SARS-CoV-2 Vaccine Safety

Subjects: Immunology

Contributor: Rashika El Ridi

Recovery from SARS-CoV-2 infection requires a solid first line of innate immunity defense, namely release of interferon-alpha and beta, which interfere with viral replication. These critical defense factors are produced upon encounter of the RNA of the virus that succeeded in host cell invasion with the cytoplasmic innate immunity receptors, notably retinoic acid-inducible gene I (RIG-1). A second line of defense would be the host generation of neutralizing and opsonic antibodies capable of preventing virus entry and virus spread, respectively. We need to avoid or dampen host generation of powerful cytotoxic T cells, which lead to destruction of the host heart, lung, kidney, and small intestine cells presenting the viral peptides on their surface membrane, and potential organ failure and destruction. We herein wish to demonstrate that the vaccine should be based uniquely on SARS-CoV-2 spike glycoprotein subunit 1 polypeptide, because that subunit is released upon virus invasion, and does not penetrate host critical cells in the heart, lung, liver, kidney and small intestine. Differently from all other viral peptides, subunit 1 peptides are not readily processed for presentation on the surface of the host structural cells, rendering them targets for the destructive action of cytotoxic T lymphocytes and natural killer cells.

Keywords: SARS-CoV-2 ; Vaccine ; Safety ; Spike glycoprotein subunit 1 ; Cytotoxic T cells

1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA genome (29,903 nucleotides) has been sequenced, revealing around 90% nucleotide similarity to a group of SARS-like coronaviruses (genus Betacoronavirus, subgenus Sarbecovirus) that had previously been found in bats, and not known to readily infect humans. The order of genes (5′ to 3′) is as follows: replicase *ORF1ab*, *spike* (*S*), *envelope* (*E*), *membrane* (*M*) and *nucleocapsid* (*N*) [1][2]. Upon host invasion, the virus infects neutrophils, macrophages, and dendritic cells via phagocytosis or endocytosis. Viral peptides antigen presentation in association with HLA class I and II leads to activation of the humoral and cellular arms of the adaptive immune response, including antibodies and cytotoxic T lymphocytes [3]. For invasion of human structural cells, the virus interacts with the angiotensin-converting enzyme 2 (ACE-2) present on the cell surface membrane in the lungs, arteries, heart, kidney, and intestine. Viral key amino acid residues necessary for binding to ACE-2, allowing virus entry into the cell, were found in the SARS-CoV-2 spike glycoprotein [4][5]. The spike protein is a transmembrane (TM, with cytoplasmic tail, CT, inside the virion), homotrimeric, fully glycosylated protein, which mediates a) receptor recognition; and b) fusion with the host cell membrane.

Primary structure of SARS-CoV-2 spike protein [4] reveals an ectodomain, a trans viral membrane protein (TM), and an intra virion cytoplasmic tail (CT). At the amino end of the ectodomain, the signal sequence (SS) is followed by the amino terminal domain (NTD), then the receptor binding domain (RBD) of 223 amino acids (Chain F, 2019-nCoV Receptor Binding Domain; 223 aa protein; Accession: 6M17_F GI: 1820435683), containing viral key amino acid residues recognized by the extracellular peptidase domain of ACE-2, thus allowing virus entry into the cell [1][2][4][5].

During viral infection of host structural cells, the spike protein is cleaved at the S1/S2 completely exposed protease cleavage site into the RBD-containing S1 subunit at the amino end and the S2 subunit at the carboxyl end [6]. The S2 subunit is responsible for virus/cell membrane fusion, and contains a protease cleavage site, S2', which is completely buried in the prefusion spike glycoprotein, a hydrophobic fusion peptide, and two heptad (seven amino acids) repeats (HR1 and HR2), located adjacent to hydrophobic, potentially fusion-related regions in the amino acid sequences of coronaviruses [5][7]. Upon fusion with the ACE-2 receptor, the receptor-binding subunit 1 (S1) is cleaved and shed. Despite that the virus interacts with ACE-2 via a single S1 subunit, disassociation of one S1-ACE2 from the S trimer could cause sequential disassociation of the S1 subunits from the spike trimer. Upon the start of virus infection of structural cells, the three S1 subunits from the spike dissociate from the virion and are shed outside of the cell [5][6][7].

2. INVASION OF STRUCTURAL CELLS

The fusion subunit (S2) undergoes large-scale conformational rearrangements to expose the hydrophobic fusion peptide, and bring the viral and cellular membranes close for fusion. The coronavirus spike protein binding to the cell membrane is further activated by specific cell enzymes. Genomic analyses of the new coronavirus have revealed that its spike protein differs from those of close relatives. A major difference lies in SARS-CoV-2 spike protein has on it a second protease cleavage site (S2', FP) that is activated by a host-cell enzyme called furin, found in the lung, liver, kidney, and small intestines [7][8]. Cleavage of the S2' site by host cell proteases is required for successful infection by SARS-CoV. The site (PRRARS|V) susceptible to furin starts at the spike protein amino acid 680.

Upon SARS-CoV-2 invasion of human cells, the viral and endosomal membranes fuse, releasing the viral RNA into the cytoplasm [9]. The viral RNA likely interacts with the cytoplasmic innate immunity receptor, RIG-1, leading to release of the viricidal interferon alpha and beta, and inflammatory cytokines, namely interleukin-1 (IL-1), responsible for fever [10] [11]. If the human host is able to generate antibodies that hinder and prevent virus entry into structural cells, he/or she will resist the infection, and present light or no symptoms [12].

Upon massive virus entry into the target lung, kidney, and small intestine cells, proteolytic processing of the viral proteins released intracellularly will take place, and innumerable viral peptides in association with HLA class I molecules will be presented on the surface membrane of the virus-infected cell, poised to stimulate pre-cytotoxic CD8+ lymphocytes. Viral proteins released by dying cells will be captured by macrophages and dendritic cells, and processed peptides presented on the surface membrane of the antigen-presenting cells (APC) ready to activate the helper CD4+ lymphocytes, which help in the proliferation and differentiation of killer CD8+ and antibody producing B cells [3][12]. The cytotoxic T cells will target the virally infected structural cells, are able to target them all, and lead to excessive damage of key organs such as the lung and kidney. It is best that such immune responses are never generated. Spike glycoprotein subunit 1, which is shed outside of the ACE-2-bearing cells, is managed by APC, leading to induction of specific anti-virus subunit 1 antibodies and cytotoxic T lymphocytes. Yet, the killer cells will not approach the host critical organs, which would remain spared from destructive immune reactions. Accordingly, a safe vaccine should be based on viral sequences that are released and shed before target cell entry: the spike glycoprotein subunit 1. During intracellular massive viral replication, all necessary viral proteins, including spike glycoprotein subunit 1 are synthesized. Yet, newly synthesized proteins are directed to generation of new virus particles rather than to proteolysis in the cell proteasome and processing for peptide presentation on the cell surface [13]. Accordingly, spike glycoprotein subunit 1-derived peptides are likely not or poorly presented on the surface membrane of the cells of the lungs, heart, liver, kidney, and small intestines.

SPIKE GLYCOPROTEIN SUBUNIT 1 VACCINE

Spike glycoprotein subunit 1 or RBD **protein** immunogen will be captured by APC leading to generation of specific antibodies, and T helper and cytotoxic antibodies. Upon viral challenge or infection, antibodies will neutralize the virus preventing entry into structural cells or lead to its opsonization, and fire up the memory adaptive response. Specific cytotoxic T lymphocytes or natural killer cell-activating antibodies will never approach virus-infected structural cells, which do not present the spike glycoprotein subunit 1 on their surface. The safety of such vaccine is uncompromising [14][15].

If, however, the spike glycoprotein subunit 1 or RBD immunogen is in the form of RNA-based vaccine [16][17], the RNA will penetrate every cell, and the peptides of the subunit 1 will be durably expressed on multiple cell types where the RNA has penetrated [18]. The APC-mediated adaptive immune responses will generate cytotoxic T cells and antibodies. The cytotoxic T lymphocytes may interact and kill all cells expressing the subunit 1 viral peptides. Antibodies may bind to viral peptides on the surface of the host cells and engage natural killer cells to devastating antibody-dependent cell-mediated cytotoxicity. The volunteers receiving that RNA vaccine are at risk during the vaccination regimen and even less safe after virus challenge or infection. The danger is multiplied several folds upon use of the whole spike glycoprotein or the whole virus in a protein, RNA, or DNA construct [9][19][20].

3. DISCUSSION

It is documented 1) that the SARS-CoV-2 spike glycoprotein subunit 1 is the only viral polypeptide, which does not penetrate host structural cells, and thus is not presented on their surface membrane in association with HLA class I molecules. 2) Virus-infected structural cells present on their surface numerous viral peptides, rendering them targets for cytotoxic T cells and for antibody-dependent natural killer cell-mediated cytotoxicity. 3) Any SARS-CoV-2 vaccine that elicits the generation of killer T cells or antibodies targeting the viral peptides expressed on the surface of host structural cells is not safe for volunteers even during the vaccination regimen and is even more risky thereafter. Accordingly, a safe SARS-CoV-2 safe and efficacious vaccine should 1) principally induces protective antibodies, which interfere with the

processes of viral invasion of critical host cells in the heart, lung, liver, kidney, and small intestines, and promote phagocytosis and elimination of virus particles. 2) The vaccine should specifically be tailored as to not induce powerful cytotoxic T cells targeting virus-infected structural cells, resulting into severe damage of the organs, especially the lungs, and often leading to substantial morbidity and mortality.

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