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Mudasir M Rather
Ph.D Scholar, Department of Veterinary Microbiology, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Vasavi Koppu
Ph.D Scholar, Department of Veterinary Microbiology, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Sudhir Singh
PG Scholar, Department of Veterinary Microbiology, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Bhavani Puvvala
Ph.D Scholar, Department of Veterinary Microbiology, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Tripti Pande
Ph.D Scholar, Department of Veterinary Microbiology, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Deepa Poloju
Ph.D Scholar, Department of Veterinary Microbiology, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Pradeep Chandra
Ph.D Scholar, Department of Animal Reproduction, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Corresponding Author:
Vasavi Koppu
Ph.D Scholar, Department of Veterinary Microbiology, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Epitope dampening: Potential for development of new generation vaccines

Mudasir M Rather, Vasavi Koppu, Sudhir Singh, Bhavani Puvvala, Tripti Pande, Deepa Poloju and Pradeep Chandra

Abstract
Epitope dampening, a novel concept in vaccine development, holds significant potential for the creation of next-generation vaccines. Traditional vaccines often target specific epitopes on pathogens to stimulate an immune response. However, pathogens can undergo epitope mutations, rendering existing vaccines less effective and requiring constant updates. Epitope dampening is a strategy aimed at minimizing the impact of epitope mutations by designing vaccines that target conserved regions of pathogens rather than specific epitopes. This approach offers several advantages, including broader and more durable protection against diverse strains and reduced dependence on frequent vaccine updates. By focusing on conserved regions, epitope dampening vaccines can potentially provide enhanced cross-reactivity and cross-protection against related pathogens. Moreover, epitope dampening may mitigate the risk of immune escape variants, which can arise due to epitope-specific immune pressure. This review article explores the potential of epitope dampening as a promising avenue for the development of new generation vaccines and highlights its implications for future vaccine design and disease control strategies.

Keywords: Epitope dampening, new generation vaccines, antigenic variation, immune evasion, epitope masking, immunodominance

1. Introduction

Antigenic variation is a common technique employed by pathogens to evade host adaptive immune responses. This can be accomplished by phase variation, in which antigenic proteins are turned on and off at the genetic level (a method often used by bacteria), or through random mutation within epitopes, as is common with error-prone RNA viruses. Immunodominant protein sequences that frequently undergo antigenic variation and do not have any apparent structural or functional purpose are often referred to as decoy epitopes. (Szczepanek, *et al.*, 2012) [23]. These hypervariable sequences appear to confer an advantage to RNA viruses as they keep the host's immune system one step behind the pathogen in the evolutionary arms race by inducing a vigorous immune response to a dispensable epitope, ostensibly neglecting the highly conserved, yet crucially important, epitopes. Vaccinologists have had difficulty dealing with decoy epitopes since their discovery in RNA viruses, where their detection is becoming more widespread. (Selvarajah *et al.*, 2008) [20].
Many of the most common and dangerous viruses in the world, including the influenza virus, appear to survive in the wild by establishing antibodies to genetically and phenotypically flexible epitopes (through antigenic variation). The more recent outbreak of avian H5N1 and pandemic influenza A/H1N1 viruses has highlighted the critical requirement for additional efficient and efficient vaccinations (Nara *et al.*, 2010) [17]. Furthermore, simply switching from an embryonated egg-based to a tissue culture-based production technique won't be sufficient to develop such vaccines. Furthermore, simply switching from an embryonated egg-based to a tissue culture-based production technique will be needed to develop such vaccines. In order to prevent viruses with more genetic diversity from evading the immune system, it will also be necessary to develop new conceptual frameworks for pathogen-host interactions. (Weidenbachera *et al.*, 2019) [30].
Antigenic variation and deceptive imprinting can be eliminated through immune focussing or epitope dampening. The immune system can show a high affinity for a limited number of epitopes despite its capacity to recognise a broad variety of B and T cell epitopes. The previously unknown epitopes, however, are now recognised when other potentially antigenic determinants are provided in the absence of the immunodominant ones.

It appears that it is a mechanism adopted by a wide array of pathogens to focus the immune response to less relevant or irrelevant epitopes. The pathogen "decoys" the immune response resulting in a very narrow strain specific immunity, no protection, blocking antibody, or immune enhancement. These immunodominant epitopes are immune dampened by epitope masking without significantly disrupting the complex conformation of the molecule. When the immune system is exposed to the immune-dampened antigen, it no longer pays attention to the formerly immunodominant decoying epitopes, causing antibodies and cell-mediated reactions to previously less antigenic regions all across molecule. When compared with the unaltered, non-immune dampened antigen, these newly refocused responses are linked to broader neutralisation and cell-mediated killing activities. (Mravic *et al*., 2019) [16].

2. How the virus evade host adaptive immune response

2.1 Shutdown of host macromolecular synthesis

Many viruses, soon after infection, inhibit normal transcription and/or translation of cellular proteins, and quickly disrupt the infected cell's mechanism to produce new virions. The innate immune response to the invading virus is hampered due to shutdown of host protein synthesis, which also affects the synthesis of essential proteins like class I MHC antigen and antiviral cytokines like type I IFN. As a result, the infecting virus can swiftly reproduce and spread before the host can mount an adaptive immune response if there are insufficient innate immune responses. RNA viruses frequently employ this tactic, and many of them have extremely quick reproduction cycles. (Zhang *et al*., 2001).

2.2 Avoidance of Cytotoxic T lymphocyte & NK cell mediated killing

Viral antigens must be presented on the surface of the infected cell in the context of the appropriate class I MHC molecule in order for cytotoxic T lymphocytes (CTL) to kill the virus-infected cell. As a result, viruses have evolved various tactics to suppress the normal expression of class I MHC proteins in order to prevent CTL-mediated lysis.

These strategies include

1. Shutting down host protein synthesis prevents cells from producing MHC class I molecules.
2. Virus-encoded proteins are produced that interfere with the Golgi apparatus or cell surface transport of MHC class I proteins or their normal synthesis in the endoplasmic reticulum.
3. Synthesis of virus-encoded proteins that interfere with MHC class I molecules' functionality or viability
4. Production of virus-encoded MHC class I molecules capable of binding β2 microglobulin and viral peptides but otherwise ineffective in inducing CTL activity (Westendorp *et al*., 1995) [31].

Unlike CTL-mediated lysis, which needs sufficient quantities of class I MHC antigen on the surface of virus-infected cells, Reduced amounts of class I MHC antigen on the cell surface increase NK-cell-mediated cytolysis. Also, the balance of inhibitory and stimulatory chemicals on the cell surface is also critical for NK cell function. Thus some viruses selectively inhibit cellular production and expression of molecules that provide stimulatory signals for NK cell activity. Other viruses inhibit host-cell production of both stimulatory and inhibitory molecules, such that the infected cell is somewhat protected against both CTL- and NK-cell-mediated lysis.

2.3 Interference with apoptosis

The extrinsic (death receptor) or intrinsic (mitochondrial) pathways can both be used by viruses to start apoptosis on their own. Particularly harmful to the relatively slow-growing DNA viruses, such as poxviruses, herpes viruses, and adenoviruses, because apoptosis can result in death of cells infected with these viruses before maximal levels of virus replication have been completed. As a result, these DNA viruses in particular have evolved a surprising range of tactics to enhance their reproduction by impeding the numerous apoptosis-causing processes. The different stratergies used by different viruses include

1. Serpins, which are protease inhibitors produced by poxviruses that bind to and inhibit the proteolytic action of caspases, are a key factor in the reduction of executioner caspase activity that mediates cellular harm.
2. The development of viral receptor homologs that bind TNF to prevent it from starting the extrinsic route, or compounds that specifically block the signalling cascade started by death receptor activation can all be used to reduce the expression, activation, and signalling of death receptors.
3. Synthesis of Bcl-2 and other anti-apoptotic proteins that are encoded by viruses
4. Synthesis of proteins that trap p53, a protein molecule that promotes apoptosis and builds up in cells infected with certain viruses (Wang G-H *et al*., 1997) [28].

2.4 Counter defenses against cytokines

Since cytokines play a crucial role in both innate and adaptive immune responses to viral infections, viruses have also evolved potent defence mechanisms against the actions of these crucial antiviral mediators. Some viruses have incorporated cellular genes into their genetic makeup, resulting in viral genes that produce proteins that are homologs of cytokines or their receptors. The biological effects of the cytokines can be mimicked by virus-encoded cytokine homologs (referred to as virokines), or they can can be non-functional and just block the particular cytokine receptor to nullify that activity. Similar to this, the corresponding cytokine is often bound to and neutralised by virus-encoded receptor homolog proteins. Other virus-encoded proteins disrupt the signalling pathways activated by IFN binding to its receptor or by dsRNA-stimulated pattern recognition receptor signalling pathways (such TLR3 or RIG-1) that cause the production of type I IFN and other antiviral cytokines (IFNAR). The particular combination of these virus-encoded proteins can influence the activity of a wide range of cytokines, including IL-1, IL-6, IL-8, types I and II IFN, and TNF, to the benefit of the virus' ability to replicate.

2.5 Evasion of antiviral state

Viruses have also developed sophisticated techniques to evade the action of crucial IFN-induced antiviral effector mechanisms, such as the protein kinase (PKR) and 2'-5' oligoadenylate synthase (OAS) pathways. The strategies involve the production of virus-encoded proteins or RNA molecules that specifically bind to essential enzymes or their encoding genes without activating them, thus rendering them non-functional. The viruses also stimulate pathways that

suppress the activity and function of these protective antiviral pathways. Furthermore, they produce proteins that bind to double-stranded RNA (dsRNA), which is an important co-factor for antiviral proteins PKR and OAS. Numerous virus families, both DNA and RNA viruses, have developed distinct mechanisms to circumvent the host's immune response (Levy *et al*., 2001) [12].

2.6 Virus specific gene silencing pathways

Viruses have evolved mechanisms to counter the cellular antiviral RNA interference processes by synthesizing virus-encoded proteins or small interfering mRNA (siRNA) molecules that interfere with critical stages of the cellular pathway. Moreover, certain viruses utilize RNA interference (RNAi) molecules to suppress crucial cellular genes involved in antiviral immunity release (Llave *et al*., 2000) [14].

2.7 Antigenic Variation

This antigenic variation mainly occur due to (Vakharia *et al*., 1994) [25].

2.7.1 Random Genetic Mutations

Mutations are the outcome of errors in viral nucleic acid replication (Peck, K. M. & Lauring, A. S. 2018) [19]. The majority of mutations are lethal causing the loss of crucial information that prevents them from replicating (Brown *et al*., 2002) [4]. According to estimations, DNA viruses have an error rate of 10-8 to 10-11 per incorporated nucleotide. However, the replicatory enzymes of RNA viruses lack a proofreading mechanism, and some of the viruses have mutation rates that are higher such as 10-3 to 10-4 errors per incorporated nucleotide. (Fleischmann WR 1996)Viral mutations can be site-specific, spontaneous, or induced. X-rays and UV radiation are used in physical mutagenesis. (Ikehata H, Ono T. 2011) [10]. Nitrous acid, nitrosoguanidine, and nucleotide analogues (5-fluorouracil or 5-bromodeoxyuridine) are all used in chemical mutagenesis. Small genome sizes and mutation rates are inversely correlated. The mutation rate is higher in ssDNA virus than dsDNA virus. (Duffy S. 2018) [6].

2.7.2 Error prone polymerase

Error-prone DNA polymerases refer to enzymes with reduced accuracy during the replication of an undamaged DNA template in its B-form, such as translesion synthesis (TLS) polymerases and Pols θ, β, λ, μ, ν, and Primpol. Among these, five TLS polymerases (Pol η, Pol ι, Pol κ, REV1, and Pol) are extensively studied as they play a crucial role in copying past both external DNA lesions and internal DNA obstacles, such as fragile sites (Vaziri, C *et al*., 2021) [26].

2.7.3 Genetic Reassortment

Genetic reassortment is a distinctive form of genetic recombination found only in segmented RNA viruses. It occurs when multiple viruses infect the same host cell, leading to the mixing of gene segments and the creation of new combinations in offspring viruses. Reassortment is observed in various segmented viral families, including the Bluetongue virus. However, influenza viruses have been particularly noteworthy in highlighting reassortment as a significant process for the transmission between different species and the emergence of pandemic virus strains (Vijaykrishna *et al*., 2015; Lowen, 2018) [27, 15].

2.7.4 Viral Quasi Species

According to Domingo *et al*. (2019) [5], viral quasispecies are defined as groups of closely related viral genomes that are continuously subjected to a process of genetic variation, competing among the variations produced, and selection of the best-fit distributions in a given environment. High mutation rates have a maximum that is consistent with inheritable information despite being an essential component of their replication. By going above this threshold, an RNA virus goes extinct, which forms the basis of the lethal mutagenesis antiviral design (Lauring AS *et al.* 2013) [11].

3. Deceptive Imprinting and Immunodominance

Adaptive immune system of the host has developed the ability to identify, react preferentially to biochemical entities that are considered "foreign" or "non-self." With the models suggested in this perspective, the long-standing topic of how host immune system eventually determines "foreignness" is given even greater significance. When immunodominance was first described, it was believed to be solely a phenomenon of the major histocompatibility complex (MHC) restricted response genes in inbred mouse strains. Immunodominance is defined as an enhanced and strongly favoured immune response by the host to a specific set of epitopes.

The concept of immunodominance was not easily extended to host-pathogen interactions, despite experimental immunologists noticing significant or extremely potent antibody responses in some host antigen interactions.

The theory of "deceptive imprinting" is significant because it suggests that immunodominance plays a crucial role in pathogenic organisms within a genetically diverse host population. It builds upon the concept of "original antigenic sin" or the Hoskins effect. When pathogens exploit the immunodominance of certain epitopes and establish them in the host's immune memory, the immune system tends to rely on previous infections by similar but slightly different entities. This reliance hampers the immune system's ability to mount more effective responses during subsequent infections, as it becomes trapped by its initial response to each entity. To exacerbate this effect, pathogens can combine hetero-specific immunity (hetero-reactive immunodominance) with strain-specific immunity and strategically position immunodominant epitopes alongside highly conserved functional domains necessary for viral infection. Consequently, the host's immune response predominantly targets less protective epitopes, resulting in deceptive imprinting. (Nara *et al*., 2010) [17].

3.1 How to overcome deceptive strategy?

3.1.1 Immune refocusing technology/ Immunodampening

Switching of immune response from more conserved subdominant epitopes to DI epitopes

Approaches

1. Deleting or hiding decoy epitopes in vaccine constructs
2. Mutation of immunodominant epitopes by addition of glycosylation masks or other substitutions induces more broadly protective immune response

3.1.2 Types of immuno-focusing

3.1.2.1 Epitope masking is a technique for preventing the formation of Ab by securing the immunodominant region of a protein, frequently employing unnatural glycosylation sites. (Weidenbacher PA *et al*., 2018) [30].

3.1.2.2 Epitope scaffolding
The aim of epitope scaffolding is to affix an unique conformational epitope to a special protein scaffold. In the realm of rational vaccination, protein design has raised expectations, especially in the pursuit of targeted neutralising antibody (nAb) responses. Achieving targeted antibody responses through the development of immunogens remains a difficult task, despite the discovery and detailed analysis of numerous effective neutralizing antibodies (nAbs) and their interactions with antigens. Previous efforts in structure-based immunogen design have primarily focused on modifying viral fusion proteins to enhance their stability, suppressing non-neutralizing epitopes, and directing attention to the germline origins of nAbs (Sesterhenn *et al*., 2020) [21].

3.1.2.3 Protein dissection
Protein dissection removes undesirable or immunodominant epitopes from the native antigen (He, L *et al.,* 2015).

3.1.2.4 Antigen resurfacing
Site-directed mutagenesis is employed in antigen resurfacing to introduce less immunogenic residues at locations other than the target epitope.

3.1.2.5 Cross-strain boosting
Cross-strain boosting involves chimeric proteins that differ at off-target epitopes or sequential immunizations with different strains. (Weidenbacher PA *et al*., 2018) [30].

3.1.2.6 Protect, modify, deprotect (PMD)
These immune-focusing techniques have made significant advancements. These techniques have inherent drawbacks. Unfortunately, they are difficult to generalise, which makes their application to novel antigens difficult. These immune-focusing techniques are likewise often "low-resolution," with the exception of epitope scaffolding (which necessitates considerable protein engineering) (i.e., directed towards a region of the protein that is significantly larger than a typical Ab epitope). Moreover, it can be difficult to maintain the epitope's accurate 3D shape with some of these approaches. With only minimum protein engineering, protect, modify, deprotect (PMD) offers the ability to deliver high-resolution immune-focusing in a generalised approach. The procedure creates an antigen that directs the immune system's attention to the broadly neutralizing antibodies (bnAb) epitope using the antibody as a molecular stencil. Despite the fact that bnAbs have previously been employed to inform and direct immunogen design. The PMD process involves the binding of a bnAb to protect an antigen's epitope and chemically altering exposed areas to make them less immunogenic. Finally, there is dissociation of the Ab-antigen complex to deprotect the target epitope. This results in an immunogen in which the bnAb-mapped epitope is the only unaltered area.

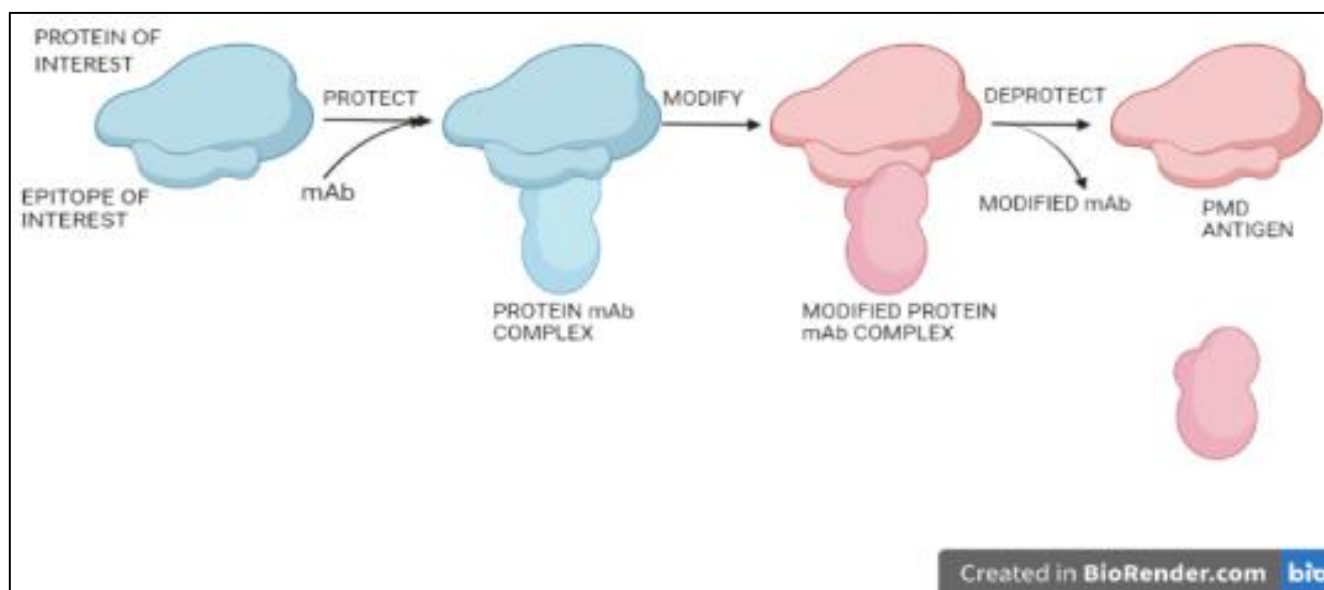

Fig 1: A general schematic representation of the PMD strategy. First, the epitope is protected by combining the mAb with the antigen (white). Then the surfaces of the protein complex are modified to render them non-immunogenic (shown as darker shading). Finally, the epitope is deprotected by removal of the mAb. (Weidenbacher PA *et al*., 2018) [30]

4. Immunodampening technology in practical use
4.1 Respiratory syncytial virus (RSV)
Researchers have explored the use of a stabilised version of the respiratory syncytial virus (RSV) fusion (F) protein as a potential vaccine against RSV infection. Through structure-based rational design, they aimed to enhance antigen presentation and concentrate antibody (Ab) responses on crucial epitopes of the pre-fusion (pre-F) protein. To further enhance the antibody response, the pre-F protein was fused with ferritin nanoparticles (pre-F-NP) and modified with glycans to cover non-neutralising or weakly neutralising epitopes. *In vivo* studies in mice and nonhuman primates (NHPs), as well as *in vitro* assessments using human cells in the MIMIC system, demonstrated that the multimeric pre-F-NP vaccine elicited robust neutralising antibody (NAb) responses. Moreover, it induced durable pre-F-specific antibodies in NHPs for over 150 days. Compared to a typical pre-F trimer, this improved pre-F-NP vaccine elicited a stronger antibody response. These findings suggest that these pre-F vaccines enhance the production of NAbs targeting the desired pre-F conformation, which is crucial for the development of an effective RSV vaccine (Swanson *et al*., 2020) [22].

4.2 Influenza virus

Mechanism employed for evasion include

1. Deceptive imprinting or clonal dominance along with steric hindrance of antibody
2. Random genetic mutation
3. Genetic reassortment

Strain specific antibodies produced against globular domain of HA

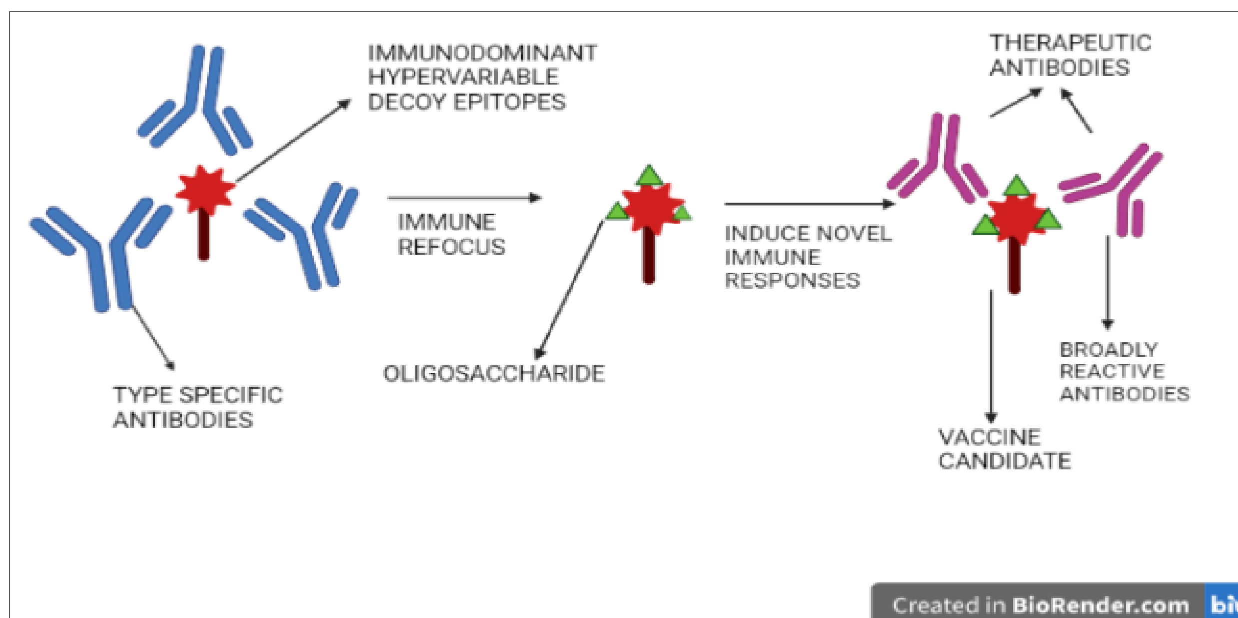

Fig 2: Picture illustrating the immunological refocusing technology and steric antibody interference

Using the influenza hemagglutinin (HA) trimer as an example, a diagram of immunological refocusing technology and steric antibody interference is presented. In its native form, HA contains decoy epitopes that elicit type-specific antibodies, represented in red in the left panel. In the modified version shown in the center panel, extra N-linked glycans are added to specific epitope locations or point mutations and deletions are introduced. This modified HA, referred to as immunological refocused HA, triggers immune responses that are broadly reactive. This characteristic can be leveraged to develop therapeutic antibodies with broad reactivity or as vaccines with enhanced effectiveness. (Tobin *et al*., 2008) [24].

4.3 Caprine arthritis encephalitis virus (CAE)

Surface glycoprotein (gp135 SU) is primary target of humoral immune response

- Low titre of cross-reactive neutralizing antibodies produced by immunization with monomeric SU (Lichtensteiger *et al*., 1991) [13]. Most immunodominant linear epitopes of SU are within the carboxy-terminal end (Bertoni *et al*., 2000) [2].
- Immune recognition of discontinuous neutralization epitopes results in generation of non-neutralization or type-specific neutralization epitopes (Garitty *et al*., 1997) [8]
- Glycan masking effectively redirected antibody response to neutralization epitopes
- Surface glycoprotein modifications are made
- Insertion of N-linked glycans (SU-M)
- Removal of mapped linear immune dominant epitope at carboxy terminus of SU (SU-T)

4.4 Porcine Reproductive & Respiratory Syndrome (PRRS)

- Glycoprotein 5 (Gp5) possess decoy epitopes in ectodomain
- There are two epitopes within Gp5

1. Epitope A- immune dominant
2. Epitope B- conserved, glycosylated, neutralizing antibodies are produced late in infection

- Initial antibody response direct towards non neutralizing epitope before significant antibody titre formed against neutralizing epitope. Strategy of viral transmission to new host (Ostrowski *et al*., 2002) [18].

4.5 Foot and mouth disease virus (FMD)

Deceptive imprinting via fast mutation within immune-dominant epitopes & Persistence through neutralizing antibody escape variants (NEVs) (Almond D, *et al*., 2010) [1].

- VP1 G-H loop is most immune-dominant antigen of the virus, and hyper variation in this region results in the generation of NEVs (Brown F, *et al*. 1999) [3].
- RGD sequence motif found in central region of G-H loop is highly conserved among isolates (Wright *et al*., 2011)
- One approach to immune refocusing involves deleting or hiding decoy epitopes in vaccine constructs.
- Another method of immune refocusing in vaccine design involves genetically altering decoy epitopes.
- Capsid based DNA vaccines bearing full or partial replacement of G-H loop with glycine residues found to enhance cross reactivity of sera (Frimann *et al*., 2007).

4.6 Human immunodeficiency virus (HIV)

4.6.1 Immune evasion mechanisms

1. Extensive glycosylation of both glycoproteins subunits gp120 & gp41 and the evolution of Hyper-variable loops (V1, V2, V3) that vary under immunological pressure (Almond D *et al.,* 2010) [1].
2. The variable loops V1 & V2 partly mask binding site for the CD4 binding site & may attract or decoy the immune

response away from other epitopes less able to vary.

3. V3 loop of HIV gp 120 appear to contain at least one decoy epitope as portions of it are highly variable and immunogenic, while the central region needed for co-receptor binding is fairly well conserved.
4. Close proximity of the hyper-variable region to the crucial co-receptor binding sequence has made HIV vaccine development quite a challenging endeavor.

5. Conclusion

Epitope mapping and dampening is a crucial process in immunology and vaccine development that involves identifying the specific regions of an antigen that are recognized and bound by antibodies or immune cells. This mapping helps in understanding the immune response and designing targeted therapies or vaccines. Epitope mapping can reveal the precise amino acid sequences or structural motifs that are recognized by antibodies or immune cells. Thus identifying a set of key epitopes responsible for the immune response, which can be used to develop targeted therapies or vaccines. Epitope mapping can provide insights into the binding preferences and specificities of antibodies. Therefore determining the epitope repertoire recognized by a particular antibody or antibody class, which can aid in understanding the antibody's function and potential therapeutic applications. It can also contribute to the development and optimization of vaccines. The identification of conserved sequence and epitopes can help us designing the broad neutralizing antibody which can target many members of the particular family of the virus.

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