Targeting and exploiting cytomegalovirus for vaccine development

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Chapter 6

Requirements for effective T cell-inducing vaccines against chronic viral infections

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ABSTRACT

For many years the focus of prophylactic vaccines was to elicit neutralizing antibodies but it has become increasingly evident that T cell-mediated immunity plays a central role in controlling persistent viral infections such as HIV, CMV, and HCV. Currently, a variety of promising prophylactic vaccines, capable of inducing substantial vaccine-specific T cell responses, are investigated in preclinical and clinical studies. There is compelling evidence that protection by T cells is related to the magnitude, breadth and quality of the T cell response as well as the type of the activated T cell subsets, and their characteristic homing properties, cytokine polyfunctionality, and metabolic fitness. In this review, we evaluate the main factors that determine the qualitative and quantitative properties of $CD4^+$ and $CD8^+$ T cell responses in the context of chronic viral disease and prophylactic vaccine development. Elucidating the mechanisms through which T cells mediate protection against chronic viral pathogens will facilitate the development of more potent, durable and safe prophylactic T-cell based vaccines.

INTRODUCTION

Our body is persistently exposed to a variety of pathogens present in the environment. The immune system is fortified with physical barriers and with diverse immune cell populations that play an integral role in protection from disease. Long-term immune protection is mediated by antigen-specific lymphocytes and antibodies that are formed upon pathogen entry. Memory B and T cells are numerically and functionally superior to their naïve-antigen precursors cells that are present prior to infection, and upon encounter with the same pathogen memory immune cells are able to induce a more rapid and powerful recall response (i.e., immunological memory) [1,2].

The majority of prophylactic vaccines against viral infections have focused on the induction of neutralizing antibodies. Indeed, potent antibody inducing vaccines against virally-induced diseases are available. Nevertheless, failures are demarcated in the case of providing long term efficacy and protection against certain complex chronic viruses. A series of studies in mice, non-human primates and humans provide evidence that effective prophylactic vaccines against chronic (low-level and high-level) replicating viruses (i.e. herpesviruses, HIV, HCV) should engage strong cellular T cell immunity [3,4,5]. The development of T cell-eliciting prophylactic vaccines has gained increasing attention despite that such vaccines are not always able to provide sterilizing immunity. The latter may relate to the fact that the immune mechanisms related to protection against chronic infections have not been clearly defined. There is still a lack of knowledge to be able to tailor vaccines to induce long-lasting $CD4^+$ and/or $CD8^+$ T cell responses of sufficient magnitude and phenotype that effectively contributes to pathogen clearance. Oftentimes, the memory cellular immune response provoked by vaccines is not sustained and frequently fades in time [6,7]. Elucidating the mechanisms through which antigen-specific T cell populations mediate long-term protection against viruses at body surfaces and (lymphoid) tissues remains an important goal, and will facilitate the development of more effective and safe prophylactic T cell-eliciting vaccines. Here we review determinants and mechanistic factors of T cell responses implicated in vaccine efficacy against chronic viral infections, and discuss how this knowledge can be utilised to maximize the possibility of creating effective vaccine platforms for persistent viral infections.

THE COMPLEXITY OF THE ANTIGEN-SPECIFIC T CELL RESPONSE DURING INFECTION

T cells acquire their activation signals as the interaction with the DCs becomes stable and reaches a duration of 12 h [8,9]. For proper activation of naïve $CD4^+$ and $CD8^+$ T cells, cognate antigenic signals through the TCR (signal 1), costimulatory signals (signal 2) and signals provided by inflammatory cytokines (signal 3) is required [10,11]. Expression

of particular chemokine receptors such as CCL19 and CCL21 by fibroblastic reticular cells (FRCs) enhance immune responses by stimulating the interactions between T cells and DCs during antigen presentation [12,13,14,15]. Additionally, the secretion of the CCL3 and CCL4 chemokines by activated DCs and $CD4^+$ T cells enhances $CD8^+$ T cell accumulation and help attract rare antigen-specific T cells [16,17]. The activation of T cells results in alteration of the expression of various molecules including integrins, selectins and chemokine receptors, resulting in modulating key intracellular signalling events that promote proliferation, differentiation and migration of T cells to inflamed tissues [18,19,20].

After resolution of the infection the majority (90-95%) of the effector T cells are eliminated by the immune system due to programmed cell death (PCD) and only a small diverse pool of memory cells remains [21,22]. Traditionally, memory T cells were classified into two major categories based on their proliferation capacity, phenotypic features and migration potential [23]. Specifically, effector-memory T (T_{EM}) cells are identified based on combined expression and/or lack of certain cell surface markers including $KLRG1^{hi}/CD44^{hi}/CD127^{lo}/CD62L^{lo}$. These cells have limited proliferation capacity upon TCR triggering, yet rapidly produce effector molecules and cytokines such as IFN-γ and TNF [24,25]. Central-memory T (T_{CM}) cells are distinguished by the expression of $KLRG1^{lo}/CD44^{hi}/CD127^{hi}/CD62L^{hi}$ surface markers, exhibit a superior proliferation capacity and produce cytokines that are directly associated with better secondary expansion such as interleukin (IL)-2. Secondary lymphoid organs are the main homing tissues of T_{CM} cells whereas T_{EM} cells are more dominantly present in tissues [26,27,28,29]. Both T_{CM} and T_{EM} cells can circulate, whereas a recently discovered new category of T cells present in tissues lacks migration capacities [30]. These cells, named tissue-resident memory T (T_{RM}) cells, permanently reside in peripheral tissues after an infection is cleared and are present in most organs and tissues. T_{RM} cells can be defined based on the expression of $CD62L^{lo}/CD44^{hi}/CD69^{hi}/CD103^{hi}$ surface markers, yet the composition of these markers depends on the tissue-specific cues [31,32,33]. Furthermore, a small subset of memory T cells exhibit advanced stem-cell like qualities and proliferation capacities compared to the conventional T cells [34]. These memory T cells, which were designated stem cell memory T cells (T_{SCM} cells), display a phenotype highly similar to naïve T cells (T_N cells), $KLRG1^{lo}/CD44^{lo}/CD127^{hi}/CD62L^{hi}/CD69^{lo}$, but they co-express stem cell antigen (Sca-1), the β chain of the IL-2 and IL-15 receptor (CD122, IL-2Rβ), and the chemokine receptor CXCR3 [34,35,36,37,38,39].

Notably, T cell immunobiology is fundamentally similar between human and mice, and the concepts of T_{CM}, T_{EM}, T_{RM}, and T_{SCM} cells are matching. Evidently, both live attenuated and synthetic or subunit vaccines are able to elicit T_{CM}, T_{EM}, and T_{RM} cells [30,33]. With respect to live attenuated vaccines, the vaccine-induced T cells subsets are in general similar to those subsets that develop upon infection [40]. However, the T cell subsets that develop upon immunization with synthetic or subunit vaccines is highly dependent on the route of administration and the adjuvant [41]. Whether sufficient

amounts of T_{SCM} can be generated with live attenuated or synthetic vaccines needs further exploration.

THE MAGNITUDE OF THE T CELL RESPONSE IS IMPORTANT FOR OPTIMAL PROTECTION

The magnitude of the viral-specific T cell responses is highly dictated by the infectious dose and route of infection [42]. Higher infectious dosages lead generally to higher peak values of effector T cells and correspondingly larger amounts of memory T cells in the circulation are found. However, if the immune system is overwhelmed and virus replication is uncontrolled this leads to immunopathology and subsequently this leads to exhaustion of T cells and poor memory formation [43].

Given the frequently observed correlation between the magnitude of T cell response and establishment of immunity during infections, in vaccination settings simply determining the magnitude of the vaccine-elicited T cell response may already serve as a predictor of efficacy. A number of studies have shown a direct association of the vaccine-elicited T cell response size and the ability for virus control [5,44,45,46]. Several parameters directly impact the magnitude of the vaccine-induced T cell response. Clearly, in case of live (attenuated) viruses the size of the initial dose of the inoculum correlates to the magnitude until a threshold is reached [47]. To reach the same level as compared to virulent virus, the inoculum sizes are, not surprisingly, higher for replication-deficient or single-cycle viral vectors. In case of synthetic vaccines, however, the saturation threshold may not be reached because of lack of sufficient inflammatory signals. However, recent discoveries in adjuvant development and synthetic (nano) particles provide promising results [48,49,50]. Besides the initial inoculum dosage, booster vaccine regimens impact evidently the magnitude of the T cell response, and are likely essential for the majority of vaccines including live vaccines [51].

MEMORY INFLATION AND THE MAGNITUDE OF RESPONSES TO RECOMBINANT VACCINES

An alternative mechanism which leads to an increased magnitude of memory T cells (especially $CD8^+$ T cells) is observed for certain specific responses following infection by CMVs - described as memory "inflation" [52,53]. Here, antigen-specific T cell responses to a subset of peptides show an unusual dynamic, whereby they expand gradually over time and are maintained at high frequencies as T_{EM} populations - as opposed to the classical expansion and contraction described above. These inflationary responses show maintained effector functions, tissue homing and can provide protection against challenge. Interestingly in the case of mouse cytomegalovirus (MCMV) there are two classes of response, a subset of inflationary responses and some which show classical

contraction and T_{CM} phenotype [52,54]. Memory inflation has also been observed for CMV-specific antibodies, which levels gradually incline over time [55]. Although the rules that determine which kind of memory have not been fully defined, it is clear that for inflation to occur viral antigen must persist long term and since CMV is reactivating from latency this condition is clearly fulfilled. Inflation appears to be restricted by antigen presentation, since peptides which are dependent on the interferon-inducible molecule LMP7 to form the immunoproteasome are not apparently presented long term, although such responses may be primed [56]. Modifying the context of the peptide can convert a classical response to an inflationary one [57].

Recombinant CMVs may provide important vectors for vaccines, although they are highly complex viruses, containing multiple immune evasion genes. In rhesus macaque experiments, the T cell responses induced against a recombinant CMV expressing SIV antigens include, in addition to $CD8^+$ T cell inflationary responses, responses mediated by class II-restricted $CD8^+$ T cells and also HLA-E restricted cells [58,59]. These unconventional responses likely arise because of the restrictions placed on normal antigen presentation by the attenuated CMV vectors used. More work is needed to identify which of these populations is critical for protection, and whether this additional protection – which can be very robust – is mediated via magnitude, function, breadth or targeting of particular peptides not normally presented.

Memory inflation is not restricted to CMV-induced responses. Similar phenomena have been seen with other viruses, including vaccine vectors. The most relevant of these are responses induced by adenoviral vectors. In mouse models, adenovirus-based vectors can lead to induction of inflationary responses which closely resemble those induced by CMV [60,61]. In this vaccine platform it is possible to generate inflationary responses against otherwise non-inflationary epitopes by removing the requirements for processing and presenting it in the form of a "minigene" [62]. Human adenoviral vectors are in use in a range of settings, including HCV, malaria and Ebola vaccines[4,63,64]. Although these do not show numerical inflation, the responses are sustained over time and phenotypically and functionally resemble those seen in mice and also those induced by CMVs [61].

THE BREADTH OF THE INDUCED IMMUNE RESPONSE IMPACTS ON PROTECTION

An increased breadth of the vaccine-induced T cell response has been found beneficial against many chronic viral pathogens [5,65,66,67,68]. Development of T cells with multiple antigen-specificities correlated with advanced capacity for virus control or even complete eradication during primary infection with HCV and superior protection upon reinfection [69]. Similarly, the breadth of Gag-specific responses was linked with low viremia as shown by analysing the $CD8^+$ T cell responses of untreated HIV-infected subjects [70].

Successful induction of potent and broad T cell responses has been reported with DNA plasmid vaccines [71,72] and adenovirus serotype 26 vector-based vaccines [73]. The latter approach incorporated a combination of subdominant and dominant epitopes of rhesus macaques SIV, known as a HIV equivalent in monkeys, in a prime-boost vaccination schedules. In parallel with these findings, synthetic long peptide (SLP) T cell based vaccines, which induce memory $CD8^+$ T cells, exhibited increased protection against mouse cytomegalovirus (MCMV), when vaccination was performed with combinations of several distinct SLPs. The efficacy of the SLP vaccines to protect against MCMV was mainly driven by the breadth of the antigen-specific T cell response rather than the magnitude of the individual SLP vaccine-induced T cell responses [5]. These findings indicate that cytotoxic $CD8^+$ T cells with a broad repertoire of specificities are more capable for effective killing of virus infected cells than T cells of a single specificity. Possible explanations are that multiple encounters with T cells of diverse specificity results directly in enhanced killing of virus-infected cells or limits immune escape mechanisms. Moreover, an increase in epitope recognition may also contribute to protection against infection with heterologous viruses via cross-reactive responses [74]. Although the underlying mechanisms are still unclear the importance of the immune repertoire diversity should be taken into account while designing prophylactic T cell-based vaccines. As a consequence selection of the correct antigens that will steer the immune response at the correct direction is a very critical step of the vaccine development process. In this respect, it also of importance to mention that competition of antigens is apparent [5], warning that antigen selection is not simply the more the better. Overall, epitope-specific T cell repertoires elicited upon vaccination might serve as an evidence of vaccine efficacy, which will apply to many infections and not be limited to chronic viruses. Furthermore, not all antigen-specific T cell populations have the same efficacy. For example, T cell populations specific for CMV antigens that provoke inflationary responses show superior protective capacity [5]. Thus antigens provoking the most effective antigen-specific T cell populations should be selected to include in designing vaccine vectors or synthetic vaccines.

While both magnitude and breadth of the T cell response is of importance there is no direct association between protection and the frequency of the T cells in the circulation [75]. In mouse models it is becoming increasingly clear that, depending on the route of infection, T cells present in the mucosal or in the tissues (Tem and/or Trm) control the infection, and sufficient numbers are required [33]. Note, however, that besides the quantity and breadth also the quality of the T cell response is of crucial importance.

CYTOKINE POLYFUNCTIONALITY OF T CELLS IS AN IMPORTANT PARAMETER FOR VACCINE EFFICACY

Cytokine production is an important effector mechanism of T cell mediated immunity. Upon most viral and bacterial infections protective immunity consists of $CD4^+$ and $CD8^+$ T cells with a Th1 cytokine profile that is characterized by (co-)production of IFN-γ, TNF and IL-2 [76]. IFN-γ and TNF are pleiotropic cytokines with direct anti-viral properties [77,78,79]. Their receptors are broadly expressed, and signal via distinct pathways, which may explain why reciprocal production of IFN-γ and TNF leads to synergistic actions [80]. The predominant assessment method of vaccine-induced responses is the frequency of IFN-γ producing T cells. However, there are many examples showing that the magnitude of the IFN-γ secreting T cell response is not a sufficient immune correlate of protection. Single positive IFN-γ producing T cells can comprise a relatively large fraction of the total cytokine-producing $CD4^+$ and $CD8^+$ T cell population after immunization. Such T cells have a limited capacity to be sustained as memory T cells and are at the final stage of T cell differentiation [81]. Vaccines that elicit a high proportion of single, IFN-γ producing T cells would not be likely protective. On the other hand, studies characterizing vaccine elicited T cell responses against HIV, CMV and HBV revealed a strong correlation between the protection level of the vaccine regiments and their capacity to induce high frequencies of polyfunctional T cells (e.g. coproducing IFN-γ, TNF and IL-2 [4,82,83,84,85]. Similar results have been described in the course of infection with hepatitis C virus, CMV, influenza or M. tuberculosis [4,86,87,88,89]. Importantly, some of these studies showed that measuring the magnitude of IFN-γ producing $CD4^+$ and $CD8^+$ T cells alone was not sufficient to predict protection, and provided evidence that measuring the quality of the $CD4^+$ and $CD8^+$ T cell response, *vis-à-vis* polyfunctional T cells, is required.

IL-2 signals through a trimeric receptor comprised of CD25 (IL-2Rα), CD122 (IL-2Rβ) and the γ_c [90]. CD25 is not constitutively expressed but instead is transiently upregulated upon activation following exposure to certain inflammatory cytokines such as IL-12 [91]. Evidence examining he role of $CD4^+$ and $CD8^+$ T cells in HIV infected showed increased levels of T cells expressing IL-2 and IFN-γ in long-term non-progressors, or those on anti-retroviral treatment, but increased levels of T cells producing IFN-γ only in individuals with high viral loads (processors)[92]. Although IL-2 has no direct anti-viral function, it promotes proliferation and secondary expansion of antigen-specific T cells [93,94,95,96,97,98]. The ability of T cells to secrete IL-2 also relates to superior survival properties of these cells [81,99]. Additionally, IL-2 increases expression of the effector proteins perforin, granzyme B and IFN-γ that are all important for mediating cytolytic function [100,101]. IL-2 signals may also enhance NK cell activity that could contribute to the early control of infection following challenge [81,102,103,104,105].

In summary, the efficient vaccine protection medicated by $CD4^+$ and $CD8^+$ T cells is moderated by multiple mechanisms. First, $CD4^+$ and $CD8^+$ T cell have the highest

secretion rate of IFN-γ per-cell. Second, T cells that secrete both IFN-γ and TNF have enhanced effector activity compared with T cells that secrete IFN-γ alone. And third, autocrine IL-2 production promotes the secondary expansion of memory T cells, and is linked to other beneficial properties. Therefore, IL-2, TNF and IFN-γ comprise a simple set of cytokines that can be used to define the quality of the vaccine-elicited response against specific infections that require T cells for protection. As will be discussed hereafter, one way to improve the polyfunctionality of vaccine-induced T cells is by targeting of T cell costimulation It remains nevertheless necessary to better understand how the cytokine polyfunctionality is regulated during the programming of $CD4^+$ and $CD8^+$ T cell responses. Further dissecting these issues might provide fundamental insights into how T cell responses are controlled and may reveal potential strategies for superior vaccine-mounted T cell responses.

IMPROVING VACCINATION BY TARGETING T CELL METABOLISM?

The transition of naïve T cells to an active effector cell and to the formation of memory cells involves dynamic and coordinated metabolic modifications [106]. This reprogramming of the cellular metabolism is not a consequence of activation but is linked to the differentiation and activation processes, and reflect the fuel and substrates necessary to support the differentiation stages of a T cell [107,108]. Both naïve T cells and memory T cells rely primarily on oxidative phosphorylation (OXPHOS) and fatty acid oxidation (FAO) for fuel. This reflects the low level yet persistent need for energy as such cells are long-lived. Effector T cells on the other hand have extraordinarily high energetic and synthesis demands. These cells have enhanced glycolysis and employ the mitochondrial tricarboxylic acid (TCA) cycle to support their demand for *de novo* proteins, lipids and nucleic acids synthesis. It is becoming increasingly clear that metabolic reprogramming plays a critical role in T cell activation, differentiation and function. The distinct metabolic demands of different T cell subsets make them exquisitely sensitive to pharmacologic inhibitors of metabolism [109]. The different metabolic requirements of T cell subsets provide us with a promising therapeutic opportunity to selectively tailor (vaccine-induced) immune responses. Thus, targeting T cell metabolism affords the opportunity to additionally regulate vaccine-induced responses.

Upon T cell activation, there is an immediate uptake of amino acids such as glutamine and leucine that is critical for proper metabolic reprogramming. This is accompanied with the upregulation of amino acid transporters involved in glutamine (SLC1A5) and leucine (SLC7A5/SLC3A2 heterodimer) [110,111]. It is essential that these processes operate well to avoid suppression of the differentiation of T_H1 effector T cells while maintaining Treg differentiation. Whether this can be improved pharmacologically *in vivo* remains however to be further examined.

CD28-mediated costimulation leads to PI3K-dependent upregulation of surface GLUT1 to facilitate enhanced glucose influx [112]. This upregulation of GLUT1 is critical for T cell function, as genetic deletion of GLUT1 markedly inhibits effector T cells [113]. Concomitant with increased expression of glucose transporters is the upregulation of key glycolytic enzymes [114]. This metabolic reprogramming occurs simultaneously with T cell activation and is facilitated by mTOR [115]. mTOR activation promotes glycolysis, fatty acid synthesis and mitochondrial biogenesis. As such, targets upstream and downstream of the mTOR signaling pathway are potential therapeutic targets. Rapamycin, although known as an 'immunosuppressive' drug due to its ability to slow down T cell proliferation, promote robust responses to vaccination by enhancing $CD8^+$ T cell memory formation [116]. Correspondingly, deletion of the mTORC1 inhibitory protein TSC2 leads to enhanced mTORC1 activity and increased effector function [117]. Targeting of TSC2 or other molecules in the mTOR pathway might accordingly enhance immunity.

Targeting of glycolysis to inhibit immune responses in the setting of autoimmune disease and transplantation rejection is evolving, and this strategy is also used to enhance anti-tumor immunity by promoting long-lived memory cells ex vivo [118]. Whether this can be used in vaccination strategies remains to be examined. Although most studies have focused on the critical role of glycolysis in promoting effector T cell generation and function, it has become clear that mitochondrial-directed metabolism also plays an important role. Memory T cells rely for their energy upon OXPHOS and FAO. Because these metabolic pathways are dependent on mitochondria, the abundance and the organization of the mitochondria are instrumental for development of fit memory cells [119]. Alterations in the mitochondrial biogenesis can influence the differentiation of T cells, thereby providing opportunity to augment T-cell mediated immunity [120,121]. The transcription factor PGC1α promotes mitochondrial biogenesis and function [122]. Hence, pharmacologically or genetically enhancing PGC1α represents a potential strategy for improving vaccine-induced T cell responses. In *ex vivo* systems, it has already been shown that enforced overexpression of PGC1α, leads to improved metabolic fitness and effector cytokine function of $CD8^+$ T cells [123]. Again, whether *in vivo* targeting is possible remains to be examined, and in this respect a major challenge may be the specificity of metabolic inhibitors/enhancers as they may affect all cells of the body. However, inhibitors of glycolysis may preferentially affect effector T cells given their enhanced glycolytic need. The future will tell if indeed metabolic targeting is possible to enhance vaccines. Nevertheless, the metabolic profiles of (vaccine-induced) T cells are surely of interest and correlate to vaccine-mediated immunity [124].

COSTIMULATION EMPOWERS T CELL ELICITING VACCINES

Costimulatory signals transduced via the CD28 family members CD28 and ICOS, and via the tumor necrosis factor receptor (TNFR) family members CD27, 4-1BB, and OX40 play dominant roles in orchestrating the required signal 2 [125]. While CD28 and CD27 are constitutively expressed on naïve T cells ICOS, 4-1BB and OX40 are upregulated upon T cell activation [125,126]. The ligands for these costimulatory receptors are highly expressed on APCs upon activation, yet expression is also found on T cells, suggesting that these molecules may also mediate communication between T cells [127,128]. Synergy between these costimulatory molecules is expected [125,129], and is confirmed in experimental models [130].

There is extensive literature addressing the influence of the TNF/TNFR family interactions during a virus specific immune response. For instance, CD28 signals are required for sufficient T cell priming during the primary phase of an infection [131,132,133,134,135], while OX40 and 4-1BB gain importance during the late effector and memory stage of antigen-specific T cells either by providing pro-survival signals or by enhancing the quality of the memory T cells [136,137,138,139,140]. Thus, although an optimal immune response is the result of many receptor-ligand interactions, costimulatory signals dominate differentially during the diverse phases of the immune response (e.g. early *versus* late) to ensure optimal expansion and contraction of primary $CD8^+$ T cells and the generation of memory $CD8^+$ T cells.

Agonistic Abs against costimulatory receptors have shown efficacy in various preventive and therapeutic preclinical vaccination settings. Enforced engagement of costimulatory molecules results in improved T cell activation, expansion, survival and establishment of long-term memory [139,141,142,143,144,145,146], and has thus the potential to serve as effective immunomodulatory components of prophylactic vaccines against chronic viruses [143,147,148]. Indeed, this has already been observed for DNA and adenovirus based vector vaccines in which enforced expression of 4-1BBL, OX40L and CD70 leads to increased T cell expansion, enhanced CTL activity and antibody response [149,150]. Strikingly, agonistic antibodies to OX40 combined with synthetic peptide vaccines prompt robust effector and memory $CD4^+$ and $CD8^+$ antiviral T cell responses, improve T cell cytokine polyfunctionality and prophylactic vaccine efficacy against lytic MCMV infection [145]. Chronic viral infections are characterized by accumulation of functionally impaired antigen-specific $CD8^+$ T cells. Studies have shown that activation via 4-1BBL alone or in combination with CD80 can enhance the generation of primary $CD8^+$ T cell responses and induce expansion of the antigen-specific $CD8^+$ T cells from this pool of impaired T cells [136,151]. Similarly, 4-1BB stimulation has been shown to enhance the generation of primary $CD8^+$ T cell responses [139,152,153] and synergizes with attenuated vaccinia virus (VACV) vectors to augment $CD8^+$ T cell responses [139] .

Targeting of inhibitory molecules on T cells such as PD-1 and CTLA-4 have been shown to restore the effector function of activated T cells in settings of chronic viral infections and cancer [154,155,156,157]. Inhibitor blockade synergizes in combination with therapeutic vaccines [158]. Targeting of inhibitory pathways during primary immunization with prophylactic vaccines may advance the vaccine efficacy as well [159,160], but whether this results in significant improved vaccine efficacy remains to be established.

Although the use of antibodies targeting costimulatory/inhibitory molecules as immunostimulatory modalities in vaccines can facilitate antigen-specific T cell responses, the use of such Abs, however, is associated with toxicity as demonstrated in selected settings in rodents and in clinical settings [157,161,162,163]. Nevertheless, given the potential benefit to significantly increase the effectiveness of vaccines, both the efficacy and safety of targeting costimulation is currently extensively examined in various immunotherapeutic approaches against persistent viral infections. Examining the timing and/or the dosing is in this respect an important aspect to not only prevent unwanted side-effects but this may also lead to improved effectiveness. In addition, CD28 costimulation modulates T cell metabolism via activation of PI3K pathways, and this is essential to control effector cytokine production [112,164]. TNFR family members are also able to metabolically program T cells [165,166]. Collectively, targeting of T cell costimulation can impact the important quantitative (magnitude, breadth) and qualitative (cytokine polyfunctionality, metabolic fitness) determinants of vaccine-induced T cells, and provides thus major opportunities for further exploration in future vaccine designs.

CONCLUSIONS AND PERSPECTIVES FOR VACCINE DESIGN

The design of vaccines that imprint T cells with the ability to boost host defense against persistent viral pathogens has gained remarkable progress. An understanding of the appropriate initial programming signals is a key step, as is how the route of priming or boosting influences the development of effective memory T cells. A combination of several metrics such as the type of the vaccine elicited T cell response, breadth, polyfunctional quality and metabolic characteristics demonstrate a valid toolbox to define when a T-cell mediated response is protective. Unanswered questions about the anatomy, activation and differentiation of memory T cells in lymphoid compared to non-lymphoid organs need to be addressed. Costimulatory signaling pathways mediate basically all of the important T cell memory properties, and may serve as interesting targets for vaccine improvement. Experimental and clinical insight into their complex synergistic or antagonistic processes may identify requisite pathways and potentially other targets for immunotherapy. Identification of the best correlations of immunity with protection for persistent viral pathogens will enable the development of effective vaccination regimes.

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