEDAVTKESFAEYTEAAA TEKDCG^{187}	Average - 1.009 Min-0.966 Max-1.050	Average - 3.539 Min-1.129 Max-5.414	Average - 1.000 Min-0.429 Max-1.560	Average - 0.880 Min-0.769 Max-1.083

4.6 Computation of a potential T cell epitope

One of the main classes of T cells, CD4+ T- helper cells, include antifungal defense mechanisms that include proinflammatory cytokine secretion and cell-cell contacts to stimulate innate immune cells, support CD8+ T cells, and aid in the production of antibodies from B cells. Fundamentally, Antigen presenting cells (APCs) processed and present antigenic peptide, 14-16 mer via MHC-class II molecule for the T-helper cells recognization. The IEDB-AR database includes the MHC-II NP tool, which predicts naturally processed MHC class II ligands by analyzing specified sequences. This tool identified five peptide sequences from the Asp f38 allergen through protease action. Protease processes peptide sequences listed in the table 4.7. Higher cleavage probability scores indicate better predictions, while lower cleavage percentile scores are more desirable.

Table 4.7. T-cell epitopes with their cleavage probability score and percentile rank from MHC-II NP tool, IEDB-AR.

S.No.	Seq. name	Peptide start	Peptide end	Peptide length	Peptide	N motif	C motif	Cleavage probability score	Cleavage probability percentile rank
1.	UYL70861.1 HYPOTHETICAL PROTEIN	67	81	15	KPEIFENKDLPERSG	TKP	SGL	1.17430	0.00
2.	UYL70861.1 HYPOTHETICAL PROTEIN	33	46	14	IPGTYTTVQAAKAA	RIP	AAA	0.83182	0.04
3.	UYL70861.1 HYPOTHETICAL PROTEIN	118	133	16	KPLYYVVQANVEYSAD	TKP	ADE	0.79812	0.09
4.	UYL70861.1 HYPOTHETICAL PROTEIN	23	35	13	NPNNVVEKVRIPG	KNP	PGT	0.74003	0.13
5.	UYL70861.1 HYPOTHETICAL PROTEIN	33	47	15	IPGTYTTVQAAKAAA	RIP	AAY	0.73955	0.17

These peptide sequence ^{67}KPEIFENKDLPERSG^{81} , ^{33}IPGTYTTVQAAKAAA^{47} and ^{118}KPLYYVVQANVEYSAD^{133} were subjected to MHC-II binding predictions. In this t-cell epitopes prediction tool, where naturally processed peptide was entered in FASTA format, selected NetMNCIIpan 4.1 EL as a prediction method, then selected species and locus (Human,HLA-DR,HLA-DQ and HLA-DPA) and in last selected MHC alleles HLA-DRB1*01:01, HLA-DRB1*03:01, HLA-DRB1*04:01, HLA-DRB1*04:05, HLA-DRB1*07:01, HLA-DRB1*08:02, HLA-DRB1*09:01, HLA-DRB1*11:01, HLA-DRB1*12:01, HLA-DRB1*13:02, HLA-DRB1*15:01, HLA-DRB3*01:01, HLA-DRB3*02:02, HLA-DRB4*01:01, HLA-DRB5*01:01, HLA-DQA1*05:01/DQB1*02:01, HLA-DQA1*05:01/DQB1*03:01, HLA-DQA1*03:01/DQB1*03:02, HLA-DQA1*04:01/DQB1*04:02, HLA-DQA1*01:01/DQB1*05:01, HLA-DQA1*01:02/DQB1*06:02, HLA-DPA1*02:01/DPB1*01:01, HLA-DPA1*01:03/DPB1*02:01, HLA-DPA1*01:03/DPB1*04:01, HLA-DPA1*03:01/DPB1*04:02, HLA-DPA1*02:01/DPB1*05:01 and HLA-DPA1*02:01/DPB1*14:01. In result, peptide sequence is depicted with their binding allele moreover their score and percentile rank. High score shows good binders.

^{67}KPEIFENKDLPERSG^{81} this peptide binds with following alleles are as follows below: HLA-DRB1*04:05, HLA-DRB1*07:01,HLA-DQA1*01:02/DQB1*06:02,HLA-DQA1*03:01/DQB1*03:02,HLA-DQA1*05:01/DQB1*03:01,HLA-DRB1*09:01,HLA-DRB5*01:01,HLA-DRB1*04:01,HLA-DPA1*02:01/DPB1*14:01,HLA-DPA1*02:01/DPB1*01:01, HLA-DPA1*03:01/DP1B1*04:02,HLA-DRB1*04:05, HLA-DRB3*01:01,HLA-DRB1*03:01,HLA-DRB1*15:01,HLA-DQA1*03:01/DQB1*03:02,HLA-DRB1*08:02,HLA-DRB5*01:01,HLA-DRB1*04:01,HLA-DRB1*08:02,HLA-DPA1*02:01/DPB1*05:01,HLA-DPA1*02:01/DPB1*01:01,HLA-DRB1*11:01,HLA-DQA1*05:01/DQB1*03:01 as mentioned in table 4.8.

Table 4.8. MHC-II binding prediction results for ^{67}KPEIFENKDLPERSG^{81} peptide.

Allele	Start	End	Length	Core Sequence	Peptide Sequence	Score	Percentile Rank
HLA-DRB1*04:05	1	15	15	IFENKDLPE	KPEIFENKDLPERSG	0.6549	1.60
HLA-DRB1*04:01	1	15	15	IFENKDLPE	KPEIFENKDLPERSG	0.4498	3.70
HLA-DRB1*13:02	1	15	15	IFENKDLPE	KPEIFENKDLPERSG	0.4392	2.90
HLA-DRB3*02:02	1	15	15	IFENKDLPE	KPEIFENKDLPERSG	0.3331	1.60

Allele	Start	End	Length	Core Sequence	Peptide Sequence	Score	Percentile Rank
HLA-DRB1*08:02	1	15	15	FENKDLPER	KPEIFENKDLPERSG	0.2781	7.10
HLA-DRB1*03:01	1	15	15	IFENKDLPE	KPEIFENKDLPERSG	0.2683	5.40
HLA-DRB1*11:01	1	15	15	FENKDLPER	KPEIFENKDLPERSG	0.1567	11
HLA-DRB5*01:01	1	15	15	FENKDLPER	KPEIFENKDLPERSG	0.1448	7.70
HLA-DRB1*15:01	1	15	15	IFENKDLPE	KPEIFENKDLPERSG	0.1061	14
HLA-DRB3*01:01	1	15	15	IFENKDLPE	KPEIFENKDLPERSG	0.0993	5.20
HLA-DRB1*12:01	1	15	15	IFENKDLPE	KPEIFENKDLPERSG	0.0978	21
HLA-DQA1*05:01/DQB1*02:01	1	15	15	IFENKDLPE	KPEIFENKDLPERSG	0.0950	19
HLA-DQA1*04:01/DQB1*04:02	1	15	15	IFENKDLPE	KPEIFENKDLPERSG	0.0872	14
HLA-DRB1*09:01	1	15	15	IFENKDLPE	KPEIFENKDLPERSG	0.0867	26
HLA-DRB1*07:01	1	15	15	IFENKDLPE	KPEIFENKDLPERSG	0.0806	18
HLA-DPA1*03:01/DPB1*04:02	1	15	15	FENKDLPER	KPEIFENKDLPERSG	0.0717	14
HLA-DPA1*02:01/DPB1*01:01	1	15	15	IFENKDLPE	KPEIFENKDLPERSG	0.0591	11
HLA-DRB4*01:01	1	15	15	IFENKDLPE	KPEIFENKDLPERSG	0.0564	14
HLA-DPA1*01:03/DPB1*02:01	1	15	15	FENKDLPER	KPEIFENKDLPERSG	0.0552	15
HLA-DPA1*02:01/DPB1*05:01	1	15	15	FENKDLPER	KPEIFENKDLPERSG	0.0525	9.90
HLA-DQA1*01:02/DQB1*06:02	1	15	15	IFENKDLPE	KPEIFENKDLPERSG	0.0504	32
HLA-DQA1*05:01/DQB1*03:01	1	15	15	IFENKDLPE	KPEIFENKDLPERSG	0.0284	42
HLA-DQA1*01:01/DQB1*05:01	1	15	15	IFENKDLPE	KPEIFENKDLPERSG	0.0215	2.20
HLA-DPA1*02:01/DPB1*14:01	1	15	15	IFENKDLPE	KPEIFENKDLPERSG	0.0209	25
HLA-DPA1*01:03/DPB1*04:01	1	15	15	IFENKDLPE	KPEIFENKDLPERSG	0.0160	16
HLA-DRB1*01:01	1	15	15	IFENKDLPE	KPEIFENKDLPERSG	0.0123	39
HLA-DQA1*03:01/DQB1*03:02	1	15	15	IFENKDLPE	KPEIFENKDLPERSG	0.0088	6.40

[33]IPGTYTTVQAAKAAA[47] this peptide binds with following alleles are as follows below: HLA-DRB1*07:01, HLA-DRB3*02:02, HLA-DRB5*01:01, HLA-DRB1*15:01,HLA-DRB3*01:01, HLA-DRB4*01:01,HLA-DQA1*04:01/DQB1*04:02 ,HLA-DPA1*02:01/DPB1*14:01,HLA-DQA1*03:01/DQB1*03:02,HLA-DQA1*05:01/DQB1*02:01 ,HLA-DPA1*02:01/DPB1*05:01,HLA-DPA1*02:01/DPB1*01:01,HLA-DQA1*01:02/DQB1*06:02,HLA-DQA1*05:01/DQB1*03:01,LA-DPA1*03:01/DPB1*04:02,HLA-DQA1*01:01/DQB1*05:01 as mentioned in table 4.9.

Table 4.9. MHC-II binding prediction results for [33]IPGTYTTVQAAKAAA[47] peptide.

Allele	Start	End	Length	Core Sequence	Peptide Sequence	Score	Percentile Rank
HLA-DRB1*01:01	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.9788	0.03
HLA-DQA1*04:01/DQB1*04:02	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.4096	0.05
HLA-DPA1*02:01/DPB1*14:01	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.3432	0.17
HLA-DRB1*08:02	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.8469	0.19
HLA-DRB1*04:01	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.8831	0.26
HLA-DRB1*07:01	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.8391	0.32
HLA-DRB5*01:01	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.6679	0.38
HLA-DRB1*09:01	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.7700	0.41
HLA-DQA1*03:01/DQB1*03:02	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.0253	0.66
HLA-DQA1*01:02/DQB1*06:02	1	15	15	GTYTTVQAA	IPGTYTTVQAAKAAA	0.6172	0.70
HLA-DRB1*11:01	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.8132	0.82
HLA-DQA1*05:01/DQB1*02:01	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.5363	0.88
HLA-DRB1*04:05	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.7756	0.96
HLA-DPA1*02:01/DPB1*05:01	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.1215	1.30
HLA-DQA1*05:01/DQB1*03:01	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.6069	1.30
HLA-DRB3*02:02	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.3794	1.40
HLA-DPA1*02:01/DPB1*01:01	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.1331	2
HLA-DPA1*03:01/DPB1*04:02	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.1981	2.30
HLA-DQA1*01:01/DQB1*05:01	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.0191	2.70
HLA-DPA1*01:03/DPB1*04:01	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.0592	4.80
HLA-DPA1*01:03/DPB1*02:01	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.1292	6.10
HLA-DRB1*13:02	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.2603	6.90
HLA-DRB1*15:01	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.1991	7.90
HLA-DRB3*01:01	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.0441	9.80

Allele	Start	End	Length	Core Sequence	Peptide Sequence	Score	Percentile Rank
HLA-DRB4*01:01	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.0713	11
HLA-DRB1*12:01	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.1156	18
HLA-DRB1*03:01	1	15	15	YTTVQAAKA	IPGTYTTVQAAKAAA	0.0127	41

[118]KPLYYVVQANVEYSAD[133] this peptide binds with following alleles are as follows below: HLA-DRB1*04:05,HLA-DRB1*07:01,HLA-DQA1*01:02/DQB1*06:02,HLA-DQA1*03:01/DQB1*03:02,HLA-DRB1*04:01,HLA-DQA1*05:01/DQB1*03:01,HLA-DRB1*09:01,HLA-DQA1*01:02/DQB1*06:02,HLA-DRB5*01:01,HLA-DRB1*04:01,HLA-DQA1*03:01/DQB1*03:02,HLA-DPA1*02:01/DPB1*14:01,HLA-DPA1*02:01/DPB1*01:01,HLA-DPA1*03:01/DPB1*04:02,HLA-DQA1*04:01/DQB1*04:02,HLA-DRB3*02:02,HLA-DQA1*04:01/DQB1*04:02,HLA-DQA1*05:01/DQB1*03:01,HLA-DRB5*01:01,HLA-DRB3*02:02,HLA-DQA1*05:01/DQB1*02:01,HLA-DPA1*03:01/DPB1*04:02,HLA-DPA1*01:03/DPB1*04:01,LA-DRB3*01:01,HLA-DRB1*13:02,HLA-DQA1*01:01/DQB1*05:01,HLA-DRB5*01:01,HLA-DRB1*15:01,HLA-DQA1*05:01/DQB1*02:01,HLA-DRB1*11:01,HLA-DRB1*12:01 as recorded in table 4.10.

Table 4.10. MHC-II binding prediction results for [118]KPLYYVVQANVEYSAD[133] peptide.

Allele	Start	End	Length	Core Sequence	Peptide Sequence	Score	Percentile Rank
HLA-DRB1*04:05	1	15	15	YYVVQANVE	KPLYYVVQANVEYSA	0.9492	0.12
HLA-DRB1*07:01	1	15	15	YYVVQANVE	KPLYYVVQANVEYSA	0.7571	0.73
HLA-DRB1*04:05	2	16	15	YYVVQANVE	PLYYVVQANVEYSAD	0.7976	0.80
HLA-DQA1*03:01/DQB1*03:02	1	15	15	YYVVQANVE	KPLYYVVQANVEYSA	0.0233	0.86
HLA-DRB1*04:01	1	15	15	YYVVQANVE	KPLYYVVQANVEYSA	0.7419	1.10
HLA-DRB1*09:01	1	15	15	YYVVQANVE	KPLYYVVQANVEYSA	0.6367	1.50
HLA-DQA1*01:02/DQB1*06:02	2	16	15	VVQANVEYS	PLYYVVQANVEYSAD	0.4700	1.70
HLA-DRB5*01:01	1	15	15	YYVVQANVE	KPLYYVVQANVEYSA	0.3986	1.80
HLA-DRB5*01:01	2	16	15	YVVQANVEY	PLYYVVQANVEYSAD	0.3797	1.90
HLA-DRB1*04:01	2	16	15	YVVQANVEY	PLYYVVQANVEYSAD	0.6222	1.90
HLA-	2	16	15	YYVVQANVE	PLYYVVQANVEYSAD	0.0164	2

Allele	Start	End	Length	Core Sequence	Peptide Sequence	Score	Percentile Rank
DQA1*03:01/DQB1*03:02							
HLA-DPA1*02:01/DPB1*14:01	1	15	15	YYVVQANVE	KPLYYVVQANVEYSA	0.1308	2.10
HLA-DPA1*02:01/DPB1*01:01	1	15	15	YYVVQANVE	KPLYYVVQANVEYSA	0.1235	2.30
HLA-DRB3*02:02	1	15	15	YYVVQANVE	KPLYYVVQANVEYSA	0.2379	2.40
HLA-DQA1*05:01/DQB1*03:01	2	16	15	VVQANVEYS	PLYYVVQANVEYSAD	0.4741	2.50
HLA-DRB3*01:01	2	16	15	YVVQANVEY	PLYYVVQANVEYSAD	0.2212	2.80
HLA-DRB1*01:01	1	15	15	YYVVQANVE	KPLYYVVQANVEYSA	0.5602	2.80
HLA-DPA1*03:01/DPB1*04:02	1	15	15	YYVVQANVE	KPLYYVVQANVEYSA	0.1746	2.90
HLA-DRB3*02:02	2	16	15	YVVQANVEY	PLYYVVQANVEYSAD	0.1779	3.20
HLA-DRB3*01:01	1	15	15	YVVQANVEY	KPLYYVVQANVEYSA	0.1696	3.40
HLA-DQA1*04:01/DQB1*04:02	1	15	15	YYVVQANVE	KPLYYVVQANVEYSA	0.1569	3.70
HLA-DRB1*07:01	2	16	15	YYVVQANVE	PLYYVVQANVEYSAD	0.4065	3.70
HLA-DPA1*01:03/DPB1*04:01	1	15	15	YYVVQANVE	KPLYYVVQANVEYSA	0.0676	4.20
HLA-DRB1*13:02	2	16	15	YVVQANVEY	PLYYVVQANVEYSAD	0.3490	4.20
HLA-DRB1*01:01	2	16	15	YVVQANVEY	PLYYVVQANVEYSAD	0.4152	4.20
HLA-DRB1*08:02	1	15	15	YYVVQANVE	KPLYYVVQANVEYSA	0.3994	4.30
HLA-DRB1*12:01	2	16	15	YVVQANVEY	PLYYVVQANVEYSAD	0.3208	4.80
HLA-DRB1*09:01	2	16	15	YYVVQANVE	PLYYVVQANVEYSAD	0.4072	4.80
HLA-DPA1*02:01/DPB1*05:01	1	15	15	YYVVQANVE	KPLYYVVQANVEYSA	0.0713	5.40
HLA-DRB1*11:01	1	15	15	YYVVQANVE	KPLYYVVQANVEYSA	0.3357	5.70
HLA-DRB1*12:01	1	15	15	YVVQANVEY	KPLYYVVQANVEYSA	0.2831	5.80
HLA-DQA1*05:01/DQB1*03:01	1	15	15	VVQANVEYS	KPLYYVVQANVEYSA	0.2816	5.90
HLA-DPA1*03:01/DPB1*04:02	2	16	15	YVVQANVEY	PLYYVVQANVEYSAD	0.1182	6.10
HLA-DRB4*01:01	2	16	15	VVQANVEYS	PLYYVVQANVEYSAD	0.1295	6.10
HLA-DQA1*01:02/DQB1*06:02	1	15	15	VVQANVEYS	KPLYYVVQANVEYSA	0.2380	6.10
HLA-DQA1*05:01/DQB1*02:01	1	15	15	YYVVQANVE	KPLYYVVQANVEYSA	0.2435	6.10
HLA-DPA1*02:01/DPB1*01:01	2	16	15	YVVQANVEY	PLYYVVQANVEYSAD	0.0759	6.40
HLA-DRB1*13:02	1	15	15	YVVQANVEY	KPLYYVVQANVEYSA	0.2476	7.30
HLA-DQA1*05:01/DQB1*02:01	2	16	15	VVQANVEYS	PLYYVVQANVEYSAD	0.2113	7.70
HLA-DRB1*08:02	2	16	15	YVVQANVEY	PLYYVVQANVEYSAD	0.2225	9.30

Allele	Start	End	Length	Core Sequence	Peptide Sequence	Score	Percentile Rank
HLA-DPA1*02:01/DPB1*14:01	2	16	15	YYVVQANVE	PLYYVVQANVEYSAD	0.0512	9.40
HLA-DPA1*01:03/DPB1*04:01	2	16	15	YVVQANVEY	PLYYVVQANVEYSAD	0.0281	9.60
HLA-DQA1*01:01/DQB1*05:01	1	15	15	YYVVQANVE	KPLYYVVQANVEYSA	0.0085	9.90
HLA-DRB1*15:01	2	16	15	YVVQANVEY	PLYYVVQANVEYSAD	0.1562	9.90
HLA-DRB1*11:01	2	16	15	YVVQANVEY	PLYYVVQANVEYSAD	0.1778	10
HLA-DQA1*01:01/DQB1*05:01	2	16	15	YVVQANVEY	PLYYVVQANVEYSAD	0.0075	12
HLA-DPA1*02:01/DPB1*05:01	2	16	15	YVVQANVEY	PLYYVVQANVEYSAD	0.0476	12
HLA-DRB4*01:01	1	15	15	YYVVQANVE	KPLYYVVQANVEYSA	0.0661	12
HLA-DPA1*01:03/DPB1*02:01	1	15	15	YYVVQANVE	KPLYYVVQANVEYSA	0.0719	12
HLA-DRB1*15:01	1	15	15	YVVQANVEY	KPLYYVVQANVEYSA	0.1335	12
HLA-DQA1*04:01/DQB1*04:02	2	16	15	YYVVQANVE	PLYYVVQANVEYSAD	0.0922	13
HLA-DRB1*03:01	2	16	15	YVVQANVEY	PLYYVVQANVEYSAD	0.0601	18
HLA-DPA1*01:03/DPB1*02:01	2	16	15	YYVVQANVE	PLYYVVQANVEYSAD	0.0300	24
HLA-DRB1*03:01	1	15	15	YVVQANVEY	KPLYYVVQANVEYSA	0.0338	25

These peptide sequence ^{67}KPEIFENKDLPERSG^{81} , ^{33}IPGTYTTVQAAKAAA^{47} and ^{118}KPLYYVVQANVEYSAD^{133} were showed the large no. of MHC class II alleles interaction with their IC_{50} value as shown in table 4.11. The T cell the epitopes' binding effectiveness with the MHC molecules is shown by the IC50 value.IC_{50} value has to be less than 50nm which is consider for more putative T-cell epitope. The binding efficiency as the IC_{50} value decreases.

Table 4.11. Interacting MHC II super type with T-cell epitope prediction from IEDB-AR.

S.No.	**Core sequence**	**Peptide sequence**	**Start position**	**Interacting MHC II allele**	**IC50 nn-align**	**Percentile rank**
1.	IFENKDLPE	^{67}KPEIFENKDLPERSG^{81}	67	HLA-DRB3*02:02	4517.2	1.60
				HLA-DRB3*01:01	4794.2	5.20
				HLA-DRB1*03:01	1762.4	5.40
				HLA-DRB1*04:05	2589.7	1.60
				HLA-DQA1*01:01/DQB1*05:01	4933.20	2.20
				HLA-DRB1*13:02	699.3	2.90
				HLA-DRB1*04:01	7179.2	3.70
				HLA-DRB1*15:01	7580.90	5.50
				HLA-DRB1*08:02	3768.00	7.10

				HLA-DRB1*11:01	7465.00	11
				HLA-DQA1*03:01/DQB1*03:02	8750.50	6.40
				HLA-DRB5*01:01	9205.70	7.70
				HLA-DPA1*02:01/DPB1*05:01	14713.9	9.90
				HLA-DPA1*02:01/DPB1*01:01	3282.3	11
				HLA-DQA1*05:01/DQB1*03:01	1235.20	11
2.	YTTVQAAKA	^{33}IPGTYTTVQAAKAAA^{47}	33	HLA-DRB5*01:01	3.30	0.32
				HLA-DRB1*07:01	65.00	0.32
				HLA-DRB3*02:02	405.00	1.30
				HLA-DRB1*15:01	1426.30	7.10
				HLA-DQA1*04:01/DQB1*04:02	1435.60	0.04
				HLA-DPA1*02:01/DPB1*14:01	1435.60	0.12
				HLA-DQA1*03:01/DQB1*03:02	3331.80	0.80
				HLA-DQA1*05:01/DQB1*02:01	3275.00	0.88
				HLA-DPA1*02:01/DPB1*05:01	6303.90	1.10
				HLA-DPA1*02:01/DPB1*01:01	3297.10	1.80
				HLA-DQA1*01:02/DQB1*06:02	32.00	1.90
				HLA-DQA1*05:01/DQB1*03:01	72.80	2.10
				HLA-DPA1*03:01/DPB1*04:02	3786.00	2.20
				HLA-DQA1*01:01/DQB1*05:01	29011.5	2.30
3.	YYVVQANVE	^{118}KPLYYVVQANVEYSAD^{133}	118	HLA-DRB1*04:05	36.30	0.12
				HLA-DRB1*07:01	348.00	0.73
				HLA-DQA1*01:02/DQB1*06:02	112.30	0.79
				HLA-DQA1*03:01/DQB1*03:02	1137.40	0.86
				HLA-DRB1*04:01	88.40	1.10
				HLA-DQA1*05:01/DQB1*03:01	114.0	1.50
				HLA-DRB1*09:01	159.30	1.50
				HLA-DRB5*01:01	266.90	1.80
				HLA-DPA1*02:01/DPB1*14:01	1692.70	2.10
				HLA-DPA1*02:01/DPB1*01:01	324.00	2.30
				HLA-DPA1*03:01/DPB1*04:02	275.30	2.50
				HLA-DRB3*02:02	137.40	3.20
				HLA-DPA1*01:03/DPB1*04:01	888.60	3.30
				HLA-DQA1*04:01/DQB1*04:02	337.60	3.70
				HLA-DRB1*13:02	154.30	4.20
				HLA-DRB1*08:02	468.00	4.30
				HLA-DPA1*02:01/DPB1*05:01	2543.60	4.60
				HLA-DRB1*12:01	754.50	4.60
				HLA-DRB1*11:01	957.00	5.70
				HLA-DQA1*01:01/DQB1*05:01	702.40	7.50
				HLA-DRB1*15:01	327.00	7.70
				HLA-DQA1*05:01/DQB1*02:01	208.70	7.70

The IEDB population coverage estimation tool is used to determine the population coverage for established T cell epitopes for the global population. This technique determines the proportion of individuals who should react to a specific set of epitopes with known MHC limitations.

Table 4.12. HLA alleles(genotypic frequency(%)) and population coverage for T-cell epitopic peptide from population coverage ,IEDB-AR.

Epitope	Coverage	HLA allele (genotypic frequency (%))																																					Total HLA hits
	Class II	(0.0)	HLA-DPA1*01:03/DPB1*04:01 (0.0)	HLA-DPA1*02:01/DPB1*01:01 (0.0)	HLA-DPA1*02:01/DPB1*05:01 (0.0)	HLA-DPA1*02:01/DPB1*14:01 (0.0)	HLA-DPA1*03:01/DP1B1*04:02 (0.0)	HLA-DPA1*03:01/DPB1*04:02 (0.0)	HLA-DQA1*01:01/DQB1*05:01 (0.0)	HLA-DQA1*01:01/DQB1*05:01. (0.0)	HLA-DQA1*01:02/DQB1*06:02 (0.0)	HLA-DQA1*03:01/DQB1*03:02 (0.0)	HLA-DQA1*04:01/DQB1*04:02 (0.0)	HLA-DQA1*05:01/DQB1*02:01 (0.0)	HLA-DQA1*05:01/DQB1*03:01 (0.0)	HLA-DRB1*03:01 (8.43)	HLA-DRB1*04:01 (1.37)	HLA-DRB1*04:05 (0.50)	HLA-DRB1*07:01 (15.22)	HLA-DRB1*08:02 (0.35)	HLA-DRB1*09:01 (1.09)	HLA-DRB1*11:01 (6.12)	HLA-DRB1*12:01 (1.66)	HLA-DRB1*13:02 (2.09)	HLA-DRB1*15:01 (8.48)	HLA-DRB3*01:01 (0.0)	HLA-DRB3*02:02 (0.0)	HLA-DRB4*01:01 (0.0)	HLA-DRB5*01:01 (0.0)	LA-DPA1*03:01/DPB1*04:02 (0.0)	LA-DRB3*01:01 (0.0)		
Epitope #1: IPGTYTTVQAAKAAA	41.78 %	-	-	+	+	+	-	-	-	+	+	+	+	+	+	-	-	-	+	-	-	-	-	-	+	+	+	+	+	+	-	16
Epitope #2: KPEIFENKDLPERSG	65.85 %	-	-	+	+	+	+	-	-	-	+	+	-	-	+	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	-	17
Epitope #3: KPLYYVVQANVEYSA	59.72 %	+	+	+	-	+	-	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	-	+	-	+	-	+	22
Epitope set	70.10 %	1	1	3	2	3	1	1	1	1	3	3	2	2	3	1	2	2	3	1	2	2	1	1	3	2	2	1	3	1	1	55

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Primarily, ^{67}KPEIFENKDLPERSG^{81} , a 15-mer peptide sequence showed highest cleavage probability score 1.17430 and lowest cleavage probability percentile rank

0.00 among five peptide sequence according to the MHC NP II results. On the other hand, this peptide showed best population coverage % in India which was found as 65.85% nevertheless IC_{50} value was found more than 200nm which determined their lower binding efficiency. Furthermore ^{33}IPGTYTTVQAAKAAA^{47}, a 15-mer peptide sequence and ^{118}KPLYYVVQANVEYSAD^{133} a 16-mer peptide sequence both found less than 50nm IC_{50} value 3.30 nm and 36.30nm and the population coverage for these two peptide were 41.78% and 59.72%, respectively as mentioned in table 4.12 and 4.13. Considering the both IC_{50} value and population coverage ^{67}KPEIFENKDLPERSG^{81}, ^{33}IPGTYTTVQAAKAAA^{47}and^{118}KPLYYVVQANVEYSAD^{133} three peptide sequences were chosen for molecular docking.

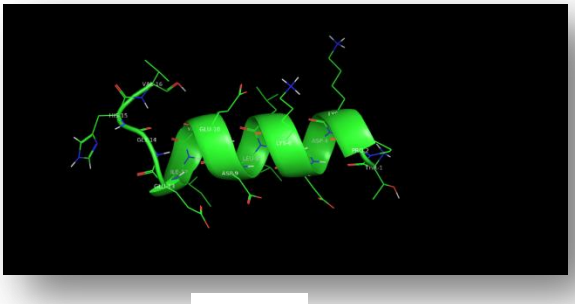
Table 4.13. Population coverage and IC50 value for the chosen peptide sequences.

S.No.	Suspected Epitope Sequences	Core Sequence	Interacting Allele	IC_{50} Value	Population Coverage
1.	^{67}KPEIFENKDLPERSG^{81}	IFENKDLPE	HLA-DRB1*13:02	699.3	65.85%
2.	^{33}IPGTYTTVQAAKAAA^{47}	YTTVQAAKA	HLA-DRB5*01:01	3.30	41.78%
3.	^{118}KPLYYVVQANVEYSAD^{133}	YYVVQANVE	HLA-DRB1*04:05	36.30	59.72%

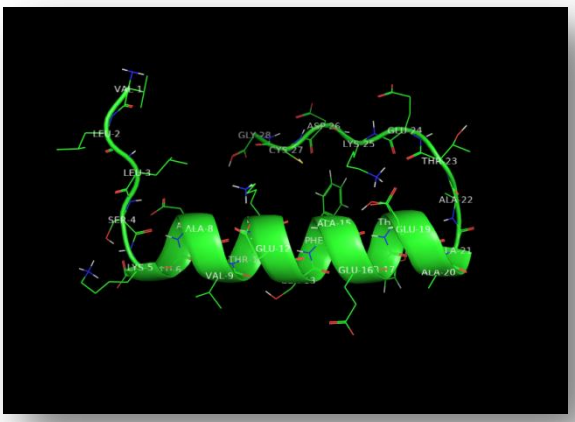
4.7 3-D structure of B-cell and T-cell epitope

PEP-FOLD server generated 3-D structure of predicted epitopes is shown in fig 4.5 and 4.6.

(A)

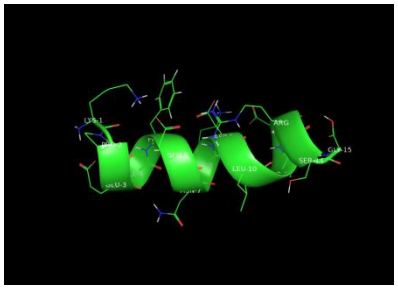


(B)

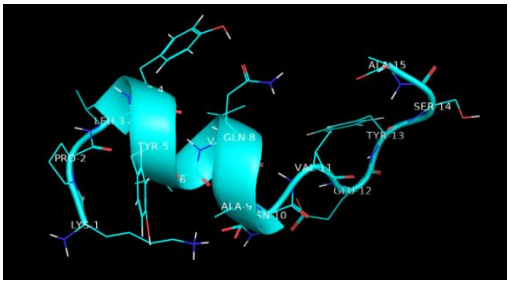


(C)

Figure 4.5 . 3-D structure of epitopic peptide for B-cell from PEP-FOLD server.(A) ^{55}GYEKEWFTTYETKPEIFENKDLPER^{79} (B) ^{101}TPKDDKLLDEVIEGHV^{116} and (C) ^{160}VLLSKEDAVTKESFAEYTEAAATEKDCG^{187} B-cell epitopes.



(A)



(B)

(C)

Figure 4.6 .3-D structure of epitopic peptide for T -cell from PEP-FOLD server.(A) ^{67}KPEIFENKDLPERSG^{81}(B) ^{33}IPGTYTTVQAAKAAA^{47}and (C) ^{118}KPLYYVVQANVEYSAD^{133}T-cell epitopes.

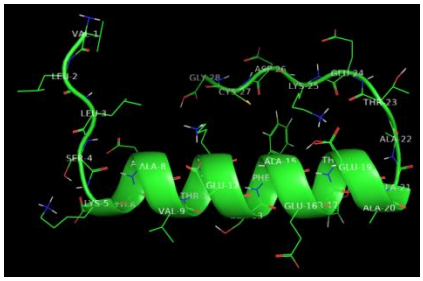
4.8 Molecular docking results

Protein- protein docking was performed through ClusPro 2.2 web server that analyzed antibody-antigen interaction by utilizing the FFT-based algorithm. Putative B-cell epitopes docked with allergic antibody, IgE (PDB ID 4J4P). Consequently, maximum 12 docked models were provided after protein-protein docking with each set of energy parameters. Moreover, the server mentioned the number of members of docked model and their lowest energy's weighted score for each docked model. In the

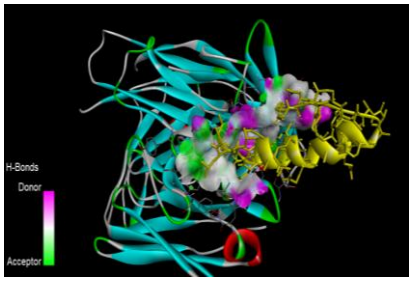
series of analysis, a comparative analysis has been done between suspected three B-cell epitopes docked clusters by comparing their lowest energy's weighted score as mentioned in table 4.14. ^{160}VLLSKEDAVTKESFAEYTEAAATEKDCG^{187} peptide has the lowest energy's weighted score -319.7 Kcal/mole among three suspected B-cell epitopes. Subsequently, the best docked cluster was visualized by Discovery studio 2025 which displays receptor (IgE)-ligand (epitope) interaction. The total number of favourable non-covalent bonds was 15 which includes total hydrogen bonds: 10, hydrogen bond: 5, salt bridge: 2, non-classical hydrogen bond: 3, charge interactions: 3 and hydrophobic bond was 2 as mentioned in table 4.15. A stable association between the ligand and the receptor protein was demonstrated by the docked complex's reduced energy and large number of interacting bonds and residues.

Table 4.14. Listed of the B-cell epitope with their docking results from ClusPro server.

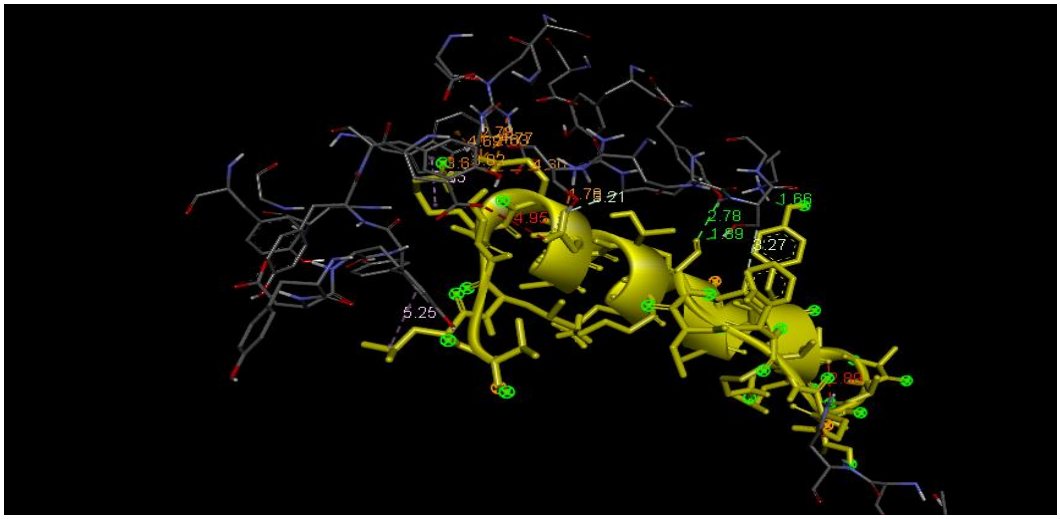
S.No.	B-cell epitopes	Members of docked models	Lowest Energy's Weighted score
1.	^{55}GYEKEWFTTYETKPEIFENKDLPER^{79}	226	-316.4
2.	^{101}TPKDDKLLDEVIEGHV^{116}	182	-315.3
3.	^{160}VLLSKEDAVTKESFAEYTEAAATEKDCG^{187}	116	-319.7



(A)



(B)



(C)

Fig 4.7(a)B-cell epitope , [160]VLLSKEDAVTKESFAEYTEAAATEKDCG[187] a 3D – structure generated by PEP-FOLD database.(b) Docking structure visulazied by discovery studio which was obtained through ClusPro server.Yellow coloured structure indicated peptide, Blue coloured sticks indicates IgE fab region and magenta to green bar scale colour indicates hydrogen bond between antibody and peptide.(c) Green coloured digits shows hydrogen bonds distance and orange coloured digits shows electrostatic bonds distance.

Non-covalent interaction between antibody and B-cell epitope shows in figure 4.7(b)and(c). Hydrogen bond depicted with magenta to green colour bar scale,whereas magenta and green region indicates donor and acceptor interaction,respectively shown in fig4.7(b). Interacting residues of antibody and epitope with their category of interaction was mentioned in table 4.15.

Table 4.15. Details of total favourable non-covalent interaction with their respective bond length between IgE and B-cell epitope.

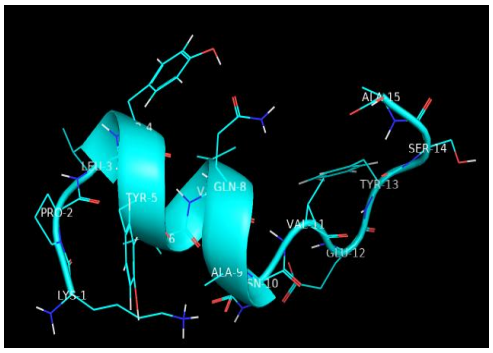
S.No.	Interacting residues	Distance(Å)	Category of Interactions
1.	C:ARG71:HH11 - :GLU6:OE2	2.634	Hydrogen Bond ;Electrostatic
2.	C:ARG75:HH12 - :ASP7:OD2	1.783	Hydrogen Bond ;Electrostatic
3.	C:ARG71:NH1 - :GLU6:OE1	4.774	Hydrogen Bond ;Electrostatic
4.	C:ARG71:NH2 - :GLU6:OE2	2.781	Hydrogen Bond ;Electrostatic
5.	C:ARG75:NH1 - :GLU6:OE1	4.602	Hydrogen Bond ;Electrostatic
6.	C: SER76:HN - :GLY28:O	2.784	Hydrogen Bond

7.	C:SER76:HG - :GLY28:O	1.885	Hydrogen Bond
8.	C:LYS77:HZ1 - :TYR17:OH	1.664	Hydrogen Bond
9.	C:ARG75:CD - :ASP7:OD2	3.210	Hydrogen Bond
10.	C:SER76:CB - :ASP26:OD2	3.270	Hydrogen Bond
11.	:LYS5:NZ - C:TYR131	3.611	Electrostatic
12.	:GLU6:OE1 - C:TYR131	3.824	Electrostatic
13.	:GLU6:OE2 - D:TRP111	4.692	Electrostatic
14.	C:TYR128 - :LEU2	5.247	Hydrophobic
15.	D:TRP111 - :LYS5	5.050	Hydrophobic

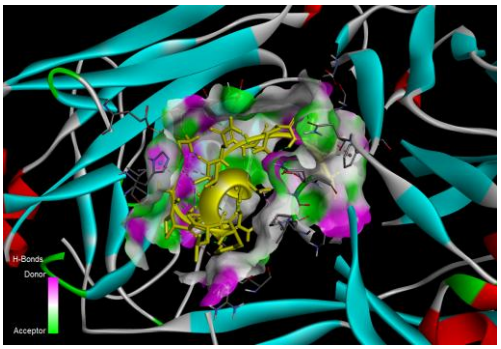
On other hand, T-cell epitopes were subjected to protein-protein docking through ClusPro server. , ^{67}KPEIFENKDLPERSG^{81} , ^{33}IPGTYTTVQAAKAAA^{47}and ^{118}KPLYYVVQANVEYSAD^{133} three suspected T-cell epitope were docked with ternary complex (PDB ID 3T0E) containing T cell receptor, a peptide with MHC molecule and CD4 receptor. By performing comparative analysis between these three epitopes, this ^{118}KPLYYVVQANVEYSAD^{133} T-cell possess lowest energy's weighted score is -889.6 given in table 4.16. Lowest energy's weighted score model further visualized in Discovery studio 2025 where non-covalent interaction could be monitored in three-dimensional structure.

Table 4.16. Listed of the T-cell epitope with their docking results from ClusPro server.

S.No.	T-cell epitopes	Number of members in docked model	Lowest energy's weighted score
1.	^{67}KPEIFENKDLPERSG^{81}	38	-565.2
2.	^{33}IPGTYTTVQAAKAAA^{47}	212	-726.4
3.	^{118}KPLYYVVQANVEYSAD^{133}	206	-889.6



(A)



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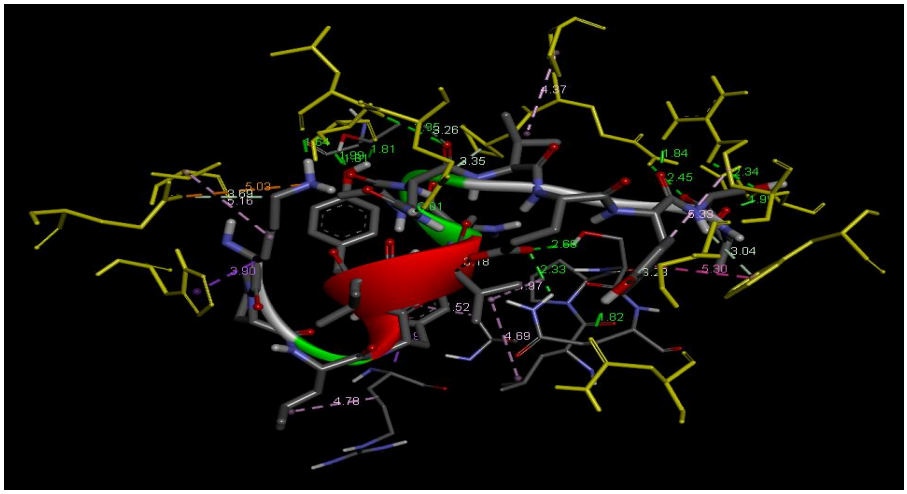

(C)

Fig 4.8 (a)T -cell epitope , ^{118}KPLYYVVQANVEYSAD^{133} a 3D –structure generated by PEP-FOLD database.(b) Docking structure visulazied by discovery studio visualizer 2025 which was obtained through ClusPro server.Yellow coloured structure indicated peptide, Blue coloured sticks indicates alpha and beta chains and magenta to green bar scale colour indicates hydrogen bond between MHC class II and peptide.(c) Green coloured digits shows hydrogen bonds distance, orange coloured digits shows electrostatic bonds distance and pink coloured digits shows hydrophobic interactions.

Table 4.17. Details of total favourable non-covalent interaction with their respective bond length between ternary complex and T-cell epitope.

S.No.	Interacting residues	Distance(Å)	Type of interaction
1.	:LYS1:NZ - D:GLU165:OE1	5.028	Electrostatic
2.	C:SER40:HG - :GLU12:OE2	2.692	Hydrogen bond
3.	C:GLN41:HE21 - :GLU12:OE2	2.332	Hydrogen bond
4.	C:THR157:HG1 - :TYR5:OH	1.813	Hydrogen bond
5.	D:LYS39:HN - :ALA15:O	1.985	Hydrogen bond
6.	D:ARG110:HE - :SER14:OG	2.342	Hydrogen bond
7.	D:LEU157:HN - :ASN10:O	2.849	Hydrogen bond
8.	D:THR172:HG1 - :ALA9:O	1.991	Hydrogen bond
9.	D:GLN175:HE21 - :SER14:O	2.449	Hydrogen bond
10.	:LYS1:HZ2 - D:LEU157:O	1.639	Hydrogen bond
11.	:TYR5:HH - D:THR172:O	1.810	Hydrogen bond
12.	:ASN10:HD22 - D:GLU156:OE2	2.014	Hydrogen bond
13.	:TYR13:HH - D:ASN107:OD1	1.820	Hydrogen bond
14.	D:ARG110:HH22 - :TYR13:O	1.838	Hydrogen bond

The following amino acids residues of ^{118}KPLYYVVQANVEYSAD^{133} T-cell epitope are LYS1, GLU12, TYR5, ALA15, SER14, ASN10, TYR13, TYR4, ALA9, VAL6,

VAL11, LEU3 and VAL7 positively interact with MHC class II molecule, compiling all the type of interaction, this docking found total 30 non-covalent interaction which consists of total 19 hydrogen bonds, 13 hydrogen bonds, 6 non- classical hydrogen bonds, 1 electrostatic interaction and 14 hydrophobic interaction as shown in table 4.17. Strong hydrogen bond has bond length is 1.5-2.5 (Å). Mostly hydrogen bond has their bond length in between 1.5-2.5 (Å) concluded that strong interaction between T cell epitope and ternary complex as shown in fig 4.8(c).

CHAPTER 5

CONCLUSION

T-cell and B-cell epitope of *Aspergillus fumigatus* are identified and characterized by using immunoinformatics approach as discussed in methodology. Epitope for T-cell and B-cell fundamentally provides cell- mediated and humoral (antibody medicated) immunity which is a part of adaptive immunity. Allergic bronchopulmonary aspergillosis is a fungal infection cause by inhalation of *A. fumigatus* spores (allergens) which triggers the hypersensitivity reaction. Moreover, it releases the cytokines interleukin (IL)-4, IL-5, and IL-13, which raise IgE and blood and airway eosinophils. Thus, IgE category of antibody is prominently neutralized the fungal antigen (allergen). B-cells secrets the IgE antibody through V-DJ recombination after the recognition of antigen and simultaneously developed memory cell against fungal allergen .The production of memory cells make the secondary immune response stronger and faster than the primary immune response which results in a more successful removal of pathogens. This is the consequence of vaccination.

In this study, B-cell and T-cell epitope is predicated against Allergic Bronchopulmonoary Aspergillosis. B-cell epitope, ^{160}VLLSKEDAVTKESFAEYTEAAATEKDCG^{187} possess the strong interaction towards the IgE antibody and it's all other epitopic's characteristic features like flexible regions, hydrophilic (surface – exposed) regions, surface- accessible regions and beta-turn regions are also ≤ to threshold value which made it more accessible for epitope- based peptide vaccine. Helper T cells, or Th2 cells, are crucial to the hypersensitive reaction brought on by the *A. fumigatus* antigen. T-cell epitope, ^{118}KPLYYVVQANVEYSAD^{133} is exhibits putative peptide sequence, the core sequence is YYVVQANVE, interacting to the 22 different MHC class II alleles with the low IC_{50} value of 36.30 nm and population coverage is 59.72%. Protein –protein docking possess the strong and stable non-covalent interaction between a^{118}KPLYYVVQANVEYSAD^{133}T-cell epitope and ternary complex of TCR, MHC and CD4 molecule. Although ^{33}IPGTYTTVQAAKAAA^{47} T-cell epitope exhibits IC_{50} value is 3.30 nm that means the binding affinity towards MHC molecule is higher than other two epitope. Binding affinity value and IC_{50} value has inversely proportional relationship. However, the population coverage for this epitope is 41.78% which relatively low. Multiple epitope based vaccine

could utilize both T-cell epitopes, one epitope possess the least IC_{50} but population coverage is low and another one epitope which possess low IC_{50} value along high population coverage. For improving immunogenicity of putative epitope adjuvants are required to introduce in vaccine formulation. The development of novel adjuvants is therefore particularly significant to vaccines based on epitopes. Further Molecular Dynamics stimulation and laboratory based experiments will be performed that after epitopes are approved. All things considered, the peptides that emerged from our research might be experimentally prioritised in order to investigate allergy immunotherapy to combat *Aspergillus*-mediated illnesses.

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Anjani

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Objective

A highly motivated final-year M.Sc. Biotechnology student with a strong foundation in molecular biology, microbiology,biochemistry, immunology and bioinformatics. Silver medalist in B.Sc(H) Biotechnology , GATE BT 2025, GATE BT and XL , IIT JAM , GAT- B AIR - 42 , CUET PG - Life sciences and Biochemistry 2023 qualified. I am seeking Ph.d or a research opportunity which allows me to continue learning and perfecting my skills as I provide high-quality work, and encourages me to flourish as a biotechnologist.

Experience

- **Cytogene Research And Development , Lucknow** April 2022 - May 2022
  Trainee
  One month training in Advance Biochemistry Techniques:-
  - Isolation of proteins from different sources.
  - SDS PAGE .
  - Native SDS PAGE.
  - Protein estimation by lowry's method.
  - Separation of amino acids by thin layer chromatography.
  - Separation of compounds by paper chromatography.
  - Purification by column chromatography.
  - Estimation of sugars by DNS essay.
  - Estimation of phenols.
  - Estimation of flavonoids.
  - DNA isolation from plant leaf.
  - Quantifications of nucleotides.
  - Agarose gel electrophoresis.

- **DNA Labs- a centre for applied science ,Dehradun ,Uttarakhand** March 2023 - May 2023
  Project intern
  - Developed essential skills and techniques through diligent practice.
  - Entered Enfield the documents and the record into system.
  - Update documentations and reports for records.
  - Work with senior personnel to troubleshoot and diagnose issues with equipments
  - Prepared report sum rising results from evaluation assessments after Eid session concluded.

Education

- **Delhi technological University** 2023-25
  Master's of Science in Biotechnology
  1st year- 8.3 CGPA, 3 sem- 8.8 CGPA

- **U.P. Pt. Deen Dayal Upadhyaya Veterinary Science University and Cattle Research Institute , Mathura** 2020-23
  Bachelor's of Science (Hons.)in Biotechnology
  78.8% , Silver medalist

- **L P S B V M Inter College, Bulandshahr** 2020
  Intermediate Examination (U.P. Board)
  79.6%

- **L P S B V M Inter College ,Bulandshahr** 2018
  High school examination(U.P. Board)
  80%

Skills

- Data analysis .
- Plant Tissue culture -Micropropagation.

- Animal tissue culture - primary cell culture of embryonic cell of chick.
- Sterilization.
- Semi-automatic biochemistry analyser.
- DNA isolation from plant , blood and bacteria.
- Agarose gel electrophoresis.
- PCR.
- ELISA.
- Immunoelectrophoresis .
- Radical Immunodiffusion .
- Isolation of microorganisms (bacteria, fungi and yeast).
- Identification of microorganisms.
- Staining techniques for microorganisms.
- Basic of Bioinformatics.
- Molecular docking
- Immuninformatic
- Microsoft powerpoint
- Nanoparticles synthesis

Projects

- **The study of different types of genotypes of ACE 2 receptor in cardiovascular diseases.**
  AIM:
  The study of different types of genotypes of ACE 2 receptor in cardiovascular diseases.
  OBJECTIVES:
  I.To performs biochemical parameters (Lipid profile, Kidney function test, Liver function test) related to cardiovascular diseases .
  II.DNA extraction from whole blood using standard kits (HUWEL Nucleic acid extraction kit).
  III.Standardization of End point PCR Thermal Cycler (Gradient PCR Machine) for detection of different genotypes of ACE-2 Receptor and analysis of bands by using agarose gel electrophoresis.
  IV.To establish the correlation between Cardio Vascular Disease and the angiotensin converting enzyme (insertion/deletion) gene polymorphism.
  Result and Conclusion:
  In conclusion, our data suggested that the DD genotype of ACE gene might be a significant risk factor for the development of CAD in suspected paitents. Polymorphism of ACE gene as the genetic predisposition of cardiovascular disease.
  The ACE I/D gene of the DD genotype and the D allele have been linked to an increased risk of developing hypertensive CVD complications.
  This study shows an association between the DD genotype and D allele of the ACE gene I/D polymorphisms and the occurrence of hypertensive CVD complications. In order to diagnose CVD early, identify it, and avoid its CVD consequence, the ACE gene I/D polymorphism may be employed as a biomarker. Additionally, CVD was linked to higher TC, TG, VLDL-C, SGOT, SGPT, BLOOD CREATNINE, UREA, URIC ACID, SODIUM and lower HDL-C values. These findings further support the key significance of the cardiovascular diseases.

Achievements & Awards

- Silver medalist in B.Sc.(Hons.) Biotechnology.
- GATE - BT and XL 2023 ,IIT JAM ,GAT - B AIR- 42 and CUET PG- Life science and Biochemistry 2023 qualified.
- GATE BT 2025

Reference

- **Dr. Priyambada Singh - College of Biotechnology, U.P. Pandit Deen Dayal Upadhyay Veterinary Science University and Cattle Research Institute ,Mathura**
  Coordinator and Teaching Associate
  priyambiochem@gmail.com

- **Dr.Narotam Sharma - DNA Labs- A Centre for applied science, Dehradun, Uttarakhand**
  Scientist and head Laboratories
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Seminar

- **World Zoonoses Day**
  'Zoonoses: Current Scenario '

Certificate

- One month training in Advance Biochemistry
- International Day of immunology
- The Study of Different Genotypes in ACE2 Receptor in Cardiovascular Diseases
- Three months training in Biochemistry and Molecular Biology.
- Technical Hands- On Workshop in Bioinformatics