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Review

Protein engineering strategies for the development of viral vaccines and immunotherapeutics

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ABSTRACT

Vaccines that elicit a protective broadly neutralizing antibody (bNAb) response and monoclonal antibody therapies are critical for the treatment and prevention of viral infections. However, isolation of protective neutralizing antibodies has been challenging for some viruses, notably those with high antigenic diversity or those that do not elicit a bNAb response in the course of natural infection. Here, we discuss recent work that employs protein engineering strategies to design immunogens that elicit bNAbs or engineer novel bNAbs. We highlight the use of rational, computational, and combinatorial strategies and assess the potential of these approaches for the development of new vaccines and immunotherapeutics.

1. Introduction

The introduction of viral vaccines during the 20th century has led to a significant decrease in viral disease burden worldwide [1]. Most viral vaccines are thought to work by inducing the production of antibodies that block infection or reduce viral load, thereby providing host protection or blunting infection such that cellular immunity can be effective [2,3]. Antibodies can participate in host defense in several ways, including opsonization, the coating of viruses to enhance uptake by phagocytic cells, or activation of the complement family of proteins that can directly destroy pathogens or enhance phagocytic uptake. Here, we will focus on neutralizing antibodies, which bind the virus and prevent infection. Neutralizing antibodies are protective against many viruses in both animals and humans [4–11]; therefore there has been much interest in their identification and characterization for potential use as immunotherapeutic agents, or to serve as templates for immunogen design. Neutralizing antibodies have historically been identified by immunization of animals with viral components, or from B-cell repertoires of human vaccinees or survivors [11–17]. In recent years, an increasing amount of structural information about neutralizing antibodies – and their mechanisms of activity – has shifted focus toward structure-based design of immunogens to elicit such antibodies and of the antibodies themselves [18–34].

Neutralizing antibodies are thought to abrogate viral infectivity by three major mechanisms (Fig. 1): (i) by blocking virus attachment to host cells; (ii) by inhibiting viral uncoating or conformational changes in viral envelope glycoproteins needed for cell entry; or (iii) by inducing the formation of non-infectious viral aggregates that cannot enter cells. In the case of enveloped viruses, those surrounded by a lipid bilayer, the primary neutralization targets are the virus envelope glycoproteins that are responsible for mediating membrane fusion between the viral and host cell membranes, a critical step for infection [35]. During the course of natural infection or vaccination, neutralizing antibodies against many viruses, such as polio, mumps, and measles, are elicited in both humans and animals. However, induction of effective neutralizing antibodies is rare or does not occur against some viruses, notably those with high antigenic diversity such as the human immunodeficiency virus-1 (HIV-1), hepatitis C virus, and influenza virus. Not surprisingly, this antigenic variation is reflected in the diverse sequences of the virus envelope glycoproteins among strains or clades, and thus antibodies that do not bind conserved epitopes have a narrow spectrum of activity.

Various strategies have been employed to develop vaccines that elicit neutralizing antibodies for these high diversity viruses. In vaccination trials, the use of adjuvants to enhance the quality of antibody response to vaccination [36], nucleic-acid based methods for the delivery of antigen [37–40], and the administration of more than one type of vaccine to boost immunogenicity [41–43] have been attempted. However, effective vaccines for these viruses remain elusive. A major hurdle appears to be that the immunodominant antibody responses are directed against the most variable parts of the envelope glycoproteins, and therefore most neutralizing antibodies are narrowly strain-specific. An

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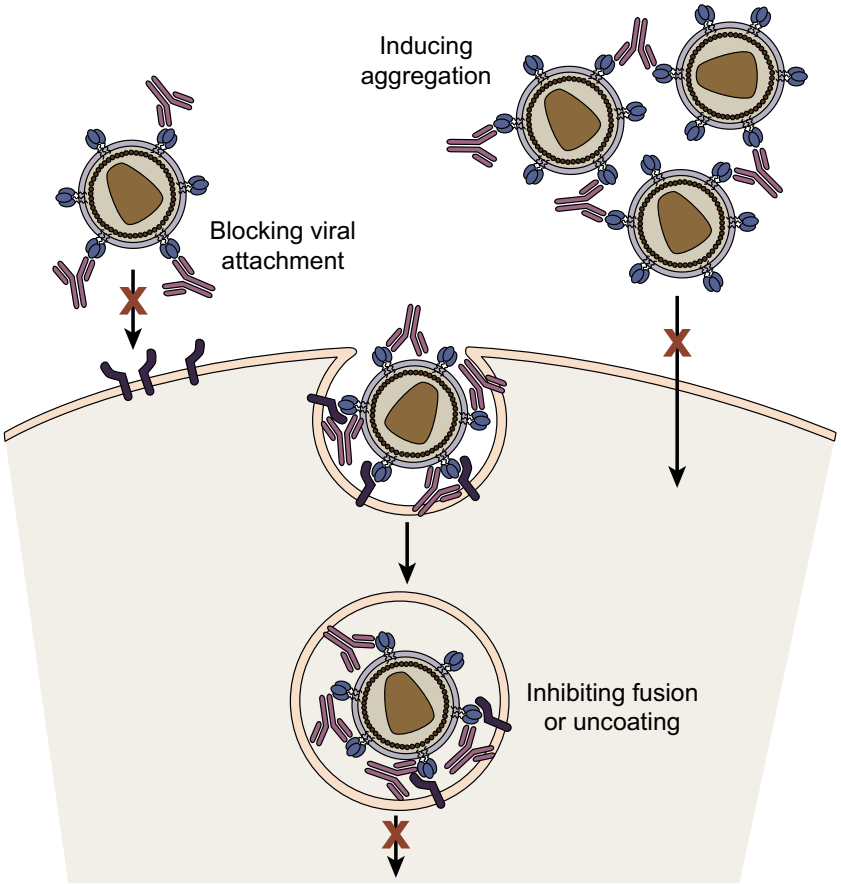

Fig. 1. Mechanisms by which neutralizing antibodies block viral infection. Neutralizing antibodies are thought to abrogate viral infectivity by blocking virus attachment to host cells, inhibiting viral uncoating, blocking conformational changes in viral envelope glycoproteins needed for membrane fusion or prematurely triggering the fusion machinery, or by inducing the formation of non-infectious viral aggregates that cannot enter cells.

effective vaccine should be able to elicit "broadly neutralizing" antibodies (bNAbs) that engage conserved, less variable domains and can therefore protect across a spectrum of genetic isolates. Likewise, immunotherapeutics for these viruses should be directed at conserved viral epitopes or infection pathways. In this review, we highlight recent work that utilizes novel protein engineering strategies for the development of effective vaccines and immunotherapeutics against highly variable viruses and viruses for which a bNAb response does not arise during the course of natural infection.

2. Viral antigen design to elicit broadly neutralizing antibodies

One promising strategy for the generation of bNAbs by vaccination is "reverse engineering," where structural information gleaned from the binding of bNAbs raised in the course of natural infection is used to guide immunogen design [3,44]. In theory, translation of this antibody binding information into an immunogen designed to display specific, critical epitopes should allow production of antibodies with similar broad neutralization capacity in vivo, provided that the immunological evolution pathway of the bNAb can be induced by vaccination. Thoughtful modification of the immunogen to reflect the specific, three-dimensional antibody-binding site is required (Fig. 2). Since the goal of reverse engineering is to develop a peptide or protein scaffold that mimics the natural epitope, most strategies have utilized rational, combinatorial, or computational methods. Here we discuss several recent examples in which these methods were used to develop and evaluate immunogens.

2.1. Conformational mimicry of linear epitopes from HIV-1 gp41 and gp120

HIV-1, a lentivirus, enters host cells by fusing its lipid bilayer with the host cell plasma membrane. This fusion is facilitated by the viral envelope glycoprotein, Env, which consists of a surface subunit, gp120, and a transmembrane subunit, gp41 [35]. Infection is initiated by gp120 binding to CD4 and a co-receptor on host cells, triggering large-scale conformational changes in gp41 that eventually lead to membrane fusion. Antibodies directed against Env have the potential to be neutralizing, but the generation of bNAbs has proven to be extremely challenging. This is likely because of the hypervariability encoded in the Env gene, the extensive glycosylation of the surface of the Env protein, and structural heterogeneity associated with gp120 that is critical for its function as the triggering molecule for membrane fusion. During the course of chronic infection by HIV-1, ~10% of patients develop bNAbs, suggesting that a vaccine approach to prevent HIV-1 infection is possible [12,45,46]. A number of HIV-1 bNAbs target linear epitopes in the V3 region of gp120 or the membrane-proximal external region (MPER) of gp41. Structures of these bNAbs bound to peptide epitopes have demonstrated that these segments contain well-defined secondary structure when bound to the bNAbs. It is therefore hypothesized that immunogens designed to elicit antibodies that bind these segments in such conformations would be critical for a successful vaccination strategy.

Immunogens based on the V3 loop have been designed and have so far met with some limited success. Antibody 447-52D was isolated via hybridoma methods from a subtype B