OVERVIEW OF PRE-CLINICAL TECHNIQUES FOR PREDICTING THE IMMUNOGENICITY OF THERAPEUTICS IN DRUG DEVELOPEMENT.

C.Musanabaganwa[1,*], F. Byiringiro[2]

[1]Rwanda Biomedical Center , Medical Research Center, Rwanda
[2]Rwanda Neurosurgery Center, Kigali University Teaching Hospital, Rwanda

ABSTRACT

Immunogenicity testing is a vital component of drug development as it leads to drugs that are safer and more effective. This review provides an overview of the pre-clinical models that can be used to predict the immunogenic potential of novel protein therapeutics prior to administration in humans. Tools important for the prediction of the immunogenicity of protein therapeutics include animal models, in vitro cell assays, and in silico techniques. Animal models including rodents, transgenic mice, and non-human primates are reviewed. Among the immunoinformatics tools commonly used to predict immunogenicity include the Structural Epitope Database, Immune Epitope Database and Analysis Resource (IEDB), The MHCBN database, Dana-Farber Repository for Machine Learning in Immunology, and TEPITOPE. Identification and subsequent removal or inhibition of epitopes and MHC agretopes minimizes immunogenicity. Strategies for minimization of immunogenicity in biotherapeutics including epitope and MHC agretope removal, improvement of solubility, derivatization with polyethylene glycol (PEG), and use of chimeric antibodies are also discussed.
Immunogenicity testing is an important part of the drug development process as it leads to drugs that are safer and more effective. Animal models including rodents, transgenic mice, and non-human primates; in vitro cell assays; and immunoinformatics tools are used to identify epitopes and MHC agretopes which are then eliminated or inhibited so as to minimize immunogenicity.

Keywords: immunogenicity - immunogenicity testing - biotherapeutics - animal models - immunoinformatics.

RESUME

Les tests d'immunogenicité sont des facteurs majeur dans le développement des médicaments en vue d'élaborer molécules plus sures et plus spécifiques. Cetteétude dispose d'une analyse globale des modelespréclinques qui prévoient un pouvoir immunogenique des nouvelles moléculesthérapeutiquesavant leur utilisation sur l'homme. Les éléments essentiels dans la prédiction de cet immunogenicité des molécules thérapeutiques sont les cobayes utilisés tels que les animaux (in vivo), l'échantillonnage de cellules (in vitro), et d'autres techniques utilisées in silico. Les modèles des cobayes utilisés (les rongeurs, les souris transgéniques, et les primates) sont revus et analysés. Les outils informatiques les plus utilisés dans l'immunogenicité sont les '' Structural EpitopeDatabase'', ''Immune EpitopeDatabase and Analysis Resource (IEDB)'', ''MHCBN database, Dana-FarberRepository for Machine Learning in Immunology'', et le ''TEPITOPE''. L'identification suivie de la suppression ou le blocage des épitopes et des agretopes MHC diminuent le risque immunogène. Cette étude discute des stratégies de réduction du pouvoir immunogenique utilisées en biothérapie qui sont entreautre la suppression des epitopes et les agretopes MHC, l'amélioration de la solubilité, la production des nouvelles molécules thérapeutiques à partir du polyethylene glycol (PEGylation), et l'utilisation des anticorps chimériques.

Mots-clés: Immunogenicité, tests d'immunogenicité, Biotherapie, Cobayes, Immunoinformatique

INTRODUCTION

It has been revealed that Protein therapeutics such as streptokinase, tissue plasminogen activator (TPA) and other thrombolytic agents, anticoagulants, peptides, vaccines, monoclonal antibodies, and recombinant DNA hormones such as insulin have revolutionized healthcare. According to estimates, the market share of protein therapeutics is up to 30% of all marketed drugs [1]. The success of protein therapeutics has largely been attributed to their high efficacy and safety profiles. They are not only versatile but they also lack intrinsic toxicity arising from harmful metabolites since products of their degradation are amino acids. Their versatility has also enhanced their use [2, 3].
Despite their obvious advantages, therapeutic proteins are encumbered by the problem of immunogenicity. Immunogenicity refers to the ability of an antigen to induce an immune response. The process of immunogenicity has been well clarified. Once administered, therapeutic proteins are taken up and processed by antigen presenting cells (APCs). The antigens are then processed. The APCs then interact with the T cell receptors (TCRs) of naïve T cells via the major histocompatibility complex (MHC), forming a peptide-MHC-TCR complex. Formation of the complex is induced by co-stimulatory signals arising from inflammation or infection. T cells mature and interact with B cells to form peptide-MHC-B cell complexes. The B cells mature and this is followed by the attachment of antibodies to the therapeutic protein [4,5,6]. (Figure1).

* *Correspondence to:* *Musanabaganwa Clarisse*
Medical Research Center,
Rwanda Biomedical Center, Kigali
Tel: (+250) 784010813
Email: mussaclasse2007@yahoo.fr

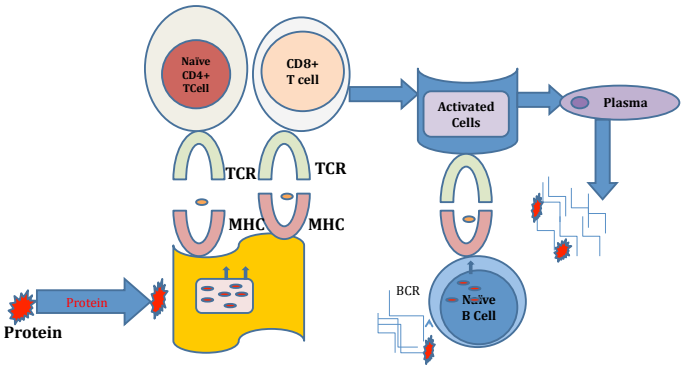

Figure 1: process of immunogenicity

Immunogenicity is affected by both patient-related factors and product-related factors and can be caused by a variety of factors. Among the factors which may predispose to immunogenicity are structural alterations, storage conditions, patient's immune status, formulation, route, dose and frequency of administration, genetic background, and differences in the sequences of endogenous and therapeutic proteins [7].

Immunogenicity is a problem as it may cause transient antibody responses that are not of clinical importance or may lead to lethal reactions with fatal consequences. Safety concerns arising due to immunogenicity of therapeutic proteins include anaphylaxis, lowered efficacy of the medicine, autoimmunity, cytokine release syndrome, infusion reactions, and anaphylactoid reactions [8].

Anaphylaxis is a potentially fatal allergic reaction that is accompanied by low blood pressure, hives, dyspnoea, difficulties in swallowing or talking, and swelling around the throat and on the face. Immunogenicity can lower the efficacy of the therapeutic protein due to cross-reactions between the drug and the endogenous proteins. For instance, administration of therapeutic erythropoietin can lead to aplasia of the red blood cells due to neutralizing antibodies which neutralize both the therapeutic erythropoietin and the red blood cells [9]. Autoimmunity arising from immunogenicity can be life-threatening[10] as can be non-acute immune reactions e.g. immune complex disease. The cytokine release syndrome occurs when many monoclonal antibodies are used and is characterized by dyspnoea, fever, rash, tachycardia, low blood pressure, asthenia, chills, scratchy throat, and chills. The symptoms arise due to cytokine release from cells targeted by the antibody and from local immune effectors cells [11]. Immunogenicity in therapeutic proteins is also undesirable as it increases therapeutic costs [3].

Due to the adverse effects associated with immunogenicity, regulatory agencies require that therapeutic products be tested for immunogenicity during the drug development stages. Immunogenicity testing involves measuring the circulating antibodies to the therapeutic product since the immune response directed against the biotherapeutics is usually humoral. Factors for prediction of immunogenicity include non-epitopes on altered proteins, immunogenicity incidence in those affected, clinical repercussions of antibody development, tolerance breaking, and comparative immunogenicity between products [3].

Several strategies have been developed to predict immunogenicity. Traditional methods involved the use of animal models to test the level of immunogenicity in therapeutic products. However, new techniques involving in vitro cell culture and in silico prediction have been designed and these have helped to enhance the safety and efficacy of therapeutic products. Despite these new developments, prediction of immunogenicity in therapeutic problems is still a complex task. This paper evaluates the different models that are currently available for testing the immunogenicity of therapeutic proteins.

ANIMAL MODELS AND IMMUNOGENICITY TESTING

Preclinical testing for immunogenicity has traditionally been done by assaying the formation of antibodies in non-human primates and rodents [6]. Animal models have been widely used as predictive tools to study immunogenicity. Among the animals favored in testing immunogenicity are transgenic mice, rodents, and non-human primates. Transgenic mice are commonly used to evaluate the immunogenicity of proteins which are poorly conserved between humans and mice [7]. Non-human primates are thought to have the highest predictive value among all the animal models since the genetic makeup of non-human primates most closely resembles that of humans. Human xenografts are also being increasingly used in immunogenicity testing. The choice of animal models for immunogenicity testing is also guided by the factor that needs to be predicted [1].

While much favored in the past, animal models have fallen off the pecking order of immunogenicity predictive tools. This is because of their many disadvantages, the most glaring of which is their inability to predict immunogenicity in humans. Inability of animal models to predict immunogenicity in humans is due to lack of homology between animal and human protein sequences, validation difficulties [1-3], and the species-specificity of tolerance [1-6].

Regarding species-specificity, humans are usually unable to raise immune responses to endogenous proteins unless they break tolerance. On the other hand, these proteins are foreign in animal models. Additionally, MHC agretopes of different species are dissimilar and this means that immunogenicity in animal models may not necessarily predict immunogenicity in humans[6]. Whereas the low predictive value of animal models is associated with animal models, the European Medicines Agency (EMA) recommends that animal models should be factored in when conducting immunogenicity studies [1-12]. Species differences lower the predictive value of animals in the assessment of immunogenicity. Perhaps the most valuable use of animal models is in uncovering the mechanisms behind antibody responses to biotherapeutics [13].

Transgenic mice are the animal models of choice when performing in vivo testing of the immunogenicity of therapeutics. These models are preferred because they are immune tolerant and are not encumbered by strict ethical regulations unlike non-human proteins. Disadvantages associated with use of transgenic mice are nevertheless manifold. One, homology between mice proteins and human proteins is not 100% and therefore differences between human and animal models can be significant. Secondly, the mechanism behind immunogenicity is not very well known. Thirdly, patients usually exhibit altered immune responses attributable to the disease state or therapy. Fourthly, the low genetic diversity between animal models is a factor that greatly contributes to species-related immunogenicity. This is because the mice that are commonly used for immunogenicity testing are usually inbred meaning that their genetic makeup is almost similar. This contrasts with humans who have great genetic diversity8. Finally, according to findings by Ottesen et al [14], mice can fail to generate antibody responses against some biotherapeutics due to their genetic background [14].

Of all the animal models, non-human primates such as rhesus monkeys have the highest predictive value when testing for immunogenicity. This is because their proteins

share significant homology with human proteins. This explains the high tolerance non-human primates have for most human proteins [8]. As such, non-human primates are useful in predicting the immunogenicity of therapeutics, determining neo-epitopes, evaluation of relative immunogenicity, and evaluation of neutralizing antibodies. They are however unsuitable for predicting immunogenicity of some proteins the notable one being human interleukin-1 (IL-3). The conclusion therefore is that predictive value of animal models depends on the protein being assayed.

Besides immunogenicity testing, animal models are also used to understand the mechanisms behind the immunogenicity of biotherapeutics. Knowledge on how immunogenicity of therapeutic proteins comes about is important as it can help in the formulation of tools that can be used to originate proteins with minimal immunogenicity. In this regard, transgenic animal models are of utmost utility [8].

IN VITRO T-CELL CULTURE SYSTEMS IN IMMUNOGENICITY TESTING

Very few in vitro methods are available for estimating the immunogenicity of a protein [15]. Besides, these in vitro methods do not entirely capture the immune response of humans. This necessitates the monitoring of patients for immunogenicity during clinical trials for long periods [6]. In vivo T cell assays involve exposing isolated APCs and T cells from human blood to protein or peptide antigens. Induction of T-cell activation will be observed if there are immunogens in the blood. Detection of any immunogens present is by measuring the cytokines that are generated using the ELISA technique. Since only a small number of naïve T cells will react with most therapeutic proteins, this immunogenicity test can be enhanced by stimulating the sample repetitively using antigen-pulsed APCs (figure

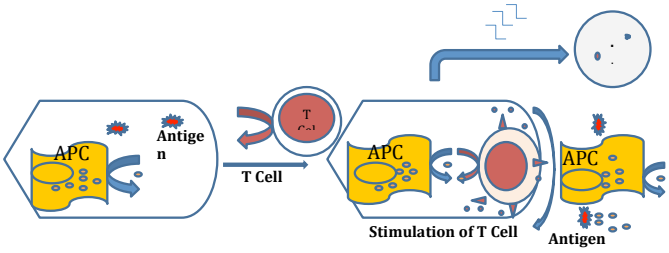

Figure 2: process of in vitro T cell assays

Several studies have investigated in vitro systems that can be used to estimate a protein's immunogenicity. Egger et al [15].describe an in vitro technique for assessing the immunogenicity of therapeutic proteins. Their technique utilizes the association between the immunogenicity of a protein and its susceptibility to proteolysis by endolysosomes. They used mass spectrometry and gel electrophoresis to compare the composition of proteins and proteolytic activity of endolysosomal fractions derived from the bone marrow of mice and dendritic cells of humans on one hand with those from the dendritic cell line JAWS II. Their findings indicated that the composition of proteins as well as specificity and activity of proteins from the endolysosomal fractions obtained from rat and human dendritic cells were similar. Their conclusion is that endolysosomal fractions made from the JAWS II cell line can be used to accurately estimate the immunogenicity of human or mouse proteins in vitro. Not only is the technique fast to use and reliable but it can also be used to assess a protein's immunogenic characteristics and be used to replace, reduce, and improve animal experiments [15].

Gaitonde & Bayu-Iyer [16] describe an in vitro tool that can be used to assess the immunogenicity of proteins. They evaluated the immunogenicity of Erythropoietin-alpha (rHuEPO) and recombinant Factor VIII (rFVIII). The maturation level of dendritic cells was determined using flow cytometry and the enzyme-linked immunosorbent assay (ELISA) used to assess secretion of immunomodulatory cytokines following challenge with free rFVIII. Their findings indicate that this method can be used to accurately determine the immunogenicity of therapeutic proteins in a pre-clinical setting [16].

The ELISA assay may be indirect, direct, or bridging. Besides ELISA and flow cytometry, immunogenicity assays are conducted using radioimmune precipitation assays, electrochemiluminiscence, surface plasmon resonance, capillary electrophoresis, Meso Scale Discovery (MSD ®) assays, mass spectrometry, nuclear magnetic resonance (NMR), affinity purification, solid-phase extraction with acid dissociation (SPEAD), and acid dissociation techniques[16, 17].

IN SILICO METHODS - IMMUNOINFORMATICS

Immunoinformatics involves the use of computer-based methods to identify T-cell and B-cell epitopes hence predict the immunogenicity of therapeutic proteins [18, 19]. Unlike conventional techniques where pathogens are grown and the antigenic proteins extracted, immunoinformatics techniques are rapid and they reduce the time needed to develop biotherapeutics. Immunoinformatics methods are also cheaper, highly accurate, and less laborious [18]. They can however be bogged down by the problem of over prediction [19].Computer tools used for prediction of immunogenicity include algorithmic tools for predicting B cell and T cell epitopes, databases of immune-related molecules and epitopes, tools for comparing the structures of immune-related molecules, and tools for the design of biotherapeutics [20]. A proliferation of computer-based immunoinformatics tools has been witnessed over the past few years. Some of the most commonly used immunoinformatics tools are discussed in the following paragraphs.

The Dana-Farber Repository for Machine Learning in Immunology (available at http://bio.dfci.harvard.edu/DFRMLI/) provides preprocessed and scaled immunological data sets suitable for use in machine learning applications. It contains a repository of HLA Binding peptides which includes the MHCPEP dataset and datasets for specific MHC molecules. The MHCPEP dataset is a database of peptides that bind to MHC. MHCPEP contains entries with peptide sequences of the MHC binding molecules, the specificity of the MHC, date of availability, experimental techniques, source protein, binding affinity, anchor positions, observed activity, and references. The dataset has however been inactive for the past 14 years [20,21]. The DFRMLI also has a repository of T cell epitopes including datasets of

tumor antigens, virus antigens, and CEF control peptide pool. The tumor antigens dataset has 718 T cell epitopes that have been experimentally validated and which were obtained from human tumor antigens. The virus antigen dataset has 44 HLA-2 restricted T cell epitopes [21].

Another valuable tool is the MHCBN Database (available at http://www.imtech.res.in/raghava/mhcbn/) which is a much more comprehensive database. The MHCBN Database is curated and stores data on allele-specific MHC binding proteins, MHC non-binding proteins, T-cell epitopes, and TAP binding and TAP non-binding molecules [22-24].Unlike the MHCPEP database, the MHCBN database is updated and current. The MHCBN web tool also has antigenic and MHC blast which allows query sequences to be compared and epitopes identified on the fly. It also has structures of MHC proteins and MHC-peptide complexes that have been archived in the Protein Data Bank. At the moment, the latest version of MHCPBNis version 4 and the database has 25,852 peptide entries with 20,717 MHC binders, 4022 MHC non-binders, 6,722 T-cell epitopes, and 1,053 TAP binders and non-binders. Also contained in the MHCBN Database are 13,910 non-redundant peptides comprising of 12,722 MHC binding peptides and 1,566 MHC non binding peptides. Other non-redundant proteins in MHCBN are 470 TAP binders and non-binders and 2,602 T-cell epitopes. Miscellaneous data in the MHCBN Database includes 119 protein structures related to MHC, 841 protein structures with matching peptides, 1,420 proteins of MHC alleles, 1,519 references for published literature, and 3,754 protein sequences.

The Immune Epitope Database and Analysis Resource (IEDB) is an updated and curated database (available at http://www.iedb.org/). The IEDB stores data related to antibody and T cell epitopes for rodents, non-human primates, humans, and other animal species. The IEDB can be used to predict T-cell and B-cell epitopes and perform epitope analysis. It has BLAST tools that allow query sequences to be entered and homologous proteins identified. Query sequences can also be used to determine peptide structure. The database has 104,592 peptidic epitopes and 1,932 non-peptidic epitope data for all infectious diseases. It also has 209,093 T-cell and 162,947 B-cell assays, 8,113 MHC ligand elution assays, 247,857 MHC binding assays, 3,055 epitope source organisms, 653 restricting MHC alleles, and 15,232 references [25].

The Structural Epitope Database (SEDB) describes the 3D structures of B-cell and T-cell epitopes, MHC binding epitopes, linear epitopes, discontinuous epitopes, and non-peptidic epitopes and provides information about the intermolecular contact of antibodies with antigens. Important information such as sequence data and methodology are also provided. SEDB has an epitope BLAST tool [26].

MHCPred is a tool that performs the heteroclitic calculation of peptides. It enables immunologists to obtain high affinity peptides by mutating one or two positions of an interested peptide in silico. Calculation is based on the additive method.

MMBPred helps to predict mutated high affinity and promiscuous MHC class-I binding peptides from protein sequence. It is used to identify mutated antigenic peptides in the absence of putative vaccine candidates and predict promiscuous mutated MHC class I binding peptides by introducing mutations at one, two or three positions. These peptides are important in overcoming the barriers of MHC restriction and subunit vaccine design. It is also used in epitope enhancement where mutated high affinity MHC binding peptides as compared to native peptides are predicted. MMBPred also predicts nonamer peptides having one, two, or three amino acids mutated at random or user defined position.

Other important databases useful for immunogenicity testing include the HIV CD8+ T cell database for epitopes, The Los Alamos HIV/HCV databases, and the SYFPEITHI database, among others. The table below summarizes important immunoinformatics tools used in preclinical immunogenicity testing.

DISCUSSION

There are several strategies which are used to reduce the immunogenicity of therapeutic proteins. Recombinant DNA technology is one such strategy where drugs obtained from non-human sources are replaced with those having human sequences. However, recombinant DNA technology is not adequate to prevent immunogenicity [6]. Strategies that are more successful in minimizing immunogenicity include improvement of manufacturing processes to reduce impurities to a minimum, and increasing the content of human sequences through use of chimeric antibodies consisting of human constant and mouse variable regions. Use of chimeras is however reported not to be adequate in preventing immunogenicity [6].

Improving the properties of solutions can go a long way towards minimization of immunogenicity. A higher degree of immunogenicity in therapeutic proteins is usually caused by protein aggregates which have a much higher degree of immunogenicity than soluble proteins. The solution therefore is to enhance the solubility of therapeutic proteins through such measures as optimization of the conditions used during expression, purification, and formulation. However, this seldom solves the problem completely. This problem can better be resolved through the use of rational solubility engineering. Rational solubility engineering helps in the identification of mutations that can potentially reduce protein aggregation to a minimum [27].

Removal of antibody epitopes is also a strategy that is in use for minimizing immunogenicity. It is now possible to quickly detect antibody epitopes due to advances in mass spectrometry. Once identified, the important residues of the antibody epitopes can then be altered and this helps minimize binding of antibodies hence reduce immunogenicity [27].Immunogenicity can also be minimized through the identification and removal of class II MHC agretopes. Identification and removal of MHC ii agretopes can help to minimize immunogenicity by preventing the formation of high affinity IgG antibodies [6]. This is because immunogenicity is caused by IgG antibodies. The MHC II agretopes can be identified using either experimental or computational tools [27].

Finally, immunogenicity can be minimized by PEGylation. PEGylation involves derivatization of therapeutic proteins with polyethylene glycol. This reduces immunogenicity as it inhibits antibodies from attaching, enhances protein solubility, and reduces dosing frequency [6].

Database name	URL	Output Summary
Structural Epitope Database	http://sedb.bicpu.edu.in/	3D structures of B-cell and T-cell epitopes, MHC binding epitopes, linear epitopes, discontinuous epitopes, and non-peptidic epitopes, information about the intermolecular contact of antibodies with antigens
Immune Epitope Database and Analysis Resource (IEDB)	http://www.iedb.org/	peptidic epitopes, non-peptidicepitopes,T-cell and B-cell assays,MHC ligand elution assays, MHC binding assays, epitope source organisms, restricting MHC alleles, and references
The MHCBN database	http://www.imtech.res.in/raghava/mhcbn/	allele-specific MHC binding proteins, MHC non-binding proteins, T-cell epitopes, and TAP binding and TAP non-binding molecules
Dana-Farber Repository for Machine Learning in Immunology	http://bio.dfci.harvard.edu/DFRMLI/	HLA Binding peptides, T cell epitopes including datasets of tumor antigens, virus antigens, and CEF control peptide pool.
ELF	http://www.hiv.lanl.gov/content/sequence/ELF/epitope_analyzer.html	Epitope location finder for HIV
TEPITOPE		For predicting promiscuous class II epitopes
TAPPred	http://www.imtech.res.in/raghava/tappred/	Prediction of TAP binders binding affinity
SYFPEITHI	http://www.syfpeithi.de/	Database of MHC ligandsand peptide motifs. Contains data on Peptide sequences, anchor positions, MHC specificity, source proteins, source organisms and publication references. Used for epitope prediction and identification of motifs, ligands, and epitopes
ProPred-1	http://www.imtech.res.in/raghava/propred/	Server for predicting MHC II binding peptides
NetMHC	http://www.cbs.dtu.dk/services/NetMHC/	Uses artificial neural networks to predict binding of peptides to different HLA alleles
NetChop	http://www.cbs.dtu.dk/services/NetChop/	Uses artificial neural networks to predict cleavages sites of the human proteasome
NetCTL	http://www.cbs.dtu.dk/services/NetCTL/	Prediction of CTL epitopes in protein sequences
MHCPred	http://www.ddg-pharmfac.net/mhcpred/MHCPred/	For the heteroclitic calculation of peptides

Non-human primates have the highest predictive value among all animal models. Immunoinformatics tools are popular because they are rapid, accurate, less laborious, and cheaper. Besides removal of epitopes and MHC agretopes, immunogenicity can also be minimized through recombinant DNA technology, use of chimeric antibodies consisting of human constant and mouse variable regions, improving the properties of solutions, and PEGylation.

CONCLUSION

Immunogenicity testing is an important part of the drug development process as it leads to drugs that are safer and more effective. Animal models including rodents, transgenic mice, and non-human primates; in vitro cell assays; and immunoinformatics tools are used to identify epitopes and MHC agretopes which are then eliminated or inhibited so as to minimize immunogenicity.

REFERENCES

1. FDA. (2013). Guidance for Industry Immunogenicity Assessment for Therapeutic Protein Products.Retrieved on 20th September, 2013 from http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM338856.pdf
2. Clarke JB. Mechanisms of adverse drug reactions to biologics. Handbook Exp Pharmacol. 2010: 453–74.
3. Brinks V., Jiskoot W., and Schellekens H. (2011). Immunogenicity of Therapeutic Proteins: The Use of Animal Models. Pharm Res. 2011 October; 28(10): 2379–2385.
5. Pieters J. MHC class II-restricted antigen processing and presentation. Adv. Immunol. 2000; 75:159–208.
6. Subramanyam M. Immunogenicity of biotherapeutics-an overview. J. Immunotoxicol. 2006; 3:151–156.
7. Chirino A.J., Ary M.L., and Marshall S.A. (2004). Minimizing the immunogenicity of protein therapeuics. DDT. 9(2): 82-91.
8. Beers MM, Sauerborn M, Gilli F, Hermeling S, Brinks V, Schellekens H, Jiskoot W. Hybrid transgenic immune tolerant mouse model for assessing the breaking of B cell tolerance by human interferon beta. J Immunol Methods. 2010; 352:32–37.
9. Schellekens H. Factors influencing the immunogenicity of therapeutic proteins. Nephrol Dial Transplant. 2005 Jun; 20 Suppl 6:vi3-9.
10. Boden S.R. and Burks A.W. (2012). Anaphylaxis: a history with emphasis on food allergy. Immunol Rev. 2011 July; 242(1): 247–257.
11. Parker B.M., Reynolds H.N., Lumicisi B., and Bryson C.J. (2010). Immunogenicity of protein therapeutics: the key causes, consequences and challenges. Lands Bioscience. 1(4):314-322
12. Breslin S. (2007). Cytokine-release syndrome: overview and nursing implications. Clin J Oncol Nurs. 2007 Feb; 11(1 Suppl):37-42.
13. EMA. Guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins. 2007. Ref Type: Conference Proceeding.
14. Sauerborn M, Brinks V, Jiskoot W, Schellekens H. Immunological mechanism underlying the immune response to recombinant human protein therapeutics. Trends Pharmacol Sci. 2010;31:53–59. doi: 10.1016/j.tips.2009.11.001.
15. Ottesen JL, Nilsson P, Jami J, Weilguny D, Duhrkop M, Bucchini D, Havelund S, Fogh JM. The potential immunogenicity of human insulin and insulin analogues evaluated in a transgenic mouse model.Diabetologia. 1994;37:1178–1185. doi: 10.1007/BF00399790.
16. Egger M, Jürets A, Wallner M, Briza P, Ruzek S, et al. (2011) Assessing Protein mmunogenicity with a Dendritic Cell Line-Derived EndolysosomalDegradome. PLoS ONE 6(2): e17278.
17. Gaitonde P., and Balu-iyer, S.V. (2012). In Vitro Immunogenicity Risk Assessment of Therapeutic Proteins in Preclinical Setting.Methods in Molecular Biology in Drug Design and Discovery: Methods and Protocols. 716:267-280.
18. Matthews JB, Davidson AJ, Beynon RJ. (2004). The application of mass spectrometry to identify immunogenic components of excretory/secretory products from adult Dictyocaulusviviparus.Parasitology. 2004;128Suppl 1:S43-7.
19. Flower D.R. (2003).Immunoinformatics and the in silico Prediction of Immunogenicity:An Introduction IN Immunoinformatics: Predicting Immunogenicity In silico. 409:1-15.
20. Tomar N., and De R.K. (2010). IMMUNOINFORMATICS: An Integrated Scenario. Immunology, 131: 153-168.
21. Korber B., LaBute M., and Yusim K. (2006). Immunoinformatics comes of age. PLoSComput. Biol. 2(6):e71.
22. DFRMLI. (2013). Dana-Farber Repository for Machine Learning in Immunology. Retrieved on 20th September 2013 from http://bio.dfci.harvard.edu/DFRMLI/index.php
23. Lata S, Bhasin M, Raghava GP. MHCBN 4.0: A database of MHC/TAP binding peptides and T-cell epitopes. BMC Res Notes. 2009 2:61.
Bhasin M, Singh H, Raghava GP. MHCBN: a comprehensive database of MHC binding and non-binding peptides. Bioinformatics. 2003 19(5):665-6
24. MHCBN. (2013). Retrieved on 20th September, 2013 from http://www.imtech.res.in/raghava/mhcbn/
25. Vita R, Zarebski L, Greenbaum JA, Emami H, Hoof I, Salimi N, Damle R, Sette A, Peters B. The immune epitope database 2.0. Nucleic Acids Res. 2010 Jan; 38(Database issue):D854-62. Epub 2009 Nov 11.
26. Sharma O.P., Das A.A, Krishna R, Suresh K.M and Mathur P.P. Structural Epitope Database (SEDB): A Web-based Database for the Epitope, and its Intermolecular Interaction Along with the Tertiary Structure Information. J Proteomics Bioinform 2012, 5:3.
27. Nagata S., and Pastan I. (2009). Removal of B cell epitopes as a practical approach for reducing the immunogenicity of foreign protein-based therapeutics.Adv Drug Deliv Rev. 2009 September 30; 61(11): 977–985.