Blood Spotlight

Vaccination against critical SARS-CoV-2 epitopes may require targeted conjugate vaccination

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Abstract (80 w)

It is evident that SARS-CoV-2 succeeds at evading immunity, resulting in long recovery times and associated deaths. Antibodies against the viral spike, a homotrimer of the S glycoprotein, can neutralize the virus and prevent entry into host cells. Apparently, the virus evades immunity by proteolytic and conformational changes in S. Targeted conjugate vaccination approaches to break immune tolerance, e.g. the recently developed immuno-Boost (iBoost) technology, can be instrumental in the development of a prophylactic strategy against SARS-CoV-2.

Introduction

Over the last two decades three coronaviruses crossed the species barriers to cause deadly pneumonia in humans: the severe acute respiratory syndrome (SARS) coronavirus SARS-CoV in 2002, the Middle-East respiratory syndrome MERS-CoV in 2012 and the current SARS-CoV-2 (2019-nCoV), which started in 2019 and grew out to a pandemic [1]. The latter virus has exceeded both earlier viruses in its rate of human-to-human transmission [2]. There are currently neither efficient therapeutics for SARS-CoV-2 disease (COVID-19), nor an immediate outlook to a prophylactic. From epidemiological information on the current pandemic it can be observed that building immunity against SARS-CoV-2 is rather slow or incomplete, due to the structure of the virus' spike protein S and the machinery applied to enter host cells. This accounts for the long recovery time after infection and related deaths, which at the time of this writing accumulated to >100.000 worldwide. Obviously, the virus has evolved mechanisms to evade immune recognition. Strategies that can overcome this evasion have to be developed.

SARS-CoV-2 immune escape mechanisms

Coronaviruses have four structural proteins, being the spike- (S), envelope- (E), membrane- (M) and nucleocapsid (N) proteins. A homotrimer of S proteins forms the viral spike that is protruding from the viral surface and which is responsible for attachment and entry into host cells (Figure 1). Although viral pathogens should easily be recognized by the host immune system, the above-mentioned coronaviruses have evolved mechanisms to escape host immunity. It was found that upon infection of host cells by SARS-CoV-2 virus, viral proteins were not sufficiently presented on major histocompatibility complex (MHC) class I molecules (HLA-A, -B and -C) [3]. Relatedly, some patient groups infected with SARS-CoV appeared to lack appropriate HLA molecules to present the viral peptides [4]. For MERS-CoV infected cells it was demonstrated that both MHC-I and MHC-II molecules were downregulated [5]. Failure of proper presentation of foreign pathogen peptides on MHC complexes impairs the priming phase of an immune response and thereby proper T cell and antibody responses, required for acquired cellular and humoral immune responses to combat the infectious agent. Recent evidence shows that specific T cell responses against SARS-CoV-2 are important for the recognition and killing of virus-infected cells in the lungs of patients [6]. Innate type I interferon (IFNα and IFNβ) immune responses are essential to suppress viral replication and dissemination in the early infection phase [6–8]. The SARS-CoV nucleocapsid (N) protein was observed to inhibit type I interferon production, thereby modulating the host's innate anti-

viral immune response [9,10]. However, whether SARS-CoV-2 applies a similar host immune modification is currently unknown, but seems likely. Next to evading innate and T cell responses, coronaviruses also apply mechanisms to escape host antibody recognition, through conformational masking and glycan shielding of their antigens [11–14]. In viral infections antibody responses provide a protective role by limiting infection at a later stage of the disease and preventing future re-infection [6,15,16]. For SARS-CoV it was found that recovering patients showed higher and more sustainable levels of neutralizing antibodies than those that died from the disease [17]. Similar results were expected for SARS-CoV-2 but, counterintuitively, it was observed that in certain cases high antibody titers were associated with a worse clinical classification, although their neutralizing effect was not studied [18]. As it has been observed before, non-neutralizing antibodies may aggravate disease symptoms by enhancing viral infectivity [16] and/or by promoting IL-8/MCP-1 production and inflammatory macrophage accumulation [19,20]. As such, it is of the utmost importance to promote protective anti-SARS-CoV-2 antibody responses.

Humoral immune responses against SARS-CoV-2

Although different coronaviruses can recognize different cellular receptors, SARS-CoV and SARS-CoV-2 both use angiotensin converting enzyme-2 (ACE-2) as the cellular receptor on the host cell [1]. Engagement of the S protein with ACE-2 is mediated by a 190 amino acid sequence in S, referred to as the receptor binding domain (RBD). Therefore, the S protein – and specifically the RBD sequence – is widely recognized as the main drug target for entry inhibitors, antibodies and vaccines [21–23]. Antibodies directed at the S protein, especially the RBD, can neutralize the virus and prevent entry into host cells. It is becoming evident that coronaviruses can hide critical parts of their RBD by conformational masking, thereby preventing protective antibody responses of the host's immunity [11–14]. Unlike the coronaviruses that induce harmless common colds, all highly virulent coronaviruses (SARS-CoV, SARS-CoV-2 and MERS-CoV) have spikes in which one of the S protomers in the trimer flipped up its RBD in an 'open' conformation. Only SARS-CoV-2 S trimers with one open RBD domain are able to bind ACE-2. However, the other two remain 'closed' and therefore hidden to interfering agents. The RBD domain almost fully lacks glycosylation, as there is only one N-linked glycosylation site at N_{343}, which is in a proximal non-exposed area of RBD [11]. This suggests that receptor engagement depends dominantly on protein-protein interactions. Glycan shielding is therefore unlikely to explain hiding of epitopes. It is rather the limited exposure and bioavailability of critical ACE-2-binding epitopes in the RBD – by

by keeping them in a closed position – that determines the antigenicity of the RBD. Interestingly, the S1 subunit of S, which is at the trimer apex – the exposed part of the virus spike that contains the RBD – is less conserved than the S2 subunit domain, which is more proximal to the virus membrane and involved in the fusion with the host cell membrane [11,24–30]. This entails that coronaviruses can differ in host receptor recognition, this way varying their zoonotic transmission capacity as well as their virulence. However, sequence variability also increases antigenicity and a more stringent selection pressure by the host's immune system. It seems that SARS-CoV-2 has dealt with this by structural mechanisms of changing the RBD into a closed position and the proteolytic events upon binding the ACE-2 that are necessary for the subsequent membrane fusion events [11]. Taken together, the dynamic nature of SARS-CoV-2 spike protein, resulting from cleavage and conformational plasticity, may explain why protective humoral immune responses are weak or delayed, resulting in the currently observed high morbidity and mortality rates.

Nevertheless, antibody responses have been reported after infection. Walls et al. observed that polyclonal antibodies against SARS-CoV S protein prevented SARS-CoV-2 host cell entry [11] and that these antibodies targeted the highly conserved S2 subunit (including the membrane fusion peptide region) [11,21,31]. Furthermore, it was demonstrated that other SARS-CoV neutralizing antibodies recognize the RBD and thus prevent ACE-2 receptor engagement [13,32–36], but that these antibodies are not cross-reactive with SARS-CoV-2 [21,31]. However, a conserved cryptic epitope distal from the RBD is targeted by the neutralizing antibody CR3022, which shows cross-reactivity between SARS-CoV and SARS-CoV-2, albeit at slightly different affinity, and without interference with ACE-2 binding [37]. Moreover, synergistic neutralizing effects of combinations of antibodies have been reported by multiple studies [36–38]. These observations strongly suggest that strategies for targeted vaccination, rapidly inducing polyclonal antibody responses forced towards conserved and/or exposed sequences in or near the RBD, are needed for efficient combatting of SARS-CoV-2 and its provoked illness.

Targeted conjugate vaccination against SARS-CoV-2

We recently developed the immuno-Boost (iBoost) vaccination technology against cancer, which is based on inducing polyclonal antibody responses to self-antigens in the tumor [39,40]. Vaccination against self-antigens is challenging, as our immune system is tolerant for these antigens. To break immune tolerance and induce efficient antibody responses to self-antigens, a conjugate vaccine strategy was developed (Figure 2). By conjugation of a self-antigen to a

foreign protein, the immune system will recognize the self-antigen as foreign as well, and will start to induce self-reactive B lymphocytes to proliferate and produce antibodies, and eventually give rise to memory B cells [41]. With the development of iBoost, we showed that the induction anti-self antibody titers can be strongly augmented by conjugation to *de novo* designed "foreign" peptides, consisting of sequences that enhance solubility and immune recognition [39].

Vaccination against viruses involves recognition of foreign antigens and does, normally, not require a conjugate vaccine technology. Due to the effectiveness of vaccination against non-immunogenic proteins in cancer, iBoost technology is highly suitable for targeted vaccination approaches against non- or low-immunogenic sequences in the main target protein of SARS-CoV-2. Targeted vaccination using iBoost can be approached by producing a conjugate of the designed "foreign" peptide fused to the integral RBD sequence of S. This will evoke a polyclonal antibody response, either resulting in neutralizing infection by interference with interaction with ACE-2, steric hindrance of cellular entry and/or by opsonization and complement activation. Moreover, antibody induction to multiple epitopes may overcome protection from immunity. A second strategy is to make a vaccine against selected sequences of 12-20 amino acids – separated by hinges – that are surface exposed in the RBD of the S protein. This strategy allows the induction of antibody responses against sequences that are still exposed in closed orientation of the RBD, but lack immunogenicity when present in the intact virus (Figure 1) [38]. A strategy such as iBoost can resolve this. Alternatively, one could follow a strategy to select preserved sequences, also present in other coronaviruses. As there is a 74% sequence identity between the SARS-CoV-2 RBD and that of SARS-CoV, which is still 15% with MERS-CoV, such approach can even be helpful in future outbreaks with new coronaviruses, a situation that can be expected to reoccur and appears to be cyclic [42,43]. Of course this strategy also allows the design of multi-targeted vaccines, also containing sequences of e.g. the S2 subunits, other SARS-CoV-2 proteins, or even sequences of other viruses [42,43]. When these approaches are successful and protective antibody titers can be rapidly induced, it is conceivable that these or similar strategies can even be applied in a therapeutic setting, shortening COVID-19 recovery periods.

Conclusions and future directions

There is currently a worldwide race in the development of a vaccine against SARS-CoV-2 that can limit spread of the virus. Different vaccine strategies are proposed but most of these involve presentation of natural and folded proteins, which presents the risk that critical

domains, necessary for host cell entry and infection, are cryptic or hidden in tertiary or quaternary structure. Strategies for targeted induction of antibody responses are therefore promising and may present solutions for protective immunity against SARS-CoV-2. iBoost provides a means of generating targeted antibody responses, forced towards hidden or cryptic domains in foreign and self-antigens. Furthermore, production of vaccines based on this technology is straightforward and cost-effective. This makes iBoost highly attractive as a general strategy for induction of prophylactic antibody responses against many (infectious) diseases. In addition, it is hypothesized that iBoost vaccination can even be applied as a therapeutic strategy against COVID-19.

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Figure legends

Figure 1. Targeting of the RBD in the spike protein S of SARS-CoV-2

SARS-CoV-2 virus particle including the different structural proteins: the spike- (S), envelope- (E), membrane- (M) and nucleocapsid (N) proteins. A ribbon diagram of the side view of an S protein trimer (PDB ID: 6VSB) in the 'open' conformation, with one receptor binding domain (RBD, light blue) up, is depicted (middle panel). In the right panel the RBD is further enlarged and the exposed protein sequences of the 'open' conformation of the RBD are shown. Arrows indicate the position of the targetable amino acid residues, which are numbered. Furthermore, the spike protein (amino acid 1-1237) monomer indicating its different domains is depicted linearly. NTD, N-terminal domain; SS, signal sequence; S2', S2' protease cleavage site; FP, fusion peptide; HR1, heptad repeat 1; CH, central helix; CD, connector domain; HR2, heptad repeat 2; TM, transmembrane domain; C, CT, cytoplasmic tail. The signal peptide (SS) and the HR2, TM and CT domains are not depicted in the ribbon diagram.

Figure 2. iBoost vaccination technology

The conjugate vaccine consisting of a fusion protein – in which the low-immunogenic target antigen (or self-antigen) is fused to a foreign antigen – is injected together with a potent adjuvant and 1) is internalized by antigen presenting cells (APC), e.g. dendritic cells or macrophages. The APC will present foreign-peptides and target-peptides via MHC class II (MHC-II + antigen, Ag). 2) Presented foreign peptides are recognized by the T cell receptor (TCR) on T-helper cells, which will become activated. Presented target-peptides are not (or limitedly) recognized, since the target is hidden for the immune system (in the case of self-antigens, autoreactive T cells are deleted in the thymus during embryonic development). 3) Target-reactive B cells recognize the fusion protein's target-part via their B cell receptor (BCR). These target-reactive B cells then also present foreign peptides via MHC-II. In this way the previously activated foreign peptide specific T cells provide activation help to the target-reactive B cells. The target-reactive B cells undergo clonal expansion and produce anti-target antibodies (anti-target Ab).

Figure 1

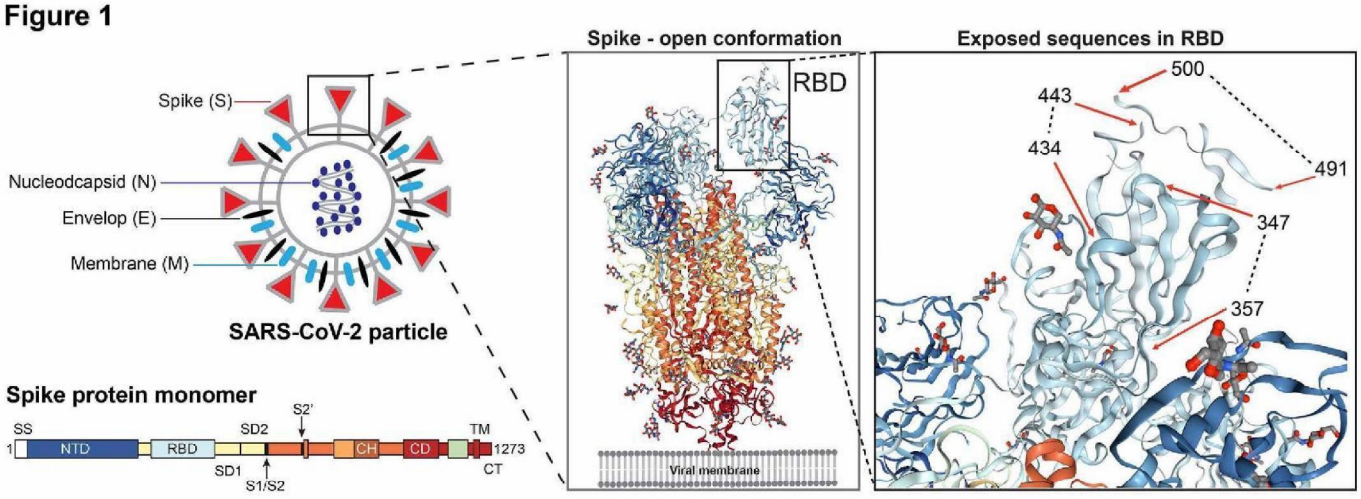
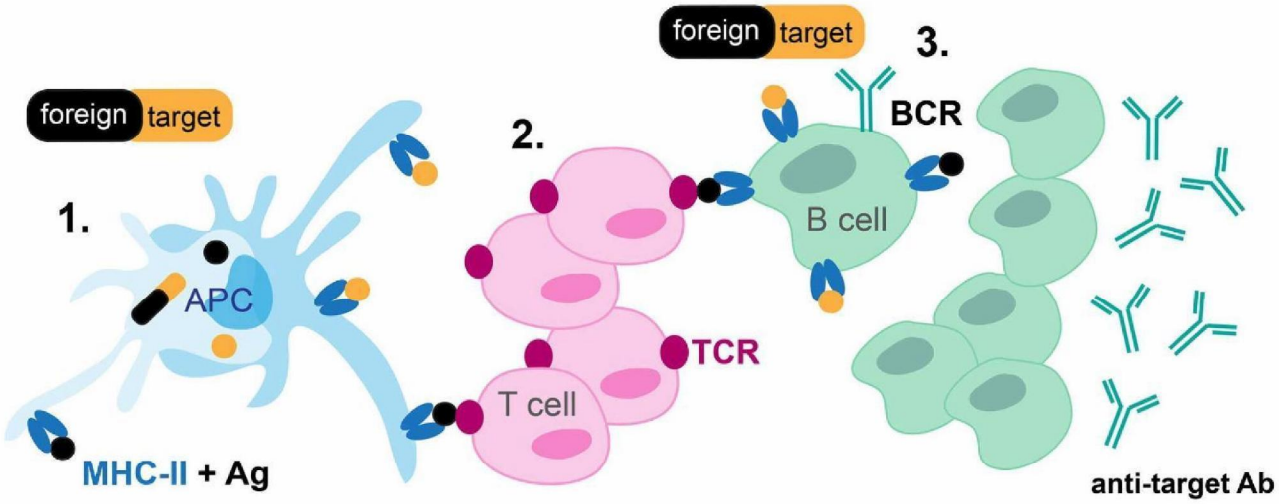

Spike (S)
Nucleodcapsid (N)
Envelop (E)
Membrane (M)
SARS-CoV-2 particle
Spike protein monomer
SS
NTD
RBD
SD2
S2'
SD1
S1/S2
CH
CD
TM
1
1273
CT
Spike - open conformation
RBD
Viral membrane
Exposed sequences in RBD
500
443
434
491
347
357

Figure 2


foreign target
1.
APC
MHC-II + Ag
2.
T cell
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foreign target
3.
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anti-target Ab