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IMMUNO-INFORMATIC APPROACH OF IN-SILICO T CELL EPITOPE PREDICTION

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Abstract:

Epitope is the defined group of amino acids which is derived from a antigen and induce response by the activating of lymphatic cell receptor either T and B- cell or both and epitope mapping is the mechanism through which we can identify where the antibody bind with antigen. For the epitope mapping we have used many type of method and divided in two group one was based on biological process and second one was based on computational method few of them are discussed in this article such as ANN, HMM, NMR, X-ray, Quadratic programming and other biological methods are fragmentation, competition etc. There were many databases available which gave us full information about the peptide sequence these are MHCBN, MHCPEP, IEDB etc. were discussed and conclude that epitope mapping is so beneficial for human there are lots of advantages in various field such as vaccine design, finding autoimmune diseases and cure of many disease such as HIV, Pneumonia, Type1-diabetes etc. by using T-cell epitope mapping.

Keywords: Epitope mapping, Prediction method, Databases.

Background

Immunoglobulins are glycoprotein molecules that are produced by plasma cells in response to an immunogen and which function as antibodies (Mayer G, 2009). It is having CDRs region that is complementary in shape to a surface structure on the antigen. Paratope and epitope are two surface features of antibody and antigen respectively and allow an induced fit due to certain amount of flexibility of them (Morris GE, 2007) and the process of locating the antibody-binding site on the antigen, known as epitope mapping (Stern LJ, 1994).

What is a T cell epitope?

Epitope is the defined group of amino acids which is derived from a protein of antigen and activating an immune response by interacting with the receptor (B-cell or T-cell) (De Groot AS, 2006) it is a complex which formed with the help of MHC molecule which is mainly use for T-cell receptor for triggering an adaptive immune response but due to unstable structure of MHC molecule it cannot bind to receptor by own self (Smith R, 2007). Structure of T-cell receptor mainly formed by two type of chain structure that are an alpha chain and a beta chain; each chain has a constant and a variable domain. A transmembrane region anchors the protein in the plasma membrane of the T-cell, which is rich in hydrophobic amino acids. The heterodimer which is noncovalently associated with an invariant set of membrane proteins called the CD3 complex (Alberts E, 1994).

Biological function of T cell in immunogenicity

T-cells are the subset of lymphocyte, which is a type of immune cell and work against the antigen by playing large role in immune response. There are two type of immunity occur in immune system one is cell mediated and another is humoral. In humoral immunity antibody production occur and in this B-cells are also include (Lundegaard C, 2007) but in cell mediated immunity antibody production does not occur instead this it produces mainly cytokine which work against the antigen it is an adaptive immune response against invasive microorganism. Cytokine productions are the specific recognition of processed antigens those bound with MHC molecule on antigen presenting cell surfaces (Singh R, 2010). T cell-mediated immunity is an adaptive process of developing antigen (Ag)-specific T lymphocytes to eliminate viral, bacterial, or parasitic infections or malignant cells but some time T cell-mediated immunity can also involve aberrant recognition of self-Ag, leading to autoimmune inflammatory diseases (Broere F) instead of the activated T cells rapidly proliferate and migrate through the tissues to the target sites (where Ag present) and perform effector functions such as cell-mediated cytotoxicity and production of various cytokines. There are mainly two type of Cytokines used such as Cytotoxic CD8+ T cells which are very effective in direct lysis of infected or malignant cells which are having the Ag, and second CD4+ T helper cells which are produce cytokines that can be directly toxic to the target cells or can stimulate other T cell effector functions and produce B cell antibody (Resch K). T cell-mediated immunity play essential role in the development of non-responsiveness toward naturally occurring self-Ag, while mounting effective immune responses against “foreign” Ag (Matzinger P, 2002). Such as cytokines which is derived from T-cell play role in down regulation of immune response in parasitic and retroviral infection (Sher A, 1992).

Type of T-cell

T cell is the type of lymphocyte and mainly divides in following subtype:

Helper T- cell:

Robert Coffman and Timothy Mossman firstly described the division of $CD4^+$ T cells in 1986, into functional subsets, which were based on cytokine production (Bluestone JA, 2009) and after activation of $CD4^+$ T-cell differentiate in two lineage of helper T cell that is T_H1 and T_H2 and both subtype having different biological function T_H1 cell support cell mediated immunity by releasing interleukin-Y and T_H2 cells produce interlukin-4 and support humoral immunity (Renier SL, 2009). But recent research found that notch pathway play essential role in T-cell mediated immunity and found that not all T_H1 cells require interlukin-12 for stimulation and if there are some essential signalling(notch signalling) exist then T_H2 cell independent from interlukin-4 (Amesn D, 2009).

Cytotoxic T-cell:

It is the subtype of T cell which is also known as killer T-cell, it is mainly related to $CD8^+$ co-receptor and has capability to kill infected cell directly by using αβT-cell receptor for antigen, $CD4^+$ also help the $CD8^+$ with the help of two mechanism first is through direct contact and second one is through dendritic cells (Smith CM, 2004) by activation of CD25 and CD127 which are present on $CD8^+$ T-cell surface. Rapid activation of $CD8^+$ T-cell is critical for some intracellular pathogen in this case $CD4^+$ play as a primary immune response (Kumamoto Y, 2011) dendritic cell play essential role for inducing CTL response by direct priming and cross priming because MHC is not sufficient to induce CTL (Melief, CJM, 2003) when infectious agent directly infect DCs when direct priming are occur and when it is not directly affect DC then cross-priming take place **(**Zinkernagel, RM, 2002).

Suppressor T-cell:

It is subpopulation of T-cell which is play crucial role in maintain immune tolerance and also known as regulatory T-cell. Although $CD4^+$ and $CD25^+$ express αβ T-cell receptor but they are enriched in suppressor activity (Sakaguchi S, 1995) and suppress T-cell in the presence of antigen presenting cells and showed increase amount of expression of TNF receptor (GITR) that shows activation of effector T-cell (Shimizu J, 2002). $CD25^+$ is not proliferate in culture except when supplemented compound like interleukin-2 was not added, in the absence of IL-2, it suppress the function of $CD4^+$ and $CD8^+$ (Shevach EM, 2002). Suppression of proliferation occur by two method direct cell contact and local secretion of cytokine (Boehmer HV, 2005). In direct cell contact APC is not required, it only require ligand and antibody to activation of supresssor T-cell and in second method cytokine such as TGFβ help in suppression of T-cells and the function of TGFβ bound cell based on inhibition of suppression by TGFβ antibody(Nakamura K, 2001).

Databases of T-cell epitope:

Database provide all information about epitope (linear and confirmational) like which peptide sequence included what is the source of protein and which pathogen group is related etc. (Saha S, 2005) and a PDB structure gives information that which amino acid shows proximity towards the epitope and by the help of these information, we can analyse new epitope or unique complex, which is provided by database (Reitmaier R). There are many databases of T-cell epitopes are used such as IEDB, JENPEP, MHCPEP, MHCBN, SYFPEITHI, Antijen database etc.

IEDB:

This is immune epitope databases it is related to immune mediated disease association. It is accessible on external site between epitope search and immune recognition site partition and this help in disease recognition (Zhang Q, 2008), for the selection of disease it provides hierarchical disease tree for the identification user can type disease name or synonyms of disease in the database (Vaughan K, 2010).

Implementation should be done for user comfort who search disease which is computationally associated with epitope (antigen). For T-cell assay Data submission tool wizard is uses presently, but this data is recommended which is short and less than 10 epitope (Newsletters, Aug 2011), Example of allergic asthma database (Vaughan K, 2010) shown in following figure.

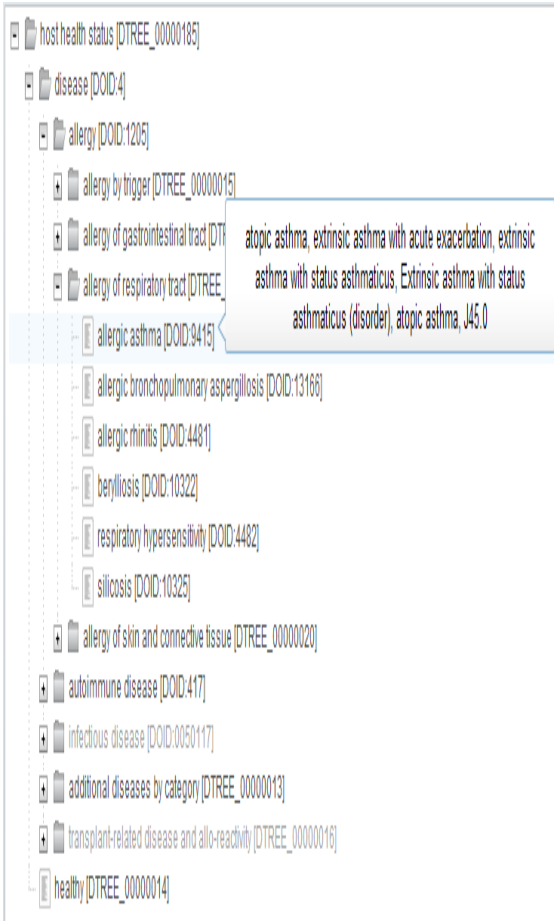

Fig.1.IEDB database of allergic asthma (Vaughan K, 2010)

JENPEP:

JenPep is a database which is mainly contain binding data for MHC1 and MHC2, TAP but it also contain a separate database for T-cell epitope and have more than 8000 sequences (Blythe MJ, 2002). JenPep currently used publish data for evaluation (Ruppert J, 1994) and the server is available at the site: http://www.jenner.ac.uk/JenPep. It is a relational database and made by using Microsoft access and graphical user interface. There is implementation in previous JenPep database and currently it is modest in size and compares more favourably with other database (Baxavanish AD, 2001).

MHCPEP:

MHCPEP is a database which is comprises with more than 13,000 peptide sequence and each entry contain full information of peptide sequences such as its binding affinity, MHC specificity, observed activity, source protein and its anchor position and identification of T cell epitope etc. (Brusic V, 1998). Present database format allows matching search by text string but it can be converted in sequence analysis packages (Wisconsin M, 1994) and entries compiled either published report or experimental data submission directly (Brusic V, 1997). MHCPEP database can be accessed by this site: http.//wehih.wehi.edu.au/mhcpep/ (Crol E, 1992). Example of database:

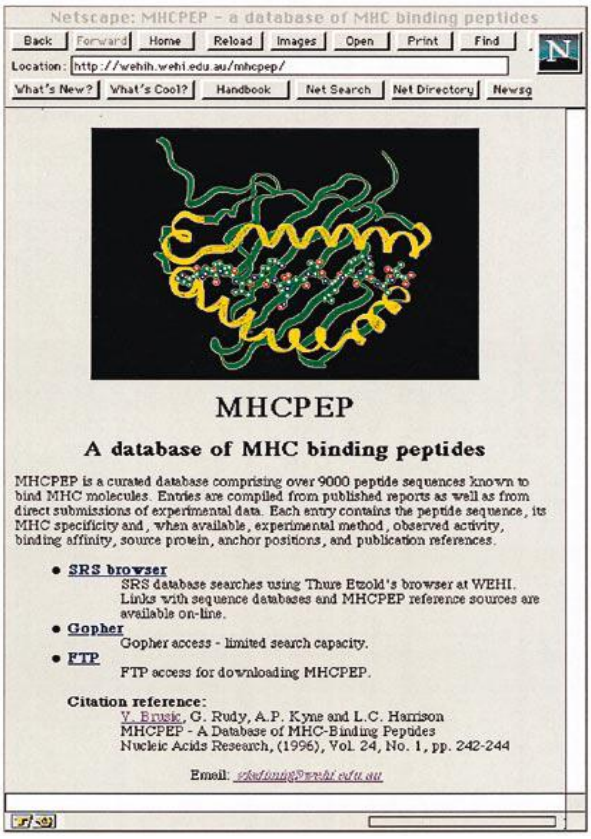

Fig.2.MHCPEP database of MHC binding protein (Home page) (Brusic et al., 1997)

MHCBN:

MHCBN is a curative database which comprises more than 23,000 peptide sequences whose binding affinity are assayed experimentally (Bhasin M, 2007) by the help of major histocompatibility complex and TAP (Transporter associated with antigen processing) (Brusic V, 1998). It provide full information of about each entry in database such as its binding affinity(Ic50), TAP etc. for this it include number of web based tool which are working (Wheeler DL, 2003) in following manner:

1.Mapping the antigenic region with query sequence.
2.Produce alleleic specific data.
3.Use BLAST for data (Altschul SF, 1997).

This database is accessible from this site: http/www.imtech.res.in/raghava/mhcbn (Bhasin M, 2007). The database also maintain 106 structure and 400 sequence which is associated with MHC molecule with other server like Swiss Prot, Genbank, PDB etc. (Bairoch A, 2000). In this database mainly entries contain mouse and human MHC molecule, the architechure of this database is following which is created by Dr. Raghava group:

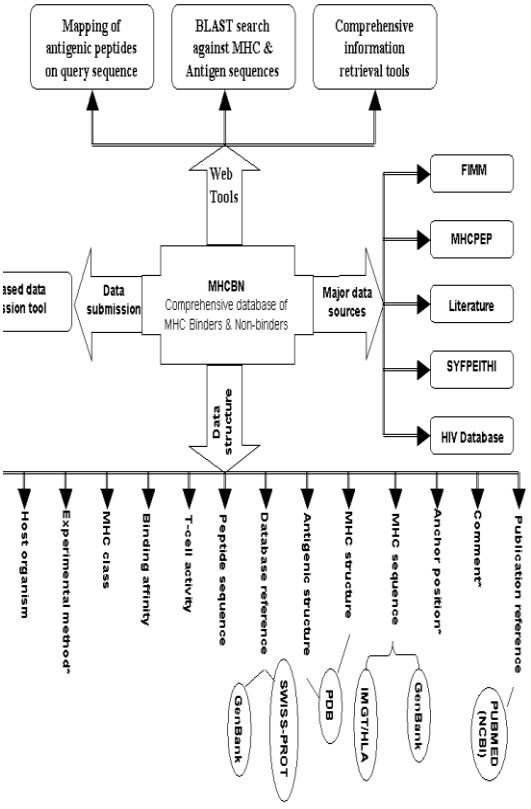

Fig.3. Architecture of MHCBN database (Bhasin et al., 2003)

Methods of T-cell epitope prediction:

There are many methods of T-cell epitope prediction used which can help prediction of epitope mapping. These method can divided in two group biological method and other one is computational method.

Biological method of prediction:

This group can include many method which is based on biological process are included such as competition binding, fragmentation, NMR, Reverse Vaccinology etc.

NMR:

NMR refer's nuclear magnetic resonance which is based on the difference in mobility between antigen and antibody in epitope mapping. It is a powerful tool for determining the epitope peptide with the help of antibodies (Rosen O, 2009), it is mainly used for study complex of antigen and antibody a crucial parameter in this is the rate of exchange between ligand's free state to bound states (Kustanovich I, 1996). By using NMR we can determine which amino acid sequence of the Ag with Abs in solution and that Ag synthesized with $_{15}N$, $_{13}C$, or $_{2}H$ is applicable. STD-NMR (saturation transfer-difference NMR) is alternative use of NMR, in which energy transfer show which atoms of antigen are in close proximity to the antibody and the proton resonance of the antigen is also important factor in the STD (Ladner RC, 2007).

Competition methods:

This method can show competition between antibody, antigen for known ligand, it show that two antibody bind with overlapping or non overlapping epitope but did not bind with same epitope by the using known ligand we can find out where the antibody bind with particular epitope means where it competes the ligand, Elisa assay (Ladner RC, 2007) is the one of example of competition method, surface plasmogen device is also used (Wassaf D, 2006). In ELISA one component is bound to surface which is either antigen or antibody but when we used polystyrene base then antibody and other protein can be denatured (Butler JE, 1992). Competitive binding is essential for linear and conformational epitope (Chou TH, 2005).

Fragmentation methods:

The use of antigen fragmenting is library approach for epitope mapping in which fragment screened on western blot with antibody, it is widely applicable but not used in case of so much assembled protein. Various type of chemical such as CNBr, idosobenzoic acid can be used in the fragmentation (Morris GE, 1996). Binding by large antigen fragment is commonly used method, in this either enzymatically or recombinantly large antigen fragments are produced and it's fragments mapping show which domain contain epitope either linear or conformational (Ladner RC, 2007). We can increase resolution of Fragment by using mutation but it should not disrupt the protein (Jin L, 1992).

Computational method of prediction:

A large variety of methods are used for epitope prediction which is based on computational method such as artificial neural network(ANN), hidden markov models(HMM), position specific scoring matrices, Gibbs sampler method (Lundegaard C, 2007) etc.

ANN:

In an ANN, three layer input layer, hidden layer and output layer are connected to a given data structure through weighted connection and all the information has collected with these layer which was trained and distributed into a computer network (Baldi P, 2001) and ANN ideally suited to recognize non-linear pattern (Adams HP, 1995). In neural networking training various type of combination of sequence encoding are used such as conventional sparse encoding, Blosum50 and other encoding (Henikoff S, 1992). The Blosum versus Sparse encoding having two different approach to represent sequence information to the neural network. In Blosum encoding network have less precise knowledge and information about a sequence, Blosum matrix contain have basic knowledge about the similar and dissimilar sequence but in sparse encoding network having precise information about the sequence means network can learn very specific knowledge about the sequence and its binding affinity (Nielsen M, 2002).

HMM:

A hidden markov model is a statistical model (Gribskov M, 1987) and it is well suited to predict peptide binding with MHC class 1 and 2 molecule and have been used to characterize biological motifs with an inherent structural compositions. The trained HMM used to discrimination or multiple alignment (Hughey R, 1995).

Basically a hidden markov model was based on the sequence in the Rammensee data set and generated for the HLA-A2 (Eddy SR, 1998) and using BLOSUM62 and FASTA format.
An epitope similarity score S for the 9-amino-acid long peptide is calculated as:

$$S=\sum_{i=1\ldots9} 2*\log(Pi/Qi) \log 2$$

where Pi is the probability for finding a given amino acid on position i in the hidden Markov model and Qi is the probability for finding the amino acid in the Swiss Prot database (Bairoch A, 2000).

Gibbs sampler method:

It is a implementation of PSSM search algorithms and based on Monte Carlo algorithm (Lundegaard C, 2007), which is provide maximal information of given motif length mainly for random selection of sample, where the optimal PSSM is determined by sequence alignment search. Although PSSM are log-odds matrices (Altschul SF, 1997) where logarithm of the ratio of observed frequency to background frequency of that given amino acids help weight matrix estimation (Lundeggard C, 2007). There are many other techniques and methods are also available which used in the construction of PSSM such as stabilization matrix method (Peters B, 2005) and evolutionary algorithm (Brusic V, 1998).

X ray:

X-ray crystallography is a structural approach to epitope mapping which can determine the complex of antigen and antibody but it should be in pure crystal form for highly confirmation (Morris GE).
X-ray structure of epitope and paratope help to prediction of epitope mapping and shows where the paratope bind with epitope and which residue contribute in the most of the binding energy and specificity (Ladner RC, 2007). X-ray crystallography can be used in the identification of inhibitory monoclonal antibody in the lymphocyte cell membrane receptor interfaces, for this we have used recombinant human antibody which is contain repeat sequence to immunize mice.

Epiphany:

This is the fully automated computational based method which is used for the high throughput analysis of protein sequences along with this we developed algorithm for epitope prediction which is integrated with this computational method, which was focusing MHC binding protein, it is mainly use prediction of medium level binding (Florea L, 2002).

Quadratic programming:

Quadratic programming prediction of epitope mapping method is based on support vector machines literature (Burgess CJC, 1998) which is employed by singh and kim method (Singh M, 2001) to predict interaction of coil in protein sequence.

For finding vector of binding constant and offset we have to minimize the difference between measured and predicted value but there some difficulties occurred because every time data was not given in binding energy term so we have to identify whether epitope bind or not and assign energy respectively and that data used as data in quadratic programming formulas (Florea L). Quadratic programming method is extension to linear regression approach but allows incorporation from source to binding half life (Parker KC, 1994).
Various software have been used by this method including Matlab, CPLEX (Florea L, 2002).

Reverse vaccinology:

Reverse vaccinology method can not identify epitope directly instead of it identify surface protein which is expressed by pathogen. This method requires whole knowledge about pathogen and the surface protein regularly attacked by killer cell and induce T-cell epitope (Smith R, 2007) and these surface proteins are compared with the BLAST (NCBI) and FASTA(EMBL-EMI) databases which helped to identification of known and unknown protein (Mora M, 2003). This method is easy to use because it is relatively simple, inexpensive and less time consuming (Pizza M, 2000).

Molecular dynamics simulation

Molecular dynamics (MD) simulations play a key role in the structural biology of membrane proteins. MDS provides flexibility to model different systems, reaction conditions and most importantly mutations simulating classical or directed evolution experiments in-silico before proceeding to actual experiment. In particular, they allow one to ‘‘transplant’’ a membrane protein structure from a crystal to a bilayer and monitor its dynamic behavior within its native environment (Philip and Mark, 2011). Simulations can provide the ultimate detail concerning individual particular motions as a function of time. Thus they can be used to address specific questions about the property of model system often more easily then the experiments on actual system (Karplus and Macmmon 2002) Molecular Dynamics (MD) is a computer simulation method that relies mainly on Newton mechanics to simulate the physical interactions and movement of atoms and molecular systems. To obtain the dynamic characteristics and the understanding of interaction mechanism at an atomistic scale, MD simulation packages like AMBER, CHARMM, GROMACS and NAMD are applied widely in the modeling of biomolecules and provide very useful informations (Ting F. *et al*., 2012). In particular, they allow one to ‘‘transplant’’ a membrane protein structure from a crystal to a bilayer and monitor its dynamic behavior within its native environment.

This method used to determine average position of atoms in the crystal, interaction energy and reveal the competitor of T-cell epitope (Davies M, 2003).

Application of T-cell epitope mapping in drug discovery:

T-cell epitope prediction has set a milestone in the field of computational immunovaccinology or computer aided vaccine design (Florea L, 2002) with the help of genome sequencing and other methods, it describes scientific discipline of sequencing, mapping and analysis of genome (McKusick VA, 1997), genome analysis is two type one is based on structure and other one is based on function. Many diseases are not inherited type so they need linkage analysis and other sequencing pattern (Lander ES, 1994) and by the using this genome analysis we can design a vaccine.

T-cell epitope prediction are used in the treatment of cancer, autoimmunity, allergy and infectious disease (Brusic V, 2004) and also in investigation of hepatitis C virus infection in this T-helper cells are use, in this investigation non-structural protein 3(NS3) play essential role and stimulate peripheral blood mononuclear cells (PBMCs) which are used to differentiate HCV patient from non HCV (Pan CH, 2002). There are many application of T-cell epitope prediction in case of disease diagnosis and vaccine design.

Mapping in Foot and mouth disease:

It is highly infectious disease which is mainly occurred in cloven-hoof animals (Rodriguez LL, 2009) and T-cell epitope prediction help in the protection against foot and mouth disease virus by using linear peptide with the help of cytokine detection method, in this TB(a peptide of B cell which is tendam with T-cell) and B-cell peptide sample was performed and found that TB was not sufficient to afford full protection against disease B cell have capability to induce full protection (Cubillos C, 2012). RT-PCR was done for the amplification of biological sample (Saiz M, 2003) and final result were calculated through statistical analysis like Man's Whiteny rank sum test (Cubillos C, 2012).

Mapping in Type 1 Diabetes:

T-cell epitope peptides having therapeutic application in autoimmune disease because some time environmental factor and other diseases induce T-cell in favour of auto immune disease, for this case 13 epitope peptides were identified in tyrosine phosphatase IA-2 (106 kd), which is a molecular target of autoimmunity in type 1 diabetes (Rabin DU, 1994) and dominant IA-2 peptide shown 75-45% identity and 88-64% similarity over 8-10 amino acid in Dengue, measles, Rota virus and HCV (Honeyman MC, 1998). The IA-2 and GAD (Glutamic acid decarboxylase) similarities increase the possibilities of T-cells activation for type 1 diabetes auto antigen along with rotavirus infection (Verge CF, 1994) induce β-cell autoimmunity due to molecule mimicry. and test was confirmed by sequence similarity analysis (Honeyman MC, 1998).

Mapping in Pneumonia:

Pneumonia is a enormous disease which is caused by streptococcus pneumonia and mainly occur in children (Nasrin D, 1999) and PspA (pneumococcal surface protein) which is conserved in streptococcus pneumonia strain and it can help to vaccine development against this strain (Singh R, 2010). PspA is a surface antigen which bind with human lectoferrin and induce the T- cell (Bignell GR, 2004) and immune response was generated by $CD4^+$ T-cell (Costantino HR, 2002) but some time it was hindered due to polymorphism of HLA(human leukocyte antigen) for avoiding this problem epitope mapping was used to identify the binding pattern of HLA and MHC2 to PspA with the help of tools like SYFPEITHI, IEDB etc (Singh, R, 2010).

Mapping in HIV:

In HIV infection $CD8^+$ T-cell receptor shows crucial response, so it can help in HIV vaccine development (Mckinnon LR, 2009) for identification of HIV specific $CD8^+$ T-cell used many parameter such as selection of overlapping peptide sequence to basic defined epitope (Frahm N, 2007), use various clade and groups of ancestral sequences (Rutebemberwa A, 2005) and evaluate the impact of HLA and TAP binding on epitope recognition. Most of pathogen process in cytosol only few is responsible for T- cell response, in HIV infection $CD8^+$ cell in large magnitude so it is essential in HIV immunity (Frahm N, 2007), but all response are not important for prevention of disease except few factor (Addo M, 2003) and for determining these factor we should use cross reactivity and OLP (Coplan PM, 2007) and by the using of epitope mapping found novel epitopes and know the reason of HIV specific immunity differences between different population which is useful for vaccine development (Mckinnon LR, 2009).

Conclusion:

Epitope mapping is very essential part of developing any new vaccine or drug against diseases. There are many databases available to know the information about the peptide sequence JenPep database have separate database for T-cell and it is advanced database compare to MHCBN, MHCPEP and SYFPEITHE databases. There was so many prediction method also available biological and computational, computational method take less time as compare to biological and give more accurate result. Epitope prediction shows that MHC-2 molecule do not always use for helper cell activation or proliferative response but it can also reveal human leukocyte antigen.

Future aspects:

Epitope mapping is the phenomena which are help in identification of binding pattern of antigen with antibody. In silico prediction of epitope mapping has various significant advantages which is so beneficial for human being in future also by using this vaccine can be designed for various infectious disease such as HIV, Type 1diabetes and cancer also. T-cell epitope mapping have included to reengineering of protein, autoimmunity, allergy and transplantation (De Groot AS, 2006) etc by the finding novel epitope know the reason of differential immunity vary with person to person which is so much helpful for effective vaccine design.

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