CD8+ T Cells in Response to mRNA Vaccination

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Coronavirus disease 2019 (COVID-19) is a respiratory disease caused by the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has led to millions of deaths globally. The rollout of SARS-CoV-2 vaccines has effectively reduced the morbidity and mortality of COVID-19, with messenger RNA (mRNA)-based vaccines being widely administrated. While neutralizing antibodies are crucial, CD8+ T cells induced by the vaccine may also play a significant role in early and long-term protection.

CD8+ T cells SARS-CoV-2 mRNA vaccination morbidity

1. Introduction

The involvement of CD8+ T cells in virologic control has been demonstrated by numerous studies. CD8+ T cells, also known as cytotoxic T lymphocytes (CTL), play a significant role in adaptive immune responses due to their unique cell-killing mechanisms, which induce sterilizing immunity. Over the last decades, there have been many studies of the CD8+ T cell response to vaccination in peripheral blood. However, in the context of SARS-CoV-2, the protective role of CD8+ T cells in response to vaccination has raised some controversy. Kent et al. addressed this controversy and noted that the role of vaccine-elicited memory CD8+ T cells in directly protecting against SARS-CoV-2 infection remains unclear [1]. A study from Koutsakos et al. demonstrated that limited Spike-specific CD8+ T cell responses were detectable in vaccinees in the first 7–10 days following breakthrough infection of SARS-CoV-2 [2], questioning the early protective role of CD8+ T cells in viral control. Moreover, the level of reactive Spike-specific CD8+ T cells and the response of those cells elicited by the original SARS-CoV-2 vaccines against other variants of concern remain variable, with a decreased reactivity against B.1.351 and CAL20.C SARS-CoV-2 variants [3]. To harness the protective abilities of CD8+ T cells in developing effective immunity against all variants of SARS-CoV-2 and address current contradictions, it is crucial to understand their role in the lymph nodes (LNs), where these cells may first encounter the vaccine/Spike protein.

2. The Protective Effect of CD8+ T Cells in Response to SARS-CoV-2 Messenger RNA Vaccine in Peripheral Blood

Although the role of vaccines is to induce immunity, some vaccines have suboptimal prophylactic effects, making it crucial to understand their mechanisms to induce immunity in order to optimize efficacy and prolonged protection. In the context of SARS-CoV-2, the rollout of vaccines and the regimen of prime-boost vaccination has substantially

reduced mortality and morbidity [4]. As reported by Polack et al., in the phase 2 clinical trial, the protective effect elicited by vaccination was induced as early as 12 days following first dose of BNT162b2 messenger RNA (mRNA) vaccine in a large study cohort, as measured by a lower rate of occurrence of COVID-19 cases observed in the vaccine group compared to the placebo group [5]. Such protective effects may be mounted by the robust response of $CD8^+$ T cells, as Spike-specific $CD8^+$ T cells were detected as early as day 7 post prime vaccination [6]. However, as outlined by Painter et al., the response of $CD8^+$ T cells induced by vaccine is more gradual and the magnitude is more variable than the $CD4^+$ T cell response [7]. The inconsistency between studies may be attributed to the different assays used to identify Spike-specific $CD8^+$ T cells, or differences in Human Leukocyte Antigen (HLA)-restricted epitopes used between study cohorts [8]. Unlike the T cell responses, NAbs elicited by vaccination were only detected 21 days after the first dose of vaccination [9]. Notably, vigorous expansions of Spike-specific $CD8^+$ T cells were elicited by the vaccine with an 11-fold increase after the first dose compared to baseline, with further expansion after the second dose [10]. Aligned with the previous studies, the study from Oberhardt et al. utilized tetramer staining and successfully observed that Spike-specific $CD8^+$ T cells were detected in the periphery at days 6–8 after initial or prime vaccination and peaked at days 9–12 following initial or the prime vaccination [11]. Moreover, those cells differentiated into effector $CD8^+$ T cells with increased expression of T-BET, TOX, and CD39, which vigorously expanded after boost vaccination [11]. Aside from the immunophenotyping markers that have been referred to, the Spike-specific $CD8^+$ T cells also displayed activation markers, such as CD69, CD154, CD137, and CD38, as well as the proliferating marker Ki-67 [6]. In addition to the massive expansion, those cells exhibited an effector function by producing IFN-*γ* and TNF, and their effector capacity was induced early after prime vaccine and remained stable after subsequent boost vaccinations [6][11]. The significance of Spike-specific $CD8^+$ T cells in the containment of SARS-CoV-2 is highlighted by compelling evidence in other vaccine studies that are not mRNA-based. Pardieck et al. used a transgenic mouse model of SARS-CoV-2 infection to demonstrate that $CD8^+$ T cells responding, in the absence of NAbs, to a synthetic long peptide (SLP)-based vaccine, containing a single $CD8^+$ T cell epitope, provided protection against lethal SARS-CoV-2 infection in mice [12]. However, full protection was only provided after the third vaccination [12]. Liu et al. observed higher viral loads in the respiratory tract of adenovirus vector-based vaccine Ad26.COV2.S vaccinated macaques that underwent $CD8^+$ T cell depletion prior to challenge with the SARS-CoV-2 virus [13]. However, the protective effect of $CD8^+$ T cells elicited by mRNA vaccine that compensates for lack of humoral immunity remains to be explored. Furthermore, the protective effect of immunity induced by current mRNA vaccines against SARS-CoV-2 in humans has been observed to be long lasting and persistent. As demonstrated by Mateus et al., mRNA vaccine induced NAbs and Spike-specific memory $CD8^+$ T cells remained detectable in vaccinees at least 6 months post second vaccination [14]. Similarly, Ozbay Kurt et al. observed that Spike-specific $CD8^+$ T cells could be detected 4–6 months after the second vaccination [15]. However, a gradual decrease in the frequency of these cells was noted 12 weeks after the booster vaccination [15]. In the same study, Spike-specific $CD8^+$ T cells showed an ability to be stimulated to expand again after third dose followed by a contraction observed a few months later [15].

3. $CD8^+$ T Cells and SARS-CoV-2 Epitopes

Over the past few years, a number of studies have been undertaken to demonstrate that Spike-specific $CD8^+$ T cells can ameliorate recovery from SARS-CoV-2 infection. Identification of dominant HLA types and SARS-CoV-2 epitopes is the fundamental task before conducting such research. An epitope is an immunogenic domain of the antigen, which binds to the MHC class I and class II molecules (HLA on human cells) and triggers an immune response by $CD8^+$ and $CD4^+$ T cells, respectively [16]. Notably, the $CD8^+$ T cell specific epitopes are shorter than $CD4^+$ T cell specific epitopes, composed of only 8 to 11 amino acid residues [17]. Hence, the activation and effector function of $CD8^+$ T cells can be easily compromised by a single mutation on the epitope [18].

To date, scientists have identified over 1000 SARS-CoV-2 $CD8^+$ T cell epitopes with the utilization of peptide stimulation and peptide-tetramer staining [17]. There are ten HLA class I alleles dominant for SARS-CoV-2 $CD8^+$ T cell epitopes, including HLA-A*01:01, -A*02:01, -A*03:01, -A*11:01, -A*24:02, -B*07:02, -B*08:01, -B*15:01, -B*40:01, and -C*07:02 [19]. In the study by Nelde et al., 81% of pre-pandemic donors had T cell responses to cross-reactive SARS-CoV-2 epitope compositions, which was shown to be similar to common cold coronavirus [19]. Interestingly, the HLA-B*07:02-restricted nucleoprotein $(N)_{105-113}$ epitope ($B7/N_{105}$) is more dominantly targeted by $CD8^+$ T cells [20]. Nguyen et al. identified SARS-CoV-2 specific $CD8^+$ T cells in pre-pandemic populations and patients infected with SARS-CoV-2 by using peptide-HLA tetramers. $CD8^+$ T cells specific for $B7/N_{105}$ were detected in higher frequencies in pre-pandemic unexposed individuals and COVID-19 patients than $CD8^+$ T cells specific for three subdominant epitopes [21]. Notably, $B7/N_{105}$ tetramer-specific $CD8^+$ T cells displayed a naïve phenotype in pre-pandemic populations [21]. However, whether individuals with the HLA-B*07:02 allele are less susceptible to severe disease is not clear from this research. It is worth considering that this research was conducted with a small study cohort of 61 subjects within the local area. In fact, another research study analyzing 4361 subjects indicated that there was no correlation between HLA types and SARS-CoV-2 susceptibility [22]. What is even more intriguing is that specific alleles could induce a substantially greater T cell response compared to others. Gao et al. pointed out that although both HLA-A*02:01- and HLA-B*40:01-restricted Spike epitopes induced a more than 35-fold rise in the frequency of Spike-specific $CD8^+$ T cells, the magnitude of the HLA-B*40:01-specific T cell response was inferior to that of the HLA-A*02:01-specific response [6].

4. Vaccine-Elicited $CD8^+$ Memory T Cells

The key to long-term protection from vaccination is the establishment of immunological memory. Immunological memory reflects the development of memory subsets within $CD8^+$ T cells that are specific to particular antigens, allowing for faster recognition and stronger recall immune responses upon re-exposure to the antigens [23][24]. Apart from NAbs, many vaccines also aim to generate subsets of memory $CD4^+$ and $CD8^+$ T cells that persist to provide durable protection.

There are four main types of memory $CD8^+$ T cells identified in previous studies, including central memory T cells (T_{CM}; $CD45RA^-$ $CCR7^+$ $CD27^+$), effector memory T cells (T_{EM}; $CD45RA^-$ $CCR7^-$ $CD27^-$), stem cell memory T cells (T_{SCM}; $CD45RA^+$ $CCR7^+$ $CD27^+$ $CD95^+$), and terminally differentiated effector memory $CD45RA^+$ T cells (T_{EMRA}; $CD45RA^+$ $CCR7^-$ $CD27^-$) [10][11][23][25][26][27][28][29]. Vaccine-induced memory $CD8^+$ T cells circulating in the periphery can be rapidly induced as soon as one month after prime-boost vaccination. In the study by

Papadopoulou et al., they used specificity and cytotoxicity assays to show that the vaccine-induced memory $CD8^+$ T cells can be rapidly activated and exert a protective function following the challenge of the virus even 8 months after first vaccination [30]. A Spike-specific memory precursor pool of $CD8^+$ T cells expressing memory markers CD127 and T cell factor 1 (TCF1) was found in circulation at day 6–8 after the first vaccine dose [11]. As reported by Kondo et al., a population of antigen-specific $CD8^+$ T cells expressing early activation markers HLA-DR and CD38 was detected 21 days after boost, which is an indicator for the generation of memory $CD8^+$ T cell subsets [31]. Notably, those memory $CD8^+$ T cells were composed of a considerable proportion of T_{EM} [31]. This result aligns with the studies of Oberhardt et al. and Gao et al., where they also identified a high frequency of $CD8^+$ T_{EM} cells in vaccinated subjects at the early timepoints after prime-boost vaccination [6][11]. Rapid and potent immune response recall is the main characteristic of T_{EM}. These T_{EM} populations progressively contracted with an expansion of T_{CM} cells during the later phase of the immune response to vaccination [6]. These memory subsets form a stable memory cell pool that can promptly recognize and eliminate infected cells when virus is re-encountered. In addition to the identification of a high proportion of T_{EM} cells post prime-boost vaccination, a considerable proportion of T_{EMRA} were identified in the periphery of vaccinees after 2 months post boost vaccination [31]. T_{EMRA} are the most terminally differentiated effector memory $CD8^+$ T cells that re-express CD45RA and have potency of effector function [11]. However, the SARS-CoV-2 specific T_{EMRA} have been described as non-typical T_{EMRA}. In the study by Neidleman et al., the phenotype of SARS-CoV-2 specific T_{EMRA} was compared to T_{EMRA} specific for Cytomegalovirus (CMV) [28]. The majority of SARS-CoV-2 specific $CD8^+$ T_{EMRA} cells expressed high levels of CD27 and CD28 compared to the CMV-specific T_{EMRA} cells, indicating that these cells are less terminally differentiated [28]. Interestingly, as the phenotypic characterization of SARS-CoV-2, specific $CD8^+$ T_{EMRA} cells identified in this research showed similarity to previously reported Epstein-Barr virus (EBV)-specific $CD8^+$ T_{EMRA} cells [32]. Neidleman et al. proposed that the SARS-CoV-2 specific $CD8^+$ T_{EMRA} cells are cytotoxic and long-lived [28], but further research will be needed to validate this hypothesis. The T_{SCM} subset has been suggested to be associated with long-term T cell immunity, and a SARS-CoV-2-specific T_{SCM} cell subset persistent across a 6-month study has been identified by Guerrera et al. [10]. More importantly, Reinscheid et al. showed that the T_{SCM} pool was expanded after the first vaccine dose and remained stable with subsequent vaccinations [29]. Interestingly, a high proportion of SARS-CoV-2 specific T_{SCM} was also detected in SARS-CoV-2 infected subjects a few months after symptom onset [33]. This research also emphasized the ability of T_{SCM} cells to self-renew and differentiate into diverse memory subsets, including T_{EM}, T_{EMRA}, and T_{CM} [33]. Additionally, T_{SCM} cells have been previously studied in other vaccine contexts. In the study by Fuertes Marraco et al., they reported the induction of a population of yellow fever-specific $CD8^+$ T cells with a naïve-like phenotype ($CD45RA^+$ $CCR7^+$) after yellow fever vaccination [34]. With further investigation, they found that this population expressed CD58, CD95, and CXCR3, which was phenotypically distinct from “bona fide” naïve cells [34]. Remarkably, this T_{SCM} population was sustained for more than 25 years [34].

It is interesting to note that the distribution of Spike-specific memory $CD8^+$ T cell subsets elicited by vaccination and natural infection were distinct even at the same time point post vaccination/infection. In the study by Oberhardt et al., T_{CM} and early differentiated (T_{ED}; $CD45RA^+$ $CCR7^+$ $CD27^+$ $CD11a^+$ $CXCR3^+$) $CD8^+$ T cell subsets specific for SARS-CoV-2 spike peptide-loaded HLA class I tetramers (HLA-A*01-restricted Spike $(S)_{865,}$ (HLA-A*01/S_{865})

and HLA-A*02/S_{269}) and transitional memory (T_{TM}; $CD45RA^+CCR7^-$ $CD27^+$) $CD8^+$ T cells specific for HLA-A*03/S_{378} were more dominant in convalescent individuals after 80 days of natural infection, while T_{EM} were more prevalent in vaccinees at the same timepoint [11]. The differences in memory $CD8^+$ T cell pools induced by vaccination and natural infection may be attributed to variations in the routes of antigen exposure between infection and inoculation and the induction of only Spike proteins as antigens in current mRNA vaccines. More importantly, SARS-CoV-2-specific T cell responses elicited by natural infection were shown to be more durable than vaccine-induced responses. Two other studies reported that SARS-CoV-2 specific $CD4^+$ and $CD8^+$ T cell responses remained detectable more than 10 months post infection [33][35]. The difference in memory subset composition may contribute to the more durable T cell responses in convalescent individuals. However, further research into vaccine-elicited memory $CD8^+$ T subsets is required to clarify the correlation between duration of protection and memory T cell subset composition, which could inform optimization of vaccination.

As noted earlier, there has been some debate among researchers regarding the extent to which vaccine-elicited $CD8^+$ T cells protect against SARS-CoV-2 infection. This controversy was partly due to the lack of sufficient data linking the level of circulating memory $CD8^+$ T cell subsets to the degree of protection conferred against infection in vaccinees [1]. According to Kent et al., this data gap can be partly attributed to the absence of a standardized assay for measuring T cell responses [1]. Utilizing tetramers or multimers has facilitated the successful identification of antigen-specific $CD8^+$ T cells, whilst its limitation cannot be neglected. Since this method heavily relies on the binding affinity between the peptide-tetramer complex and TCR, it sometimes fails to entirely detect the full range of functional T cell clonotypes, resulting in an underestimation of the antigen-specific $CD8^+$ T cell population [36]. Therefore, the protocol for using tetramers or multimers should be carefully optimized before the commencement of research. Alternatively, activation induced marker (AIM) assays that utilize a wider pool of overlapping peptides are an effective and sensitive tool for the identification of antigen-specific $CD8^+$ T cells [37][38].

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