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Research paper

Are synthetic peptides real toxic allergens? a comprehensive literature review and update on therapeutic and adverse toxic immunogenic potentials of peptides -truth or dare

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Abstract

In the pharmaceutical industry, there is much debate about how to translate current guidelines on biotherapeutic immunogenicity testing into a real-life strategy that meets the regulatory requirements of newly discovered pharmaceutical peptides. This paper will present a consensus view on the essential elements for biotherapeutic immunogenicity consideration for pharmaceutical synthetic peptides to ensure patient safety and allow successful market entry. This paper's scope is limited to aspects relevant to the biotherapeutic synthetic peptide pharmaceuticals and does not include necessary academic immunogenicity studies. Medical research shows little support for antigenic or allergic reactions to short synthetic peptides. These negligible results warranted a detailed literature review to examine antigens and allergens linked to peptides.

Keywords: *Mutagenicity; Peptides; Skincare Products; Amino Acids; Skin.*

1. Introduction

Therapeutic proteins and peptides have been revolutionizing the treatment of many diseases for nearly 40 years. Different classes of biotherapeutics, such as antibodies, hormones, enzymes, growth, and blood factors, have become available to allow significant progress in treatment. It became apparent, however, that many of these products are at risk of some unwanted reactions, one of which is immunogenicity. A multitude of product-related, process-related, and patient-related factors that must be routinely analyzed during the therapeutic agent's clinical development to ensure the adequate benefit and risk assessment defines the immunogenic profile of therapeutic proteins and the consequent hazard to patients. Clinical immunogenicity effects vary from no effect, reduction, or loss of therapeutic effectiveness to severe complications due to neutralization of natural counterpart or general immune system reactions. Such unusual incidents often have a frequency of less than 1% of patients under therapeutic synthetic peptide treatment and only become evident at the late stage of or after approval of phase III studies. (I.C. Büttel et al. 2011)

Cosmetic science is a combined discipline of different scientific and technical subjects, such as genetics, chemical engineering, chemistry, psychology, and more. All novel synthetic peptides should be developed based on understanding skin and hair biology, as these are the primary objectives of cosmetic or personal care products. Water is the essential component of the human body, comprising 60 –70 percent of the body weight. Next are proteins of around 20%, which not only act as the structural foundation for cells and organs but also participates in specific biochemical processes within cells to keep the body healthy. Maintaining water in the body is an essential function of skin, the largest organ of the human body, and the stratum corneum (SC), the outmost layer of only about 15-20 mm thickness, plays the crucial role of the barrier function. (Yamashita Y., 2015)

In the pharmaceutical industry, there is much controversy about how to turn the current guidelines on biotherapeutic immunogenicity testing into a research technique that meets the specific requirements of individual drug candidates. The structure and role of the over 400 biotherapeutics approved by therapeutic regulatory bodies in different parts of the world vary widely. (Walsh, 2010). These include peptides, proteins of low molecular weight such as insulin that mostly lack immunogenic reactions, in addition to the bigger complex, heterogeneous globular proteins such as monoclonal antibodies (mAb), as well as proteins of conjugation and fusion. Most large molecular biotherapeutics might cause undesirable immune reactions with possible safety and efficacy implications and differ in frequency and severity of their adverse effects. (Bhogal, 2010; Buttel et al., 2010; Schellekens and Casadevall, 2004). Although anti-drug antibodies (ADA) may be life-threatening to specific biotherapeutics such as erythropoietin or thrombopoietin with non-redundant endogenous equivalents, immune responses to others, such as most mAb products, are generally regarded as low risk to patient safety. (Berger and Niesner, 2011; Bhogal, 2010; Casadevall et al., 2002; Coiffier et al., 2008; Getts et al., 2010; Li et al., 2001; Niebecker and Kloft, 2010).

2. Peptides

Peptides are organic molecules composed of monomers of amino acids paired with amide covalent bonds. The shortest are dipeptides, consisting of two amino acids that are joined by a single amide bond, followed by tripeptides, tetrapeptides, etc. There are only three amino acids in the smallest natural peptide, a thyrotropin-releasing hormone. Peptides are molecules with a total of 50 amino acids or less. Medical research shows little support for antigenic or allergic reactions to short synthetic peptides. These negligible immunogenic effects, on the other hand, still mandate a detailed literature review to clarify possible antigenic and allergenic impacts encountered with some large complex peptides. (Sato et al., 2006; McGregor, 2008; Kovalainen et al., 2015).

Recently, a new era of disease care mandated groundbreaking medicinal compounds such as pharmaceutically active peptides as insulin. Nevertheless, due to some of the peptides' characteristics, such as low oral bioavailability and metabolic susceptibility to approximately 600 molecularly different proteases in the human body, industrial researches about novel peptides was neglected compared to nonpeptide small molecular weight drugs. Low molecular weight drugs were much more comfortable to manufacture, to administer (oral pill rather than insulin injection) and, superior in pharmacodynamic properties. (Pan et al., 2011; Uhlig et al., 2014).

Nonetheless, interest in the potential of peptides as diagnostics and therapeutics encourages some studies to improve productivity, change and develop synthetic variants to minimize peptide metabolism and allow alternative routes of peptide administration using different formulations that have led to an increased number of peptide-based drugs on the market. Besides, modern analytical methods promote the detection and identification of the medicinal potential of novel pharmaceutical peptides. (Gregoriadis et al., 2005; Vlieghe et al., 2010).

Peptides have many advantages over proteins and antibodies. Peptides are more able to cross barriers and go further into tissues, like tumors, with their smaller size. Similar to protein and free amino acids, peptides of 2–4 amino acids will be consumed more readily. (Harris et al., 2012). However, for larger chains, the possibility of crossing the intestinal barrier decreases. Besides, peptides with proline and hydroxyl proline chains are more resistant to digestive enzymes (Sarmadi and Ismail, 2010).

Additional benefits over proteins and antibodies include more excellent stability, as long-term storage at room temperature and decreased processing costs than antibodies during detection and manufacturing, and are generally less immunogenic than recombinant proteins and antibodies (Ladner et al., 2004; McGregor, 2008).

3. Skin and skin sensitization

The skin is a vital organ that not only covers the internal organs and protects them but also provides an individual with a specific personality trait. The skin color, age, and health all play a role in social interactions and one's self-perception. Biologically speaking, the skin is one of the most complex structures and the largest organ in the body. It covers an area of 1.5 - 2.0 m^2 and constitutes 16 percent of the total body weight of a human. (M.Z. Albanna et al. 2016)

The skin forms a protective barrier of the first line between the body and the outside world. It regulates the temperature of the body and prevents excessive fluid loss through evaporation (A.A. Romanovsky 2014). Several nerve endings in the skin relay pain, heat, and contact information to the central nervous system, allowing people to interact with each other and their surroundings. The composite skin structure divides into three main anatomical and functional layers: a surface epidermis forming the external environment interface, a middle dermal layer, and a deep fat layer, also referred to as hypodermis of dermal white adipose tissue. (A. Zimmerman et al. 2014)

Immune-response molecules are called immunogens and consist of both antigens and allergens. Antigens include pathogenic substances such as viruses, bacteria, helminths, parasites, or other pathogens, or even proteins produced by the host. Typically, these antigens are harmful and cause symptoms of the disease that are different for different antigens such as fever, infection, severe dehydration, or failure of the organ. Via interactions with surface-expressed immunoglobulin receptors, B lymphocytes need to recognize conformational antigen epitopes to induce an immune response. This process requires T lymphocytes for most antigens to help result in sequential steps of class switching, maturation of affinity and, the spread of epitope. A compound must, therefore, contain an antigenic determinant or epitope and must be of sufficient size to induce the activation of the lymphocyte necessary for an antibody response. (Tanabe S., 2007).

Allergens are antigens of different types. Cells that produce an antibody can class switch. They start with IgM and can cause secretion of IgA or IgG to remain in the bloodstream or IgE to bind to mast cells and release histamine. (Goldsby, R. A. et al. 2000). IgE is the antibody associated with symptoms of allergy known as allergens. Allergy shows many adverse health effects that can result from a xenobiotic triggered immune response. Allergens can be dust, pollen, insects, other foods, chemicals, drugs, and many others that are often harmless but still capable of producing unusual IgE on the surface of mast cells and basophils. The majority of allergens from the immune system, unlike a virus or bacteria, will not induce an innate response. Instead, it is a reaction that develops over time, often with no rhyme or reason as to why it occurs in some individuals and not in others. (A. Zimmerman et al. 2014)

Sensitizer potential prediction is a crucial step in risk assessment and a vital toxicological endpoint in the production and evaluation of ingredients used in cosmetic and personal care products. Skin sensitization, also known as allergic contact dermatitis (ACD), is a disease associated with skin-induced chemicals and cosmetics when a sensitive person becomes exposed to a chemical allergen. (Macmillan et al., 2016). This chemical allergen leads to an immune response in the skin, resulting in contact sensitization. Therefore, there are four main biological events underlying skin sensitization defined as (i) molecular initiation event; (ii) keratinocyte adverse outcome pathway, (iii) compromising gene expression associated with specific cell signaling pathways and inflammatory responses; (iv) dendritic cell activation through expression of specific cell surface markers, namely cytokines and chemokine. (Gerberick et al., 2004, 2007; A. Zimmerman et al. 2014, and, Natsch et al., 2015).

4. Review of literature

Most tetrapeptides are less than 700 Daltons, and there is little evidence to suggest that they can activate an immune response on their own effectively. Theoretically, based on their physicochemical properties and sequences of amino acids, peptides have the versatility required to be immunogenic. (Natsch et al., 2015) However, most 1000-2000 Dalton synthetic peptides in size are recognized poorly as well by the immune system and are weak immunogens. Many tetrapeptides are less than 800 Daltons, and there is little evidence that such tetrapeptides haptens can activate the immune response effectively. (Macmillan et al., 2016)

A hapten is a molecule that interacts with different antibodies but is not immunogenic on its own, but can only be rendered immunogenic by conjugating to an acceptable carrier. Only when paired with a larger carrier, such as a protein, haptens may produce antibodies. Short peptides are haptens, so they obviously can not confer immunogenicity on their own without the aid of a more significantly large carrier

protein. It is reasonable to say that such a compound is unlikely to be an allergen when it is unable to elicit an immune response. Nonetheless, No recent literature studies proved that short synthetic peptides (less than seven residues) are self-immunogenic without a carrier's assistance. In one research work, the researchers tried an IRGERA hexapeptide that corresponded to a linear epitope located at the histone H3 C-terminus and found that this peptide was not immunogenic when administered in the absence of a carrier and adjuvant. (Frisch B et al., 1991)

In contrast to the few studies available on immunogenicity, many studies suggest short peptides of both natural and synthetic origins can be anti-allergic or suppress/inhibit the allergic inflammatory response, and below are some examples. Thymic immunosuppressive pentapeptide (TIPP) is a new pentapeptide obtained from calf thymic immunosuppressive extract. It significantly inhibits the increase in Th2 cytokines and OVA-specific IgE production. Overall, TIPP effectively suppresses the allergic and inflammatory responses in allergic mice via blocking MAP kinases/NF-B signaling pathway with the potential to become an anti-allergic and anti-inflammatory therapeutic (Lian Q. et al., 2015).

Another study found that synthetic hexapeptide inhibits immediate hypersensitivity reactions, e.g., passive cutaneous anaphylaxis and mast cell degranulation in rats and antigen-induced bronchoconstriction in actively sensitized guinea pigs in a dose-dependent manner, and such activity was more potent than the clinically used drug disodium cromoglycate (DSCG). It also blocks egg albumin-induced histamine release in chopped lung tissues of sensitized guinea pigs, suggesting that the anti-allergic hexapeptide has a potent inhibitory effect on immediate hypersensitivity reactions (Singh R. et al., 2001). A seven amino acid submandibular gland peptide-T (SGP-T), TDIFEGG, effectively inhibits lipopolysaccharide-induced hypotension (Mathison RD et al., 1997). Structure-activity relationship revealed the C-terminal of SGP-T, the tripeptide FEG and it's D-isomer feG, also have a significant reduction in the severity of intermediate hypersensitivity reactions and inhibition of neutrophil chemotaxis and superoxide production. (Mathison RD et al., 1998)

Human IgE pentapeptide (HEPP) is another excellent example. Application of 0.5% IgE pentapeptide intranasal solution appears to be safe and effective in the treatment of allergic rhinitis. (Prenner BM., 1987) Another finding suggests that tetrapeptide DYLK, when administered locally, can effectively inhibit the airway inflammation and airway remodeling of TDI-sensitized/challenged mice via down-regulation of VEGF, implicating a role in the treatment of allergic reaction in asthma (Ahn MH et al., 2008)

The skin is a unique immunological organ that integrates the external environment with the systemic immune response. To perform sensitization testing is an efficient way to predict allergens. The process of becoming allergic to a particular substance by an animal or human body is called sensitization. (Lian Q. et al., 2015) The most common form of immune toxicity found in humans is skin sensitization, which contributes to the development of an allergic reaction. The GPMT (Guinea Pig Maximization Test) and the Occluded Patch Test had been the most commonly used for the detection of allergens in the past. The murine local lymph node assay (LLNA), had become the commonly used and validated tool to replace the standard guinea pig test methods since the early 2000s. The LLNA quantifies the response activation process by calculating the proliferation of lymphocytes as a skin sensitization predictive biomarker. (Wilbur Johnson, Jr., 2013)

In recent years, regulations have created a strong incentive in the cosmetics and chemical industries to create non-animal alternatives. There were three research methods validated: the direct peptide reactivity assay (DPRA), the KeratinoSens, and the human Cell Line Activation Research (h-CLAT). It is reasonable to assume that if a compound passes guinea pig maximization or LLNA test, or has been negative in two or more of the three above-mentioned in vitro sensitization assays, it is unlikely to be an allergen. Of the few released studies, peptides, including palmitoyl oligopeptide, palmitoyl tetrapeptide-7, palmitoyl dipeptide-18, palmitoyl pentapeptide-4, palmitoyl tripeptide-38, all lack the potential for dermal sensitization. However, most cosmetic peptide sensitization data are not available because vendors and manufacturers are not prepared to publish these details. (Wilbur Johnson, Jr; Cosmetic Ingredient Review, 2014 and, H. Kojima 2017)

It is worth noting that the most well-known culprits of allergens are fragrances and preservatives in personal care products. Most of the allergic reactions occur on category-specific products such as hair coloring and other hair care products, nail cosmetics, sunscreens, as well as antioxidants, vehicles, and emulsifiers. There seems to be a negligible risk of immunologically serious or allergic reactions associated with short cosmetic peptides. The American Academy of Dermatology Cosmetic did not list peptides as common allergens. (Cosmetic Ingredient Review, 2014) Numerous peptides have entered the personal care market over the past twenty years. In many ways, these peptides have improved product efficiency, including anti-aging, skin-soothing, skin-brightening,anti-acne, and other beneficial functions. Peptides remain an essential ingredient that provides results-oriented goods for both the industry of personal care and dermatology. (Lau, J. L 2017)

Work has provided important information on immunogenicity pathogenesis, including' classical' immune responses to neo-antigens as well as the breakdown of self-antigens immune tolerance. At the same time, technology for manufacturing and analytical characterization has evolved and continues to evolve. Regulatory agencies are, therefore, faced with a highly dynamic operation. (Kovalainen, M. et al., 2015) Legislation and guidelines established to support drug developers and regulators are employed to preserve the safety of the therapeutic proteins. Such guidelines must be sufficiently precise and updated to take into account ongoing technical and scientific progress. Experts from industry, academia, and regulatory agencies worldwide in the field of unwanted immunogenicity discussed the phenomenon of unwanted immunogenicity from various angles. (Wilma F. et al. 2014) Many other researchers aimed at bringing the regulatory evaluation of immunogenicity danger to the next level: first, to improve the dialog on the implementation of regulatory guidelines on recognizing and managing the risks associated with undesirable immunogenicity, and second, to examine how to manage unwanted immunogenicity effectively to achieve an acceptable overall benefit-risk calculation. (I.C. Büttel et al. 2011 and, Wilma F. et al. 2014) Research studies in the mid-1990s presented the first proof that synthetic T-cell sensitive peptides could induce tolerance, and thus they were used to suppress IgE-mediated diseases such as rhinitis and asthma induced by cats and ragweed. (Briner TJ et al. 1993 and Peter Socrates 2014) Other researchers have shown that peptides have not led to an increase in the response of the antibody, suggesting immune tolerance. (Wallner BP et all 1994, Norman PS et al. 1996 and, Peter Socrates 2014) A critical benefit of peptides lies in their smaller size; i.e., molecules of insufficient duration to cause IgE cross-linking on mast cells and basophils can provide an advantage by potentially reducing the risk of IgE-mediated allergic reactions such as asthma, urticaria, or anaphylaxis. (Verhoef A et al. 2005, Larche M 2007, Campbell JD 2009 and, Larche M 2011)

Recently, a rise in the regulatory approval of peptide products is frequent. (R. Lax et al. 2012) Technological breakthroughs for peptide bioprocessing have evolved. Nowadays, multiple development methods are employed for the processing of peptide products. Some of these processing methods (e.g., enzymatic protein hydrolysis and microbial protein fermentation) are relatively low cost and can, therefore, tackle the relatively high cost of synthesis. (D. Agyei 2011) Besides, combinatorial production strategies such as chemoenzymatic methods can lead to high solubility peptide production and improved profiles of absorption, distribution, metabolism, and excretion (ADME). (K. Fosgerau et al. 2015) Advances in bioinformatics and silico methods to model binding mechanisms and peptide metabolic profile when researching and linking peptide structure to their activities. (C.C. Udenigwe 2014 and P. Vlieghe et al. 2010) Development and promotion of peptides' use as biopharmaceuticals should be openly revealed to users. With such regulatory advancements, peptides are expected to show amplified use in treating many health conditions as well as success in the pharmaceutical market. In the manufacture of polypeptides,

including tetrapeptides, state-of-the-art developments in the modification, formulation, and delivery of peptide drugs are used. (K. Fosgerau et al. 2015)

Peptides do not exhibit idiosyncratic toxicity, although due to excessive pharmacology, most adverse effects occur. This nontoxic effect is endorsed typically to the fact that peptides are unable to enter cells and are unable to communicate with large numbers of intracellular molecular targets with unpredictable consequences. Besides, since most peptide drugs are very potent, pharmacologically active plasma concentrations tend to be well below 100 nM and do not seem to tax excessively the organs of elimination (liver, kidneys). (H. Kojima 2017)

The structural complexity and versatility of peptides offer the medicinal chemist several opportunities to choose the necessary selectivity, structural complexity, and size needed for each desired medicinal use. The necessary selectivity, which, together with low plasma concentrations, reduces the possibility of off-target effects and drug-drug interactions through transporters or metabolizing enzymes (cytochromes or proteases). The presence of injection site reactions following subcutaneous administration is an adverse effect that needs to be addressed early in early clinical trials. Many large and complex peptides and drugs of protein nature have the ability to induce degranulation of mast cells and release of histamine, particularly given that concentrations in the mM range can be found in the solutions injected. (McNeil, B. D et al. 2015) Fortunately, these reactions are temporary in most cases and not too extreme to stop treatment. Generally speaking, data on peptide immunogenicity is not usually reported in the general literature and must be retrieved from regulatory documents. A comparative study on the mutagenicity of various licensed products should be done and released to consumers to help them feel safe using industrially sound peptide products. (Madsbad, S. 2016)

In general, peptides are less likely to encounter unexpected safety issues during clinical studies. (Cathelijne Kloks et al. 2015) Although the median time from initiation of clinical development to approval is not very different from therapeutic chemical molecules, around ten years, from any stage of development to regulatory approval, they have a higher probability of success than such molecules. (Lau, J. L., and Dunn, M. K. 2017)

5. Conclusions

Most synthetic peptides whose size is in the range of 1000 to 2000 Dalton are poorly recognized by the immune system and are typically weak immunogens. Most of the tetrapeptides are less than 800 Daltons in size, and there is little evidence that such tetrapeptides can activate the immune response, and hence they are known as haptens. A hapten is a molecule that interacts with a particular antibody, but on its own, is not immunogenic. Nevertheless, it can only be rendered immunogenic by conjugating to an appropriate carrier. A significant development in the area of immunogenicity leads to substantial improvements in both knowledge and methodology. To conclude, a flexible approach of regulatory guidelines is a good platform from which researchers can continue with the immunogenicity assessment of therapeutic proteins and peptides. It seems appropriate to supplement this basis with more clear and specific recommendations on distinct product classes, new technologies, and state-of-the-art technologies and approaches for peptides' manufacture, use, and safety issues.

References

[1] A. Zimmerman, L. Bai, D.D. Ginty. (2014): The gentle touch receptors of mammalian skin, Science 346 (6212), 950- 4. https://doi.org/10.1126/science.1254229.

[2] A.A. Romanovsky (2014): Skin temperature: its role in thermoregulation, Acta Physiol 210 (3) (March 2014) 498-507. https://doi.org/10.1111/apha.12231.

[3] Ahn MH, Park BJ, and Kwon JH, et al. (2008): Asp-Tyr-Leu-Lys tetrapeptide inhibits airway inflammation in toluene-2,4-diisocyanate-induced asthma mice. Clin Exp Allergy 2008 Jun; 38(6):1025-32. https://doi.org/10.1111/j.1365-2222.2008.02977.x.

[4] Berger, C. and Niesner, U. (2011): Immunogenicity of microbial digestive enzymes for oral replacement therapy in pancreatic exocrine insufficiency. In: Tovey, M.G. (Ed.), Detection and Quantification of Antibodies to Biopharmaceuticals: Practical and Applied Considerations. John Wiley & Sons, Inc., Hoboken, NJ, USA. https://doi.org/10.1002/9781118075685.ch20.

[5] Bhogal, N. (2010): Immunotoxicity and immunogenicity of biopharmaceuticals: design concepts and safety assessment. Curr. Drug Saf. 5, 293. https://doi.org/10.2174/157488610792246037.

[6] Briner TJ, Kuo M-C, Keating KM, et al. (1993): Peripheral T-cell tolerance induced in naive and primed mice by subcutaneous injection of peptides from the major cat allergen Fel d I. Proc Natl Acad Sci- U S A, 90:7608–7612. https://doi.org/10.1073/pnas.90.16.7608.

[7] Buttel, I.C., Voller, K., Schneider, C.K. (2010): Immunogenicity and its impact on benefit/risk considerations in the authorization of biopharmaceuticals. Curr. Drug Saf. 5, 287-11 https://doi.org/10.2174/157488610792245993.

[8] C.C. Udenigwe (2014): Bioinformatics approaches prospects and challenges of food bioactive peptide research, Trends Food Sci. Technol. 36:137-143. https://doi.org/10.1016/j.tifs.2014.02.004.

[9] Campbell JD, Buckland KF, McMillan SJ, et al. (2009): Peptide immunotherapy in allergic asthma generates IL-10-dependent immunological tolerance associated with linked epitope suppression. J Exp Med, 206:1535. https://doi.org/10.1084/jem.20082901.

[10] Casadevall N., Nataf J., and Viron B., et al. (2002): Pure red-cell aplasia and anti-erythropoietin antibodies in patients treated with recombinant erythropoietin. N. Engl. J. Med. 346-469. https://doi.org/10.1056/NEJMoa011931.

[11] Cathelijne Kloks, Claudia Berger, Pierre Cortez et al. (2015): A fit-for-purpose strategy for the risk-based immunogenicity testing of biotherapeutics: a European industry perspective. Journal of Immunological Methods 417: 1–9 https://doi.org/10.1016/j.jim.2015.01.003.

[12] Coiffier B., Lepretre S. and Pedersen L.M et al. (2008): Safety and efficacy of ofatumumab, a fully human monoclonal anti-CD20 antibody, in patients with relapsed or refractory B-cell chronic lymphocytic leukemia: a phase 1- 2 study. Blood 1011- 94. https://doi.org/10.1182/blood-2007-09-111781.

[13] Cosmetic Ingredient Review expert panel (2014): 131st Meeting findings. June 9-10, 2014. Accessed online, 27th December 2019.

[14] D. Agyei, and M.K. Danquah (2011): Industrial-scale manufacturing of pharmaceutical-grade bioactive peptides, Biotechnol. Adv. 29: 272-7. https://doi.org/10.1016/j.biotechadv.2011.01.001.

[15] Daniel A.B, Strickland J, and Allen D, et al. (2018): International regulatory requirements for skin sensitization testing. Regul Toxicol Pharmacol, 95:52-65. https://doi.org/10.1016/j.yrtph.2018.03.003.

[16] Frisch B, Muller S, and Briand JP et al. (1991): Parameters affecting the immunogenicity of a liposome-associated synthetic hexapeptide antigen. Eur J Immunol. 1991 Jan; 21(1):185-93. https://doi.org/10.1002/eji.1830210128.

[17] Getts, D.R., Getts, M. T., McCarthy, D.P. et al. (2010): Have we overestimated the benefit of human(ized) antibodies?. MAbs; 2(6): 682– 94. https://doi.org/10.4161/mabs.2.6.13601.

[18] Goldsby, R.A., Kindt T.J., and Osborne, B.A. (2000): T-cell maturation and the thymus, in Immunology, 4th ed, Ch. 10, pp. 239–67 (NewYork, W. H. Freeman Company).

[19] Gregoriadis G., Sanjay Jain, and Ioannis Papaioannou et al., (2005): Improving the therapeutic efficacy of peptides and proteins: a role for polysialic acids. Int. J. Pharm. 300 (1–2), 125– 30. https://doi.org/10.1016/j.ijpharm.2005.06.007.

[20] H. Kojima. (2017): Safety Assessment of Cosmetic Ingredients. National Institute of Health Sciences (NIHS), Tokyo, Japan. Cosmetic Science and Technology: Theoretical Principles and Applications. (Chapter 51): 793-803. https://doi.org/10.1016/B978-0-12-802005-0.00051-3.
[21] Harris, R.C. Hoffman JR, Allsopp A, Routledge NB. (2012): L-Glutamine absorption is enhanced after the ingestion of alanyl-glutamine compared with the free amino acid or wheat protein. Nutr. Res. 32 (4), 272– 7. https://doi.org/10.1016/j.nutres.2012.02.003.
[22] I.C. Büttel, P. Chamberlain, Y. Chowers et al. (2011): Taking immunogenicity assessment of therapeutic proteins to the next level. Biologicals 39;100 -109. https://doi.org/10.1016/j.biologicals.2011.01.006.
[23] K. Fosgerau and T. Hoffmann (2015): Peptide therapeutics: current status and future directions, Drug Discov. Today 20: 122-128. https://doi.org/10.1016/j.drudis.2014.10.003.
[24] Kimber I, Dearman RJ, Scholes EW, et al. (1994): The local lymph node assay: developments and applications. Toxicology: (93):13–31. https://doi.org/10.1016/0300-483X(94)90193-7.
[25] Kovalainen, M., Mönkäre J, and Riikonen J. et al. (2015): Novel delivery systems for improving the clinical use of peptides. Pharmacol. Rev. 7, 541–61. https://doi.org/10.1124/pr.113.008367.
[26] Larche M and Moldavar D: (2011): Immunotherapy with Peptides. Allergy, 66:784–791. https://doi.org/10.1111/j.1398-9995.2011.02610.x.
[27] Larche M: (2007): Immunotherapy with Allergen Peptides. Allergy Asthma Clin Immunol, 3:53–59. https://doi.org/10.1186/1710-1492-3-2-53.
[28] Lau, J. L., and Dunn, M. K. (2017): Therapeutic Peptides: Historical Perspectives, Current Development Trends, and Future Directions. Bioorg. Med. Chem, https://doi.org/10.1016/j.bmc.2017.06.052.
[29] Li, J., Yang, C., Xia, Y., et al. (2001): Thrombocytopenia caused by the development of antibodies to thrombopoietin. Blood 98, 3241. https://doi.org/10.1182/blood.V98.12.3241.
[30] Lian Q. Jiang W and Cheng Y et al. (2015): A novel pentapeptide originated from calf thymus named TIPP shows an inhibitory effect on lung allergic inflammation. Int Immunopharmacol. 2015 Feb;24(2):256-266. https://doi.org/10.1016/j.intimp.2014.12.019.
[31] M.Z. Albanna, I.V.J.H. Holmes, and J. Fenner (2016): Clark, Anatomy, Physiology, Histology, and Immunohistochemistry of Human Skin. Skin Tissue Engineering and Regenerative Medicine, Academic Press, pp. 1-17. https://doi.org/10.1016/B978-0-12-801654-1.00001-2.
[32] Madsbad, S. (2016): Review of Head-to-Head Comparisons of Glucagon-Like Peptide-1 Receptor Agonists. Diabetes Obes. Metab, 18(4), 317–332. https://doi.org/10.1111/dom.12596.
[33] Mathison R, Lo P, and Moore G, et al. (1998): Attenuation of intestinal and cardiovascular anaphylaxis by the salivary gland tripeptide FEG and its D-isomeric analog feG. Peptides;19(6):1037-42. https://doi.org/10.1016/S0196-9781(98)00048-5.
[34] Mathison RD, Befus AD, and Davison JS. (1997): A novel submandibular gland peptide protects against endotoxic and anaphylactic shock. Am J Physiol. Sep;273(3 Pt 2): R1017-23. https://doi.org/10.1152/ajpregu.1997.273.3.R1017.
[35] McGregor, D.P. (2008): Discovering and improving novel peptide therapeutics. Curr. Opin. Pharmacol. 8 (5), 616 – 9. https://doi.org/10.1016/j.coph.2008.06.002.
[36] McNeil, B. D.; Pundir, P.; Meeker, S. et al. (2015): Identification of a Mast-Cell-Specific Receptor Crucial for Pseudo-Allergic Drug Reactions. Nature, 519(7542), 237–241. https://doi.org/10.1038/nature14022.
[37] Niebecker, R. and Kloft, C. (2010): Safety of therapeutic monoclonal antibodies. Curr. Drug Saf. 5, 275. https://doi.org/10.2174/157488610792246055.
[38] Norman PS, Ohman JL Jr, Long AA, et al. (1996): Treatment of cat allergy with T cell epitope containing peptides. Am J Respir Crit Care Med, 154:1623 – 8. https://doi.org/10.1164/ajrccm.154.6.8970345.
[39] P. Vlieghe, V. Lisowski, J. Martinez, et al. (2010): Synthetic therapeutic peptides: science and market, Drug Discov. Today 15: 40-56. https://doi.org/10.1016/j.drudis.2009.10.009.
[40] Pan, H., Ivashyna O, Sinha B. et al. (2011): Post-formulation peptide drug loading of nanostructures for metered control of NF kappa B signaling. Biomaterials 32 (1), 231– 8. https://doi.org/10.1016/j.biomaterials.2010.08.080.
[41] Peter Socrates Creticos. (2014): Advances in synthetic peptide immuno-regulatory epitopes. Creticos World Allergy Organization Journal, 7-30. https://doi.org/10.1186/1939-4551-7-30.
[42] Prenner BM. (1987): Double-blind placebo-controlled trial of intranasal IgE pentapeptide. Ann Allergy. May; 58(5):332-5.
[43] R. Lax, C. Meenan (2012): Challenges for therapeutic peptides part 1: on the inside, looking out, Innov. Pharm. Pharmaceut. Technol. 42: 54- 6.
[44] Sarmadi, B.H., Ismail, A., (2010): Antioxidative peptides from food proteins: a review. Peptides 31 (10), 1949–1956. https://doi.org/10.1016/j.peptides.2010.06.020.
[45] Sato, A.K., Viswanathan M, Kent RB, et al., (2006): Therapeutic peptides: technological advances driving peptides into development. Curr. Opin. Biotech. 17 (6), 638–642. https://doi.org/10.1016/j.copbio.2006.10.002.
[46] Schellekens, H., and Casadevall, N., (2004): Immunogenicity of recombinant human proteins: causes and consequences. J. Neurol. 251 (Suppl. 2), ii4. https://doi.org/10.1007/s00415-004-1202-9.
[47] Singh R. Nath A, and Gupta PP, et al. (2001): Antiallergic/antiasthmatic effect of novel antiallergic hexapeptide-95/220 in various experimental models. Indian J Exp Biol. Sep;39(9):871-7.
[48] Tanabe S. (2007): Epitope peptides and immunotherapy. Curr Protein Pept Sci. Feb;8(1):109-18. https://doi.org/10.2174/138920307779941569.
[49] Uhlig, T., ThemisKyprianou, Filippo Giancarlo Martinelli et al. (2014): The emergence of peptides in the pharmaceutical business: from exploration to exploitation. EuPA Open Proteom. 4, 1–12. https://doi.org/10.1016/j.euprot.2014.05.003.
[50] Verhoef A, Alexander C, Kay AB, Larche M (2005): T cell epitope immunotherapy induces a CD4(+) T cell population with regulatory activity. PLoS Med, 2:78. https://doi.org/10.1371/journal.pmed.0020078.
[51] Vlieghe, P., Lisowski V, Martinez J, et al. (2010): Synthetic therapeutic peptides: science and market. Drug Discov. Today 15 (1–2), 40–56. https://doi.org/10.1016/j.drudis.2009.10.009.
[52] Wallner BP, Gefter ML: (1994): Immunotherapy with T-cell reactive peptides derived from allergens. Allergy, 49:302–308. https://doi.org/10.1111/j.1398-9995.1994.tb02272.x.
[53] Walsh, G. (2010): Biopharmaceutical benchmarks. Nat. Biotechnol. 28, 917-25. https://doi.org/10.1038/nbt0910-917.
[54] Wilbur Johnson, Jr. (2013): Safety assessment of palmitoyl oligopeptides ingredients as used in cosmetics. CIR expert panel meeting March 18-21.
[55] Wilma F. Bergfeld, Donald V. Belsito, and Curtis D. Klaassen et al. (2014): Tentative Report for Panel Review. Safety Assessment of Tripeptide-1, Hexapeptide-12, their Metal Salts and Fatty Acyl Derivatives, and Palmitoyl Tetrapeptide-7 as Used in Cosmetics. Cosmetic Ingredient Review. Accessed online on 27th December 2019.
[56] Yamashita Y, Sakamoto K. (2015): Role of urocanic acid in the stratum corneum. In: Sakamoto K, editor. Amino acid and peptide at the forefront of cosmetic ingredients. Tokyo, Japan: CMC Research K.K.; 2015. p. 260-72 [Chapter 25].