Chapter 7
Strategies and Implications for Prime-Boost Vaccination to Generate Memory CD8 T Cells

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Abstract Generating a large population of memory CD8 T cells is an appealing goal for vaccine design against a variety of human diseases. Indeed, experimental models have demonstrated that the overall number of memory CD8 T cells present at the time of infection correlates strongly with the ability to confer host protection against a range of different pathogens. Currently, the most conceivable approach to rapidly generate a large population of memory CD8 T cells is through the use of prime-boost vaccination. In addition, recent experimental findings have uncovered important principles that govern both the rate and magnitude of memory CD8 T cell formation.

Thus, this has resulted in novel prime-boost vaccination strategies that could potentially be used in humans to generate protective populations of memory CD8 T cells.

7.1 Introduction

The use of vaccination to protect against human diseases can be traced back to 1796, when Edward Jenner pioneered the field after successfully protecting his "test subjects" from smallpox by infecting them with the less virulent cowpox virus. Unknown to Jenner at the time, his method led to the formation of immune cell memory, thus protecting the individual from subsequent infection. Now, over two centuries later, our ability to manipulate the immune system through vaccination has allowed the human race to essentially eradicate a number of its most deadly

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diseases. It should come as no surprise that successful vaccinations are considered one of public health's greatest achievements [1].

Although a number of successful vaccines have been developed, many human diseases remain for which no vaccine is currently available. Because of this, efforts to maximize immunization strategies that elicit a strong CD8 T cell response are appealing because of this immune cell's unique ability to specifically kill cells infected with intracellular pathogens. In addition, CD8 T cells also have the potential to be utilized in the development of various "cancer vaccines" against tumor antigens that are either over-expressed or expressed in a mutated form. Thus, a thorough understanding of how memory CD8 T cells are generated and maintained has direct relevance in modern day medicine.

Recent studies have begun to elucidate many of the factors that contribute to the generation of memory CD8 T cells. Although qualitative differences in memory CD8 T cell populations may be relevant when targeting different pathogens, it is clear that the overall number of memory CD8 T cells correlates strongly with host defense [3, 5, 27, 33, 34, 54]. Because of this, the following will discuss how memory CD8 T cells are initially generated, strategies of effectively using prime-boost vaccinations to generate large memory CD8 T cell populations, and the impact that prime-boost vaccination has on the resulting memory CD8 T cell population.

7.2   Generation and Characteristics of Memory CD8 T Cells

Following their development in the thymus, naïve CD8 T cells enter the blood and lymphoid compartments of the body where they continue to re-circulate in their quest for foreign antigen. Because of the great diversity of T cell receptors (TCRs) required to ensure protection against a wide variety of pathogens, finding naïve CD8 T cells that are capable of recognizing a specific foreign peptide is an extremely rare event. In mice, the number of naïve precursors specific for a given foreign peptide ranges from as few as ten to as many as a couple thousand [11, 12, 35, 43, 45]. However, these naïve CD8 T cells have a tremendous proliferative capacity, and once activated, undergo clonal expansion resulting in a dramatic change in numbers, whereas one naïve CD8 T cell can give rise to 10,000 daughter cells (>13 divisions) [27, 34]. This amazing and dynamic biological process is the start of the CD8 T cell response that will eventually lead to the generation of a stable memory population and the fundamental basis of vaccinations.

Before a CD8 T cell can become activated and undergo proliferation, it must first receive appropriate stimulation from antigen-presenting cells of the innate immune system. It is widely accepted that a naïve CD8 T cell requires stimulation through the TCR, co-stimulatory molecules (such as CD28), and be exposed to inflammatory cytokines in order for an appropriate T cell response to occur [25, 42]. Following infection, dendritic cells (DCs) become activated through a variety of pattern recognition receptors (PRRs) such as toll-like receptors (TLRs), which recognize a wide assortment of molecules found on foreign pathogens including peptidoglycan, lipopolysachharide, double-stranded RNA, and unmethylated CpG DNA motifs.

Once activated, these professional antigen-presenting cells acquire foreign antigen, migrate into secondary lymphoid compartments, and present pathogen-derived peptides on major histocompatibility class I (MHC-I) to CD8 T cells [29, 57]. When naïve CD8 T cells encounter stimulatory peptide-MHC-I complex while concurrently receiving co-stimulation, they undergo massive proliferation, leading to the generation of a great number of effector cells. Effector CD8 T cells leave lymphoid organs, enter the peripheral tissues, and destroy infected cells using a variety of mechanisms, including the production of interferon-γ (IFNγ) and tumor necrosis factor-α (TNFα) and through the expression of Fas Ligand (FasL) and perforin/granzymes [22]. During most acute infections, the invading pathogen is eliminated by the peak of the T cell expansion phase.

Once the number of T cells reaches a peak, most (90–95%) of the newly generated effector CD8 T cells then die. However, cells that survive this contraction phase of the immune response go on to form a stable memory pool of CD8 T cells (Fig. 7.1a). How to clearly define a memory CD8 T cell remains controversial, as no single molecular marker or functional property is represented by all cells within this memory population [32]. Although this makes it difficult to clearly label a CD8 T cell as "memory," the diversity and heterogeneity within the bulk memory population probably contributes to the high success rate of preventing re-infection of the pathogen. In addition, memory CD8 T cells are not static, and the phenotype and expression of effector molecules within this cell population changes over time (Fig. 7.1b–e). An understanding of the complexity of memory CD8 T cell populations is potentially critical when designing vaccinations against specific diseases.

One important distinguishing characteristic that separates memory CD8 T cells from naïve cells is the overall change in anatomical distribution. As mentioned, naïve CD8 T cell are confined mostly within the blood and spleen but also enter lymph nodes though the high endothelial venules (HEV) due to their high expression of the lymph node homing molecules L-selectin (CD62L) and CCR7. In contrast, memory CD8 T cells are able to traffic into peripheral tissues such as the liver, lung, and skin and can re-enter the lymph nodes and circulation via the afferent lymph (Fig. 7.2). In its most general characterization, memory CD8 T cells can be defined based on expression of CD62L and CCR7 [52, 60]. Memory CD8 T cells that express CD62L/CCR7 are defined as "central memory" (Tcm) and like naïve cells are able to enter lymph nodes through the HEV. Memory cells that lack expression of CD62L/CCR7, termed "effector memory" (Tem), are not able to enter lymph nodes via this route and tend to localize more efficiently into peripheral tissues [40]. This classification is likely an oversimplification of the diversity of trafficking patterns that occur in memory CD8 T cell populations and unique expression of chemokine receptors and integrins probably regulates distribution of specific memory cell subsets. In addition, recent evidence suggests that populations of non-circulating "resident memory" CD8 T cells can be found in certain organs such as the skin and gut [19, 38]. Thus, it is likely that future studies will uncover specific molecular mechanisms that regulate tissue distribution of memory CD8 T cells, which could potentially be used to maximize vaccine efficacy.

Survival of memory CD8 T cell populations does not require interactions between TCR and MHC-I [37, 44]. However, the cytokines IL-7 and IL-15 are critical
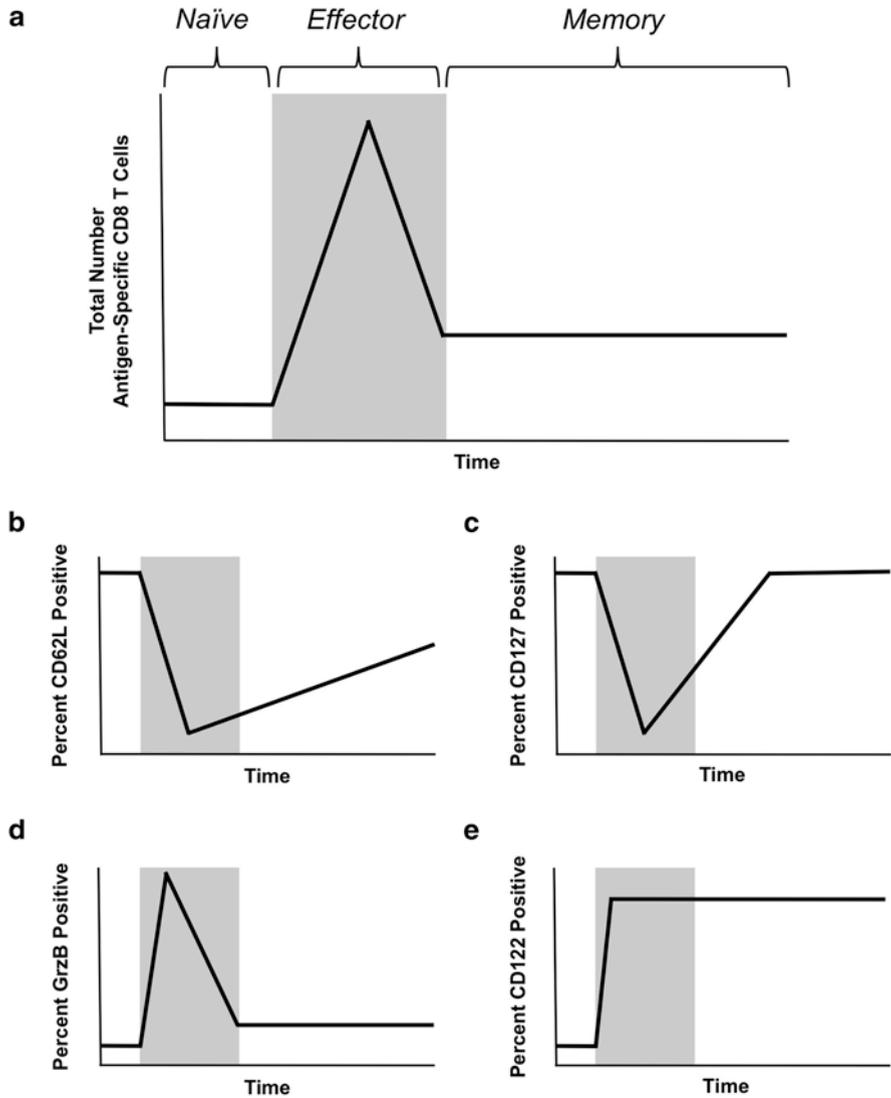
Fig. 7.1 Changes in CD8 T cell numbers and phenotype that occur following vaccination or infection. (**a**) Following activation, previously naïve CD8 T cells undergo expansion and contraction before forming a stable memory population. Based on this kinetic curve, CD8 T cells can be classified as either being naïve, effector (gray box), or memory. (**b–e**) Changes in expression of (**b**) CD62L, (**c**) CD127, (**d**) GrzB, and (**e**) CD122 that occur as CD8 T cell populations transition from naïve to effector to memory. (CD62L, L-Selectin; CD127, IL-7 Receptor α-chain; GrzB, Granzyme B; CD122, IL-2/15 Receptor β-chain)

regulators of memory CD8 T cell maintenance [58]. Both naïve and memory, but not effector, CD8 T cells express high levels of the IL-7 receptor α-chain (CD127) and this signaling pathway is necessary (but not sufficient) for the generation of memory CD8 T cells, possibly through the upregulation of anti-apoptotic molecules
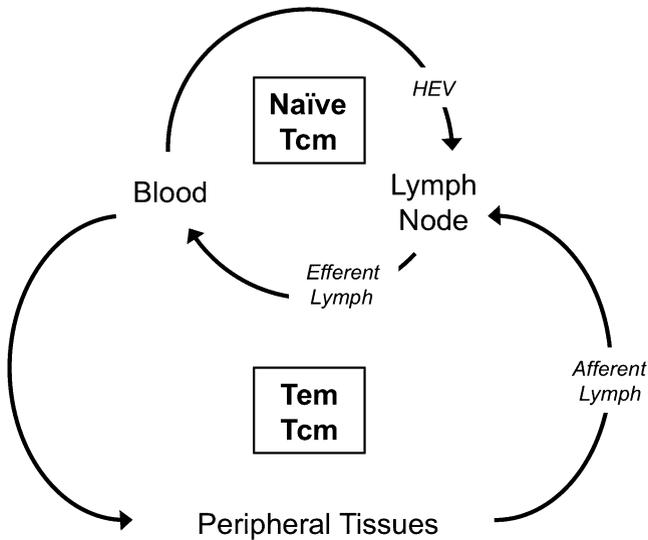
Fig. 7.2 Trafficking patterns of naïve, central memory (Tcm), or effector memory (Tem) CD8 T cells. Naïve CD8 T cells are primarily confined to the blood, spleen (not shown), and lymph node compartments of the body. Naïve and Tcm CD8 T cells are also able to enter lymph nodes via the high endothelial venules (HEV) due to high expression of CD62L and CCR7. In contrast, Tcm and Tem can also localize into peripheral tissues and then enter the lymph node through the afferent lymph. All CD8 T cells return to the circulation from the lymph nodes through the efferent lymph

including Bcl-2 [24, 26]. Interestingly, CD8 T cells express the IL-15/2 β-chain (CD122) following activation and this molecule is retained on memory populations. In contrast to the "pro-survival" impact of IL-7, signaling pathways activated in response to IL-15 drive a low level of homeostatic proliferation among memory CD8 T cells [9, 20]. Importantly, since primary memory CD8 T cell numbers remain relatively unchanged over time, this low level of homeostatic proliferation must also be accompanied by equivalent cell death. One possibility is the outgrowth of Tcm memory cells accompanied by Tem death, since the number of CD62L-expressing CD8 T cells increases with time and homeostatic proliferation is mostly confined to this subset. In contrast, there could be equal death between Tcm and Tem subsets and conversion of Tem to Tcm occurs during homeostatic proliferation. On the other hand, the eventual outgrowth of Tcm's could be the result of "out competing" their Tem counterparts for pro-survival cytokines such as IL-7 and IL-15.

In summary, following activation, naïve CD8 T cells undergo expansion and contraction resulting in the formation of a long-lived memory population. Through their differential expression of a number of trafficking molecules, memory CD8 T cells exhibit more diverse localization than naïve cells, which increases their ability to protect against invading pathogens. Importantly, following the generation of stable memory, these CD8 T cells are able to undergo a vigorous recall response in which they undergo a second round of expansion, contraction, and memory formation. This biologically relevant phenomenon is the basis for booster immunizations, where the overall goal is to maximize the number of memory CD8 T cells.

7.3 Methods of Prime-Boost Vaccination

High numbers of antigen-specific memory CD8 T cells are usually desired following vaccination, since this number strongly correlates with host protection [5, 27, 34]. Currently, the best approach known to generate these high numbers of cells is to utilize a system of prime-boost vaccination, which relies on the re-stimulation of antigen-specific immune cells following primary memory formation [51, 61]. Homologous booster immunizations that utilize re-administration of the same vaccination agent have essentially been used since the initial development of vaccines. Although this method is usually successful in boosting the humoral response to antigen, it is far less effective at generating increased numbers of CD8 T cells due to rapid clearance of the homologous boosting agent by the primed immune system [61]. Many of the pathogens for which no vaccine is currently available in humans such as human immunodeficiency virus (HIV), *Mycobacterium tuberculosis*, and *Plasmodium* species (causative agent of malaria) are highly resistant to humoral immunity generated by most traditional vaccines [55]. Thus, a successful vaccination strategy aimed at eliminating these intracellular pathogens could be aided by generation of a large memory CD8 T cell population.

In contrast to homologous prime-boost, use of heterologous prime-boost vaccination is much more effective at generating increased numbers of memory CD8 T cells (Fig. 7.3). The strategy implemented in heterologous prime-boost utilizes priming CD8 T cells with antigen delivered in one vector and then administering the same antigen in the context of a different vector at a later time point. Using this approach, the specific delivery of antigen to the CD8 T cell population and the generation of inflammatory signals during the booster immunization are maximized since the other arms of the immune system are not able to rapidly clear the vector as in homologous prime-boost vaccinations. This leads to not only a dramatic increase in the total number of antigen-specific CD8 T cells, but an enrichment of those T cells that have high affinity for antigen [18, 41]. Indeed, this strategy has been successful in generating protective immunity against a variety of pathogens in experimental models and is under evaluation in a number of human clinical trials [13, 14, 17, 30, 36, 61].

One important variable that impacts the "boosting-potential" of a CD8 T cell population is the length of time separating the primary and secondary antigen administration. In fact, following infection with common laboratory pathogens such as *Listeria monocytogenes* or lymphocytic choriomeningitis virus (LCMV), a time period of at least 40–60 days is required before optimal boosting of the CD8 T cell population is possible [27]. Indeed, this time frame is consistent with observed changes in overall gene expression of memory CD8 T cell populations, which begin to stabilize approximately 40 days after infection with LCMV [33]. Recent studies have begun to uncover the mechanisms that regulate the rate of memory CD8 T cell formation following infection or vaccination.

There is clear evidence that pro-inflammatory cytokines can dramatically impact the rate of memory formation. For example, antibiotic treatment to truncate infection following *L. monocytogenes* infection results in rapid generation of CD8 T cell memory without affecting the overall kinetics of the primary response [4, 7, 8]. This also decreases the interval that is required between initial infection and booster immunization to amplify
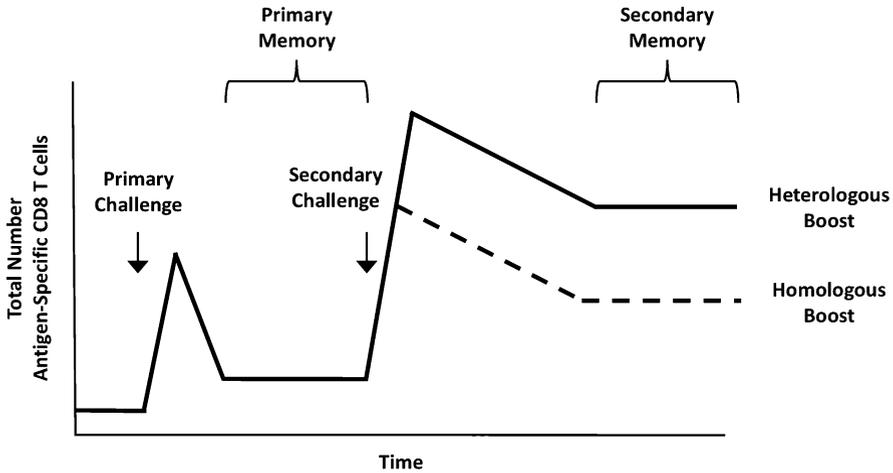
Fig. 7.3 Use of booster vaccinations to generate increased numbers of memory CD8 T cells. Following primary vaccine challenge, CD8 T cells undergo expansion, contraction, and form a primary memory population. When this primary memory population of CD8 T cells is exposed to a secondary challenge of the same vaccination (Homologous boost, dashed line), another round of expansion, contraction and formation of a larger, secondary memory population occurs. In contrast to a homologous booster vaccination, administration of a CD8 T cell antigen delivered in the context of a different vector (Heterologous boost, solid line) drives greater expansion of the primary memory CD8 T cells, resulting in a larger secondary memory population than what is seen with homologous booster vaccinations

CD8 T cell numbers. In agreement with the observation that pro-inflammatory cytokines have a negative impact on the rate of memory CD8 T cell formation, T cells genetically deficient for the IL-12 receptor progress to memory more quickly than wild-type cells [46]. Thus, limited exposure to pro-inflammatory cytokines allows for primary memory CD8 T cells to be boosted more quickly following initial antigen priming.

Another prime-boost method that leads to the rapid generation of memory CD8 T cells is by vaccinating directly with mature, peptide-pulsed DCs followed by a conventional booster immunization [6]. Using this technique, naïve CD8 T cells are exposed to antigen, co-stimulation, and localized inflammatory cytokines (from the DC), but are not exposed to overt systemic inflammation that occurs following infection. Although antigen-specific CD8 T cells still progress through a condensed effector phase, the transition to memory occurs within days without influencing the overall kinetics of the CD8 T cell response. In fact, DC-primed antigen-specific CD8 T cells can be boosted as soon as 4–7 days post infection. This protocol has been used in mice to establish lifelong CD8 T cell-mediated sterilizing immunity against the malaria parasite *Plasmodium berghei* [54]. This model has also been used to demonstrate the importance of inflammation in regulating the transition of CD8 T cells from effector to memory, as treatment with toll-like receptor agonists (such as CpG) following DC-peptide vaccination increases the length of time required for optimal boosting to occur [48]. Therefore, this form of heterologous prime-boost vaccination can be used to quickly generate a large, memory CD8 T cell population through

direct priming of the desired antigen-specific CD8 T cells in the absence of systemic inflammation followed shortly by a strong booster vaccination.

The biggest advantage of using peptide-pulsed DCs for vaccinations would be the rapid generation of large numbers of CD8 T cells following an adequate booster immunization. However, several hurdles exist that would make this strategy difficult to implement in an out-bred human population. Since this method utilizes direct priming with peptide, the appropriate immunization peptide(s) that elicit the desired CD8 T cell response will vary from individual to individual due to the diversity of MHC-I genes found within an out-bred human population. Therefore, this would require prior genetic screening of individuals, as well as the laborious task of determining immune-dominant CD8 T cell epitopes for different MHC haplotypes for any given pathogen. In addition, immature DCs would need to be isolated from the individual prior to vaccination in order to mature these cells in vitro. Utilizing DC vaccinations in humans would be both costly and time-consuming.

In order to circumvent the problems that would be encountered with individualized peptide-DC immunizations, recent studies have uncovered an alternative method of prime-boost vaccination by exploiting the immune system's ability to cross-present antigen to CD8 T cells. Uptake of apoptotic cells by antigen presenting cells occurs readily within the body every day, which will lead to the presentation of self-antigens by these cells [10]. However, no immune response is initiated to these self-antigens, most likely due to the mechanisms of both central and peripheral tolerance. When apoptotic cells are artificially coated with full length immunizing protein and used as a vaccination agent, a small primary CD8 T cell response can be detected in laboratory animals. When a conventional booster vaccination is then utilized shortly after this primary vaccination, antigen-specific CD8 T cell numbers explode to significantly higher numbers and establish long-term memory [49]. Using OVA-pulsed H-2K^b–/− apoptotic splenocytes, we verified that this priming step must occur through cross-presentation since these cells are unable to directly present peptide to CD8 T cells. These findings indicate that manipulating the immune system to cross-present foreign antigen in a low inflammatory environment leads to rapid generation of boostable, primary memory CD8 T cells.

Cross-presentation of antigen occurs much more effectively when introduced in a particulate, rather than soluble form [28]. In fact, encapsulating antigen in biodegradable particles such as poly(lactic-co-glycolic) acid (PLGA) microspheres has been explored as a potential mechanism for the delivery of antigen to the immune system [23, 53, 56]. However, it is widely accepted that use of adjuvants is required to generate any appreciable CD8 T cell response using this method. Our laboratory has also demonstrated that simply coating these microspheres with full-length protein and using them as a vaccination agents lead to the generation of a nearly undetectable CD8 T cell response. Regardless of this low starting number, these CD8 T cells undergo substantial boosting in response to secondary challenge and progress to long-lived memory cells. In fact, using this method, laboratory mice can be protected from both malaria and influenza [49]. Since the microbeads are coated using whole protein (not peptide), this strategy could be utilized in an outbred population and result in the rapid generation of boostable CD8 T cells, a technique that has the potential to be used as an effective "off-the-shelf" immunization method in humans.

Overall, the studies discussed here call into question the use of adjuvants during a primary vaccination when the ultimate goal is to quickly generate a large, memory CD8 T cell population. The methods described here that lead to the rapid generation of boostable memory CD8 T cells include primary immunizations that elicit an effector response dramatically smaller than the effector response that occurs following immunization with pathogen (Fig. 7.4). These forms of prime-boost vaccinations generate significantly higher memory CD8 T cell numbers compared to traditional
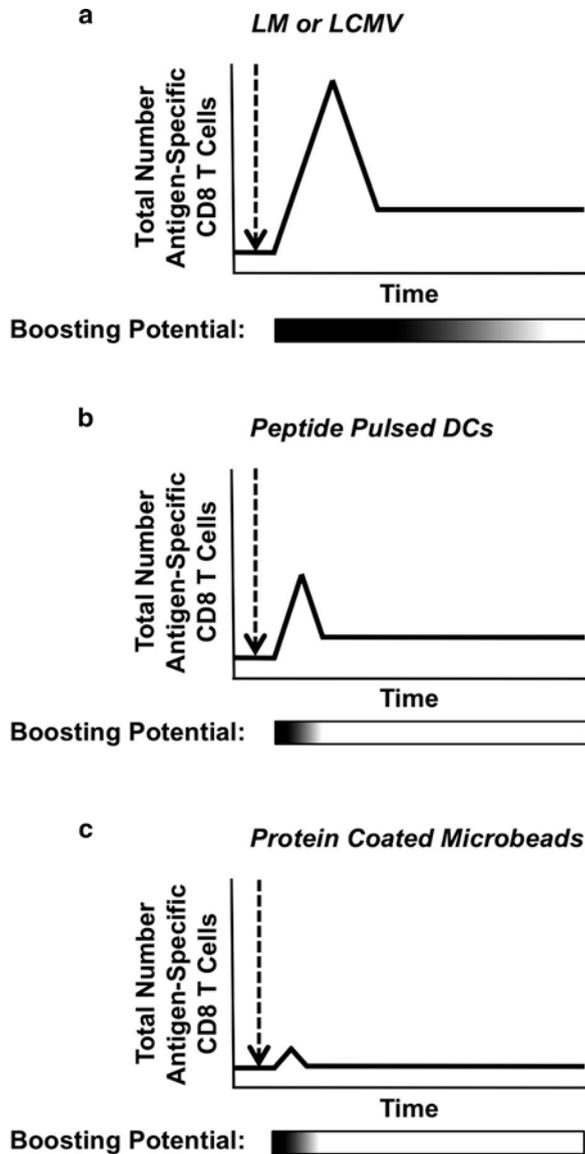
Fig. 7.4 Peptide pulsed DCs and protein coated microbead vaccinations rapidly generate boostable CD8 T cells. (**a**) Common laboratory pathogens such as *Listeria monocytogenes* (LM) and lymphocytic choriomeningitis virus (LCMV) stimulate strong primary CD8 T cell responses that are not able to undergo optimal boosting until 40–60 days following administration. (**b**) Peptide pulsed DCs and (**c**) protein coated microbeads stimulate a smaller primary CD8 T cell response, but generate memory CD8 T cells that can undergo boosting as soon as 4–7 days following administration. (Boosting Potential: *Black* – least boostable, *White* – most boostable)

primary vaccinations with laboratory pathogens. We would argue that rapidly progressing naïve CD8 T cell through a diminished effector phase and into an early primary memory phase followed by a strong booster vaccination is the most efficient way to rapidly generate a very large memory CD8 T cell population.

7.4 Impact of Subsequent Antigen Encounters on Memory CD8 T Cell Populations

Nearly all studies on the formation, maintenance, trafficking and overall characteristics of memory CD8 T cell populations have been performed by analyzing T cells during and following a primary infection or vaccination. This information has strengthened our understanding of the factors and mechanisms that influence the generation of memory CD8 T cells that originated from a naïve population. However, in all practicality, a population of memory CD8 T cells exposed to a single round of antigen rarely, if ever, exists outside of experimentally manipulated laboratory mice. Most successful vaccination regiments in humans require one or more rounds of booster immunizations in order to generate robust protective immunity against a number of pathogens [61]. In fact, recent studies demonstrate that multiple antigen encounters impact several aspects of memory CD8 T cell populations and are summarized in Fig. 7.5 [5, 21, 31, 39, 59]. Since prime-boost vaccinations would lead to the generation of "secondary" memory CD8 T cells, it is critical to determine how multiple antigen encounters change the ensuing memory populations, as this may lead to new ideas concerning ideal vaccine design and implementation.

	Naïve	Primary	Secondary
CD62L	+++	++	+
IL-2	-	++	+
CD122	-	+++	++
CD127	+++	+++	++
GrzB	-	+	++
Lymph Node Localization	+++	++	+
Peripheral Tissue Localization	-	+	++

Fig. 7.5 Impact of subsequent antigen encounters on the ensuing memory CD8 T cells. Naïve, primary memory (stimulated with antigen once), and secondary memory (stimulated with antigen twice) CD8 T cells exhibit differential phenotypes and anatomical localizations. (CD62L, L-Selectin; IL-2, Interleukin-2; CD122, IL-2/15 Receptor β-chain; CD127, IL-7 Receptor α-chain; GrzB, Granzyme B)

One of the most striking differences observed in memory CD8 T cell populations following multiple antigen encounters is the slower generation/formation of Tcm cells compared to a primary memory population [31, 39]. Because of this, secondary memory CD8 T cells localize poorly to lymph nodes and traffic much more efficiently to peripheral tissues including the lung and liver. Conceivably, this change in localization may enhance the ability of secondary memory CD8 T cells to provide immediate protection during the early stages of microbial infection. In addition to these changes in overall anatomical localization, secondary memory CD8 T cells also express increased levels of Granzyme B and exhibit more efficient in vivo cytolysis. In agreement with these findings, secondary memory CD8 T cells are more protective than an equal number of primary memory CD8 T cells during an acute infection with *L. monocytogenes* [31]. These studies suggest that boosted memory CD8 T cells take on a more "effector-like" phenotype rendering them more protective against acute infections in peripheral tissues.

As described earlier, the cytokines IL-7 and IL-15 are critical for both the survival and maintenance of a primary memory CD8 T cells population. Secondary memory CD8 T cells undergo less homeostatic proliferation and are less responsive to IL-15 than primary memory cells [31]. In addition, ensuing populations of memory CD8 T cells following multiple antigen challenges also express lower levels of CD127 [39]. These findings challenge the ideas of how pro-survival cytokines influence the generation of stable memory CD8 T cell populations. However, since contraction of T cell numbers is also protracted following secondary antigen stimulation, the observation of decreased homeostatic proliferation by this population may be due to prolonged survival of both effector and Tem cells since homeostatic proliferation is most robustly observed in the Tcm memory compartment. If this is the case, then how do Tem cells survive more efficiently following subsequent antigen encounters compared to a primary response? Could it be due to an overall resistance to apoptosis through both intrinsic and extrinsic pathways or to yet unidentified pro-survival cytokines? Clearly, our understanding of memory CD8 T cell maintenance following booster immunizations is far from complete.

Generally, booster immunizations will lead to increased memory CD8 T cell numbers with the majority of these cells exhibiting an effector phenotype and localizing to peripheral tissues. Another hurdle in utilizing prime-boost vaccination to generate memory CD8 T cells is the decreased representation of Tcm within these populations. Although the decreased localization into lymph nodes by boosted memory CD8 T cells allows for the priming of new naïve CD8 T cells during subsequent vaccinations or infection [31], in many cases, the presence of a large Tcm population may be optimal for host protection, especially against those pathogens that cause systemic or chronic infections. Many chronic/latent viruses, such as HIV and Epstein-Barr virus (EBV), undergo most re-activation and replication within lymph nodes. Since multiple antigen encounters drive memory CD8 T cells away from the lymph nodes, theoretically, this anatomical location would be most ideal for viral replication to occur in order to largely avoid the cellular arm of the immune system. In support of this idea, the majority of CD8 T cells in infected humans specific for HIV and EBV are CD62L^{Lo} [15, 16]. However, two recent studies have demonstrated that the rate of memory CD8 T cell formation may be regulated by

changes in cellular metabolism. Inhibition of mTOR (mammalian target of rapamycin), a key regulator of cellular energy homeostasis, not only impacts memory cell generation, but also augments the rate of Tcm formation following contraction [2, 47, 50]. Thus, prime-boost vaccination in combination with this type of drug treatment may be a conceivable approach to generate a large number of secondary memory CD8 T cells that exhibit a Tcm phenotype.

In summary, it is clear that multiple antigen encounters impact memory CD8 T cell populations in ways other than simply boosting the overall total numbers. Specifically, these phenotypic and functional differences that occur following booster-immunizations alter anatomic distribution, cytokine production, effector function, and protective capacity against different pathogens. It is clear that many aspects of memory CD8 T cell biology, as it relates to humans, may currently be unknown due to the lack of laboratory studies aimed at understanding key aspects of memory CD8 T cell populations that have gone through more than one antigen encounter. Future studies aimed at identifying key changes in both global and specific gene expression of multiply boosted CD8 T cells will undoubtedly provide tools for optimizing vaccine design in humans.

7.5   Conclusion

As described here, utilizing heterologous prime-boost vaccinations is a valid approach to generate very large numbers of memory CD8 T cells. Specifically, studies described here would argue that "weak" primary immunizations resulting in low inflammatory environments give rise to primary memory CD8 T cells that are able to undergo substantial boosting in response to a strong secondary immunization. Since major human pathogens including HIV and *Plasmodium* reside inside of cells, an effective vaccine against these diseases will most likely require the generation of protective memory CD8 T cells. It is also clear that learning and understanding methods that would manipulate memory CD8 T cell localization following multiple antigen encounters could potentially also increase efficacy of vaccine design. As more tumor antigens are discovered, efforts to develop "cancer vaccines" will also rely on the generation of memory CD8 T cells. By appropriately understanding the CD8 T cell biology using animal models and applying it to specific human diseases, the potential to continually develop and improve vaccinations is highly probable.

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