Vaccine 26 (2008) 6189–6199

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Deceptive imprinting and immune refocusing in vaccine design

Gregory J. Tobin[a,*], Jessie D. Trujillo[b], Ruth V. Bushnell[a], George Lin[a,c], A. Ray Chaudhuri[a], Jinxue Long[a], Jose Barrera[d,1], Lindomar Pena[d,2], Marvin J. Grubman[d], Peter L. Nara[a,e]

[a] Biological Mimetics, Inc., 124 Byte Drive Frederick, MD 21702, United States
[b] Utah Veterinary Diagnostic Laboratory, Utah State University, 950E 1400N, Logan, UT 84322, United States
[c] Department of Pathology, Brigham and Women's Hospital, Boston, MA 02115, United States
[d] United States Department of Agriculture, Agricultural Research Service, Plum Island Animal Disease Center, United States
[e] Department of Biomedical Sciences and Center for Advanced Host Defenses, Immunobiotics and Translational Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, United States

ARTICLE INFO

Article history:
Received 9 September 2008
Accepted 18 September 2008
Available online 11 October 2008

Keywords:
Immune refocusing
Deceptive imprinting
Vaccine design

ABSTRACT

A large number of the world's most widespread and problematic pathogens evade host immune responses by inducing strain-specific immunity to immunodominant epitopes with high mutation rates capable of altering antigenic profiles. The immune system appears to be decoyed into reacting to these immunodominant epitopes that offer little cross protection between serotypes or subtypes. For example, during HIV-1 infection, the immune system reacts strongly to the V1, V2, and/or V3 loops of the surface envelope glycoprotein but not to epitopes that afford broad protection against strain variants. Similarly, the host mounts strain-specific immunity to immunodominant epitopes of the influenza hemagglutinin (HA) protein. A large number of pathogens appear to exploit this weakness in the host immune system by focusing antigenic attention upon highly variable epitopes while avoiding surveillance toward more highly conserved receptor binding sites or other essential functional domains. Because the propensity of the immune system to react against immunodominant strain-specific epitopes appears to be genetically hard-wired, the phenomenon has been termed "deceptive imprinting." In this review, the authors describe observations related to deceptive imprinting in multiple systems and propose strategies for overcoming this phenomenon in the design of vaccines capable of inducing protection against highly variable pathogens.

1. Introduction

Classical technologies that rely on the production of inactivated or attenuated pathogens as well as subunit antigens for vaccine development depend entirely on the native antigenicity and immunogenicity of the pathogen and have led to the derivation of over 20 human vaccines: currently considered one of the greatest preventative medical achievements of the last century in service. The widespread use of these vaccines have brought many human and veterinary pathogens such as measles, mumps, rubella, poliovirus, brucellosis, hog cholera, bovine tuberculosis, and smallpox under increasing degrees of control or, in the case of smallpox, eradication. Vaccine programs continue to demonstrate that significant reductions in disease and subsequent gains in public health can be realized once an effective vaccine is developed. Moreover, economic analyses highlight the cost-effective expenditures to eliminate the financial burdens associated with preventable infections. However, traditional vaccine strategies have proven ineffective in inducing protective immunity against the remaining majority of human and animal pathogens. Despite great efforts expended, prophylaxis against many of the most serious global pathogens, such as human immunodeficiency virus-1 (HIV-1), malaria, tuberculosis, Foot-and-Mouth Disease Virus, and bacterial diarrheal pathogens remain out of grasp. Table 1 categorizes examples of pathogens based on our ability to produce effective vaccines.

Class I pathogens are those for which we have developed effective vaccines and Class II pathogens are those that have demonstrated resistance to classical vaccine strategies. Although the Class I and Class II categories are comprised of pathogens that appear to be very different from each other in terms of genetic makeup, phylogenic complexity, and disease outcome, distinct similarities exist within the two categories. For example, Class I pathogens generally

* Corresponding author at: Biological Mimetics, Inc., 124 Byte Drive, Frederick, MD 21702 United States. Tel.: +1 301 620 7565.
E-mail addresses: tobin@bmi-md.com (G.J. Tobin), nara@bmi-md.com (P.L. Nara).
[1] Current address: The McConnell Group, Inc., Plum Island Animal Disease Center, P.O. Box 848, Greenport, NY 11944, United States.
[2] Current address: Virginia-Maryland Regional College of Veterinary Medicine, College Park, MD 20742, United States.

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doi:10.1016/j.vaccine.2008.09.080

Table 1
Properties of Class I and Class II pathogens.

Class I	Class II
Pathogen infects narrow age range	Pathogen infects wider age range
Host exhibits spontaneous recovery	Pathogens frequently persist as latent infections
Host generates long lasting protective immunity	No or low long-lasting protective immunity
Priming with wild-type whole killed or attenuated	Priming with wild-type antigens offer little protection or strain-specific protection
Pathogen induces protection	
Pathogen is genetically stable with limited antigenic variation	Pathogen exhibits high mutation rate and tolerates high degree of variation in epitope regions
Immune responses are directed to multiple epitopes	Immune responses are limited to a smaller number of genetically variable and strain-specific epitopes and suggest early cross-reactive recall
Examples include: Measles, mumps, rubella, diphtheria, Canine distemper, rabies, poliovirus	Examples include: HIV-1, malaria, influenza, hepatitis C, FMDV, human rhinovirus

infect specific age groups of individuals, such as the very young, while Class II pathogens are associated with infection of a wide range of ages. After infection with Class I pathogens, hosts generally make long-lasting immune responses that protect them from future infections. In contrast, infection with Class II pathogens often sets up re-occurring, latent or chronic-active disease, and protection is narrowly limited to the infecting substrain or subtype of the pathogen. Perhaps most importantly, Class I pathogens exist with relatively limited variability while Class II pathogens typically are made up of multiple serotypes, strains, serovars, and subtypes. Since both Class I and Class II pathogens can have replication modes that enable high rates of mutation, it is likely that the structural requirements of successful Class I pathogens are more rigid and that Class II pathogen structures allow more fluid changes without deleterious effects on infection and replicative functions. For example, within the RNA virus Picornaviridae family two members, poliovirus (Class I) and rhinovirus (Class II), exhibit high replication rates and high levels of contagiousness yet poliovirus is genetically more stable while rhinovirus consists of upward of over 100 serotypes. In reviewing the experimental literature, it appears that immune responses against Class II pathogens are limited to a subset of immunodominant epitopes that are in regions that permit a large amount of genetic variability. Thus, the immune system makes an effective response against the infecting strain, but this response is not protective against infection by variants of the pathogen.

We hypothesized that elimination or masking of immunodominant epitopes may allow the immune system to recognize previously subdominant epitopes that induce protection against Class II pathogens [1–4]. Although it may seem at first counterintuitive to remove the most immunodominant epitopes that convey some neutralizing activity or protection, we recognized that the correlates of protection induced by most immunodominant epitopes are largely strain specific. The role of these epitopes in inducing protective immunity has likely been over-emphasized in the historical literature due to the use of homologous challenge strains in both cell-based and animal model experiments. Fig. 1 depicts immune refocusing technology as applied to masking or "dampening" immunodominant epitopes of the influenza hemagglutinin glycoprotein (HA) to engineer a more broadly protective vaccine candidate. The major epitopes of the HA trimer, as represented by small blue loops, induce type-specific antibodies, shown as red molecules. Mutation of the immunodominant epitopes by the addition of N-linked glycans or other substitutions results in an engineered antigen with immune dampened epitopes (middle panel). In the model, immunization with the dampened antigen induces more broadly protective responses. Analysis of the immune responses demonstrates that equivalent levels of antibodies are induced, but that they are now specific to previously subdominant epitopes. The immune refocusing technology can be used for the dual purposes of engineering more broadly protective vaccines and for smithing novel antigens to manufacture broadly reactive therapeutic antibodies.

In this review, we present experimental evidence that supports the theory of immune refocusing using the five examples of Class II pathogens: (1) human immunodeficiency virus type 1 (HIV-1), caprine arthritis encephalitis virus (CAEV), human rhinovirus (HRV), human influenza virus, and Foot-and-Mouth Disease virus (FMDV).

2. Human immunodeficiency virus

Despite the vast amount of time and resources poured into the development of an effective vaccine for HIV-1, none have been successful. Immune responses directed to the envelope glycoprotein (Env) are likely correlates of protection, yet classical vaccination with unmodified viral genes or proteins induces protection that is strictly limited to the strain represented by the vaccine [5–7]. Interestingly, it appears that the variable immunodominant epitopes have evolved to be adjacent to conserved functional sites and act as decoys to divert the immune system from raising responses to vital areas of the envelope. Thus, HIV-1 represents an unfortunate but perfect example of a Class II pathogen due to the two hallmarks of deceptive imprinting: (1) variability of antigenic sites and (2) the immunological hierarchy that results in the host focusing its attention on these variable sites without responding to conserved domains. HIV-1 replication results in rapid evolution of the virus, allowing it to escape immune surveillance [8]. The variable Env loops permit a high degree of variability that challenges the notion of protective prophylaxis. Perhaps most importantly, infection with HIV or immunization with Env proteins induce limited oligoclonal immune responses that target the immunodominant epitopes of the variable loops. Due to deceptive imprinting, the immune system does not react to conserved epitopes within structurally essential areas of the envelope, such as the receptor or co-receptor binding sites. During natural infection or immunization with classically designed antigens, broadly reactive neutralizing antibodies (Nab) are either not produced or are only found late and in low titer [9]. Consequently, during the natural variation of the epitopes, the virus evolves to escape immune pressure. The theory of deceptive imprinting helps explain why classical vaccine strategies have failed and may continue to fail to stimulate broadly protective immune responses [3,10]. Vaccination with Env derivatives may afford protection against the specific strain used to develop the antigen, but immunity against a variable swarm of viruses is not possible with this method. Thus, a major challenge will be to engineer antigens that elicit immune responses directed against conserved subdominant epitopes that elicit broadly protective immunity.

Studies with monoclonal antibodies suggest that humans are capable of mounting immune responses against such broadly protective epitopes. The 2G12 antibody derived from an HIV-positive patient is a particularly interesting example. The 2G12 binding site consists of an epitope determined by high mannose N-glycans on the outer domain of gp120 [11]. 2G12 binds both native and CD4-complexed Env and demonstrates broadly neutralizing activities against an expanded range of HIV strains. The IgG1 antibody b12,