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Generation and maintenance of immunological memory

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Abstract

The key feature of the adaptive immune response is its specificity and the ability to generate and maintain memory. Preexisting antibodies in the circulation and at the mucosa provide the first line of defense against re-infection by extracellular as well as intracellular pathogens. Memory T cells are an important second line of defense against intracellular pathogens, and in particular against microbes that can cause chronic or latent infection. In this article we will review our current understanding of the generation and maintenance of B cell and T cell memory.

Keywords: Immune memory; Plasma cell; Antibodies; CD8 T cells; Microbial infection

1. Introduction

Immunological memory is characterized by the ability to respond specifically and more rapidly upon a subsequent encounter with a pathogen or antigen. This rapid and specific memory response upon recall is the basis for vaccination. The concept of vaccination can be traced back to 10th century China where individuals were inoculated with dried smallpox pustules obtained from infected individuals [1,2]. This process known as 'variolation' elicited a somewhat milder form of smallpox that protected the individuals from a more virulent disease. Variolation was in common practice in Asia in the 16th century [3]. Although variolation provided protection against smallpox for some individuals, a substantial number of people succumbed to serious disease as a result of the actual variolation, and subsequently this practice was discontinued. The seminal work by Edward Jenner in the 18th century showing that protection against smallpox could be obtained by vaccinating with a related virus, cowpox, is accredited as the first demonstration that protection against an infectious disease can be obtained without contracting the actual disease [4]. Interestingly, it was not however proven until the 19th century that microbial pathogens were the causative agents of disease. This founding work by Pasteur, Koch, Ross, von Behring, Ehrlich and others laid the foundations for immunology, microbiology and for the development of modern vaccines. Of note, four of the first eight Nobel Prizes for Medicine were awarded for this work to von Behring (1901), Ross (1902), Koch (1905), and Ehrlich (1908). As Noble Prizes are not awarded posthumously Pasteur was not eligible.

The most important and effective mechanism for maintaining immunity is the periodic re-exposure to the pathogen. Such re-infections are usually asymptomatic or produce only mild symptoms and act as a natural booster to the immune system. The importance of this mechanism is shown in epidemiological studies demonstrating that protective immunity is maintained for longer periods in people living in areas where a given disease is endemic. Individuals that live in regions where, for example, malaria is endemic have higher malaria-specific antibody titers than those who have intermittent exposures.

It is well established that protective immune memory can persist for many years after the initial antigenic exposure [5–12]. Examples of long-term protective immune memory in the absence of re-exposure to antigen include; measles immunity on the Faroe Islands (65 years) [5], yellow fever immunity in Virginia (75 years) [7] and polio immunity in

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remote Eskimo villages in Alaska (40 years) [8]. It is well accepted that people who have had diseases such as measles and mumps as children are unlikely to succumb to the infection upon re-exposure.

Three groups have recently demonstrated the presence of residual immunity against the smallpox vaccine in the population [10–12]. Crotty et al. [10] and Hammarlund et al. [11] both showed that smallpox vaccine specific antibody was maintained for greater than 60 years post-vaccination. In addition Crotty et al. [10] also demonstrated the presence of smallpox vaccine specific memory B cells 60 years post-vaccination. Both the antibody level and memory B cell numbers were maintained at steady levels for decades [10,11]. The smallpox vaccine specific CD4 and CD8 T cell memory cells were detectable in some individuals up to 75 years post-vaccination [11,12]. Although these studies do not show protection against smallpox they do demonstrate that both B cell memory and T cell memory can be maintained for decades.

There are two arms of immunological memory, humoral immunity that includes preexisting antibody, memory B cells and plasma cells, and cellular immunity that includes memory CD8 and CD4 T cells. The relative importance of humoral and cellular immunity in protection against reinfection has been of high interest and has created much debate. When considering this issue one should take into account the fact that humoral and cellular immunity have evolved to provide distinct effector functions. Preexisting antibodies can directly bind virus particles, extracellular bacteria, and parasites. Their role is to provide the first line of defense by neutralizing or opsonizing invading pathogens. T cells on the other hand cannot recognize free pathogens but instead recognize infected cells by interacting with microbial antigens (peptides) bound on major histocompatibility complex (MHC) class I (CD8 T cells) or class II (CD4 T cells) molecules. The main purpose of memory T cells is to detect infected cells then exert their effector functions which is to kill the infected cell directly and/or produce cytokines to inhibit microbial growth. CD4 T cells in addition to exerting effector functions also provide help to B cells and CD8 T cells [13–18]. In this review we will focus on the development and maintenance of humoral memory and CD8 T cell memory.

2. The generation of memory B cells and plasma cells

We have come a long way in understanding many aspects of the humoral response to T-dependant antigens (Fig. 1). Naïve B cells upon encountering antigen and with CD4 T cell help are activated and proliferate at the margins of the T cell zone or periarteriolar lymphoid sheaths (Pals) in the spleen and lymph nodes. The activated B cells can then continue down one of two pathways: (1) remain in the marginal zone and differentiate into short-lived plasma cells, or (2) migrate into B cell follicles and with CD4 T cell help initiate a germinal center (GC) reaction. Within the GC reaction B cells can undergo somatic hypermutation, affinity maturation and selection resulting in the generation of high affinity memory B cells [19–24]. Long-lived plasma cells or their precursors [25] are most likely also generated in the germinal center reaction then migrate from the spleen and lymph nodes to the bone marrow where they take up residence.

It is well established that CD4 T cell help is required for the GC reaction, memory B cell development and T-dependant antibody responses. Mice that are deficient in CD4 T cells [26], or components of the CD40 [27–29], CD28 [30,31] and ICOS [32] signaling pathways have severely impaired humoral responses to T dependent antigens with the inability or markedly reduced ability to generate germinal centers

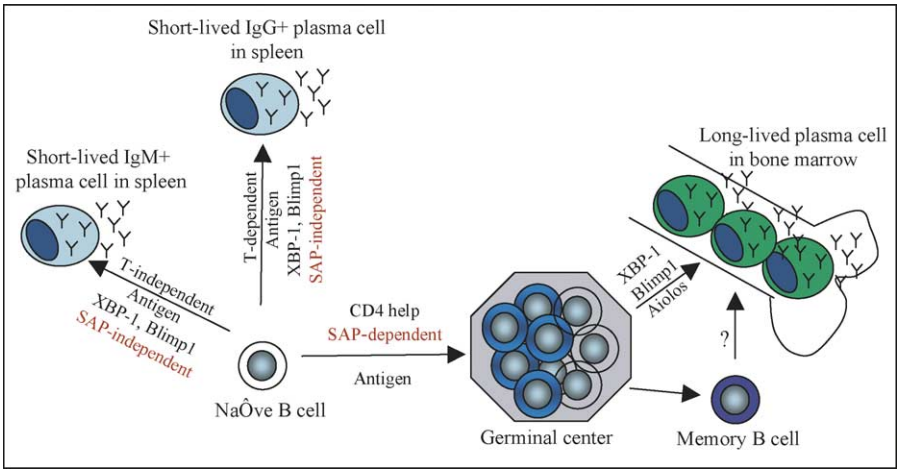

Fig. 1. The generation of memory B cells and plasma cells. After antigen stimulation naïve B cells can either, (1) differentiate into short-lived plasma cells extra-follicularly or (2) migrate into a B cell follicle and with CD4 help initiate a germinal center reaction. Out of the germinal center reaction arise memory B cells and long-lived plasma cells. The long-lived plasma cells reside in the bone marrow. In the case of a viral infection sap-independent CD4 help is required for the generation of IgG-secreting short-lived plasma cells. Sap-dependant CD4 help is required for the germinal center reaction, generation of memory B cells, and long-lived plasma cells. The transcription factors Blimp-1 and XBP-1 are required for the differentiation of both short- and long-lived plasma cells. Aiolos is required for the differentiation of long-lived plasma cells.

and memory B cells. A recent report demonstrated that there are likely to be different forms of CD4 T cell help required for the generation of the primary antibody response to T-dependent antigens versus help needed for the generation of long-lived plasma cells and memory B cells [33]. Mice that were deficient for the Slam-associated protein (SAP) had normal primary/short-lived IgG antibody responses to lymphocytic choriomeningitis virus (LCMV) but had severely reduced numbers of germinal centers, memory B cells and long-lived plasma cells. This defect was intrinsic to the SAP deficient CD4 T cells [33]. This is the first example of a defect in CD4 T cell help that delineates between help required for the acute phase (GC-independent) and later phases (GC-dependent) of the humoral response. How SAP regulates the GC reaction and the subsequent development of memory B cells and long-lived plasma cells is currently not known.

One question that still needs to be addressed in more detail is what are the cellular origins of long-lived plasma cells? Are long-lived plasma cells generated in the germinal center reaction as fully differentiated plasma cells or as a precursor? O'Connor et al. [25] using a transgenic system described a cycling post-GC plasma cell precursor in the bone marrow of mice which upon transfer into a naïve host in conjunction with immunization could differentiate into plasma cells. These findings suggest that long-lived plasma cells may be generated as a precursor. Aiolos, a member of the Ikaros family of nuclear regulators, may play an essential role in the generation of long-lived plasma cells. A recent study by Cortes et al. [34] found that mice deficient in Aiolos developed both short-lived plasma cells and memory B cells after immunization with nitrophenyl (NP)-chicken γ globulin (CGG) but did not generate long-lived plasma cells in the bone marrow. The lack of Aiolos in mature peripheral B cells lowers the threshold for B cell receptor (BCR) and CD40 signaling [35] therefore it cannot be ruled out that altered signaling within the GC may inhibit the generation of long-lived plasma cells. However, this is the first description of a defect that specifically blocks the generation of long-lived plasma cells and not memory B cells or short-lived plasma cells. It would be very interesting to determine what genes are regulated by Aiolos. The transcription factors Blimp-1 and XBP-1 are critical for both short-and long-lived plasma cell differentiation [36,37]. The expression of either Blimp-1 [38] or XPB-1 [37] in B cells is sufficient to drive differentiation into plasma cells. Conversely, B cells that are deficient in either Blimp-1 [36] or XBP-1 [37] lack the ability to differentiate into plasma cells in vivo after immunization with T-dependent or T-independent antigens. Neither Blimp-1 or XBP-1 expression was altered in Aiolos deficient mice [34].

3. Maintenance of memory B cells

Memory B cells can persist for many decades after initial infection or vaccination. Periodic re-exposure to the pathogen is a mechanism that naturally boosts the memory B cell numbers. During low-grade chronic or latent infections re-exposure to antigen also occurs. In the absence of antigenic re-exposure, however, memory B cells can still be maintained for many years [10]. What maintains memory B cells in the absence of re-exposure to the pathogen? Adoptive transfer experiments suggested the antigen was important for the long-term maintenance of memory B cells [39,40]. It has been proposed that the source of antigen for this maintenance was retained on follicular dendritic cells trapped in immune complexes [41–45]. However, recent studies have demonstrated that memory B cells can persist in mice in the absence of detectable immune complexes [46]. In a study by Hannum et al. [46] mice were engineered so that the only form of immunoglobin M (IgM) was membrane bound and thus lacked the ability to form immune complexes. Memory B cells although at lower numbers than control mice could be maintained in these mice in the absence of immune complexes. Elegant experiments by Rajewsky's group using a novel transgenic approach largely confirmed that antigen was not required for the maintenance of memory B cells [47]. After the generation of memory B cells they switched the BCR so that it could no longer recognize its cognate antigen. Memory B cells persisted for extended periods of time in the absence of specific BCR signaling.

If antigen is not required for memory B cell maintenance what is? The presence of a BCR is required for survival of all peripheral B cells [48]. It is possible that low affinity interactions with self-antigen may promote the survival of both memory and naïve B cells. Alternatively, the presence of a BCR simply may provide a B cell-autonomous maintenance signal. Recently it has been demonstrated that the tumor necrosis factor (TNF) family member B-cell activating factor (BAFF) also known as B-lymphocyte stimulator (BlyS) promotes the survival of naïve B cells in the periphery [49]. There is evidence in humans that BAFF may promote the survival of plasmablasts derived from memory B cells [50]. It still needs to be determined if BAFF also plays a role in memory B-cell maintenance. It is not known if memory B cells undergo homeostatic proliferation. The homeostatic turnover of memory CD8 T cells is regulated by the cytokines IL-15 and IL-7, and memory CD4 T cells by IL-7. Do cytokines also play a role in the maintenance of memory B cells? If memory B cells do undergo homeostatic turnover what is generated, another memory B cell, or a memory B cell and a plasma cell? These are just a few of the many interesting important questions that need to be addressed about memory B cell homeostasis.

4. Serological memory: role of long-lived plasma cells and memory B cells in maintaining antibody levels in the circulation

The long-term production of antibody is the hallmark of most effective vaccines. There are multiple mechanisms to maintain antibody levels in the circulation and at the mucosa

for up to the lifetime of an individual after vaccination or infection. Re-exposure to the pathogen or a booster vaccination is clearly the most effective mechanism to boost both the specific antibody levels and memory B cell numbers. Natural re-exposure to antigen is the most important mechanism for maintaining antibody levels against commonly reoccurring or endemic pathogens. Low-grade chronic or latent infections that provide a continuous or sporadic antigenic stimulation also drive BCR-dependent differentiation of both memory B cells and naïve B cells into antibody-secreting plasma cells. In the absence of re-exposure to the pathogen, however, antibody levels can still be maintained for many years. There are two current hypotheses that have been put forward to explain the longevity of the antibody response in the absence of re-exposure to antigen. The first proposed independently by Slifka et al. [51] and Manz et al. [52] was that antibody levels are maintained by the presence of long-lived plasma cells in the bone marrow that secrete specific antibody for extended periods, potentially for the life of an individual [51,52]. The second proposed by Bernasconi et al. [53] stating that memory B cells are continually differentiating into plasma cells in an antigen independent manner due to bystander or polyclonal activation [53]. It should be noted that although these two hypotheses are often referred to independently they are not necessarily mutually exclusive. Both mechanisms could occur concurrently.

4.1. Long-lived plasma cells

The lifespan of a plasma cell has been disputed for almost half a century. Until recently, the general conception was that plasma cells were short-lived surviving only a few days. This belief in part was based on experiments in rodents performed in the early 1960s where it was observed that most plasma cells generated after immunization only survived a few days [54–57]. The first evidence that some plasma cells could be long-lived came from experiments performed by Miller in 1964 [58] where he documented that a small number of the plasma cells generated after immunization survived for 6 months. However, given the fact that over 90% of the plasma cells died, the general consensus was that most plasma cells were short-lived and therefore were unlikely to be able to maintain antibody levels. One caveat to these early experiments was that their observations were limited to the spleen and lymph nodes. It has since been shown that the bone marrow is a major niche for long-lived plasma cells [59,60].

The question of what is the lifespan of a plasma cell was recently revisited. A population of viral-specific plasma cells in the bone marrow that were present for the lifespan of a mouse were identified [51,26]. Plasma cells could be detected in the bone marrow of mice after acute infection with LCMV 15 days after infection, peaking around day 45 and remaining at constant number for the life of a mouse [51]. To determine if the constant number of LCMV-specific plasma cells found in the bone marrow was due to longevity of the plasma cells or due to memory B cell turnover, LCMV immune mice (mice infected at least 50 days prior) were irradiated. After irradiation memory B cells that are radiation-sensitive were no longer detectable however radiation-resistant plasma cells could still be detected 250 days post-irradiation. LCMV-specific IgG titers could also be detected for 250 days. There was a slight decline in the number of LCMV-specific plasma cells with time after irradiation suggesting that memory B cells may also play a role in maintaining plasma cell numbers. In these experiments the half-life of LCMV-specific plasma cells was 94 days in the bone marrow and 172 days in the spleen. In another study Manz et al. used bromo-deoxyuridine pulse chase labeling of cells during a secondary immune response to ovalbumin to evaluate the lifespan of plasma cells [52]. Their experiments demonstrated that all of the antigen-specific plasma cells present in the bone marrow were long-lived surviving without cell division for 3 months, the timeframe of their study. The same group also demonstrated that the survival of long-lived plasma cells was independent of antigen [61]. These studies demonstrate that the long-term production of antibody seen after viral infection or vaccination is in part sustained by long-lived plasma cells present in the bone marrow.

Could a long-lived plasma cell generated during a childhood vaccination, such as smallpox vaccination, survive in the bone marrow of an individual for up to 75 years [10,11]? What mechanisms promote the survival of plasma cells in the bone marrow? It is clear that the bone marrow niche itself may provide extrinsic survival signals for plasma cells [62–64]. When removed from this niche and placed in in vitro culture plasma cells can survive no longer than a few days [64]. The factors in culture that have been shown to promote survival of plasma cells include the presence of bone marrow stromal cells, the cytokine IL-6, and interactions with the very late antigen (VLA)—4 [64]. Other molecules including IL-5, TNF-α, SDF-1, CD44, and BCMA may also be important [65,66]. In addition, bone marrow plasma cells have been shown to express the anti-apoptotic factors BCL-2 [67–69], A20 [69] and IAP-2 [69]. As mentioned earlier mice deficient in Aiolos a member of the Ikaros family of nuclear regulators generate memory B cells and short-lived plasma cells but not long-lived plasma cells [34]. It is possible that Aiolos may modulate genes involved in long-lived plasma cell survival. The transcription factors BLIMP-1 [36,38,70] and XBP-1 [37] are critical for plasma cell differentiation however it is unclear if these factors play a role in plasma cell survival.

4.2. BCR-independent differentiation of memory B cells into plasma cells

Lanzavecchia's group recently put forward an alternate BCR-independent hypothesis [53]. They proposed that memory B cells are continually undergoing antigen-independent or bystander activation and differentiating into plasma cells thereby maintaining the antibody levels [53]. Molecules that

activate innate signaling pathways such as LPS and CpG can stimulate memory B cells to differentiate into plasma cells [53,71–73]. In humans, memory B cells constitutively express high levels of several toll-like receptors (TLRs) including TLR9 that binds to unmethylated CpG DNA [72]. Naïve B cells do not constitutively express TLR9 but it can be upregulated on naïve B cells when stimulated through the B cell receptor (BCR) [72]. This differential expression of TLR9 on memory and naïve B cells in part may be a mechanism to allow only memory B cells to respond independent of BCR signaling to CpG. Bystander CD4 T cell help has also been shown in vitro to stimulate non-specific memory B cells to differentiate into plasma cells [53]. During an infection or after vaccination the number of activated cytokine-producing CD4 T cells are elevated. This increase in available CD4 T cell help could result in the bystander (BCR- and MHC class II/TCR-independent) activation of memory B cells driving differentiation into plasma cells. CD4 T cell help could be provided directly (CD40, B7-1/B7-2 signaling, etc.) and/or indirectly (cytokine signaling) and possibly in conjunction with the activation of innate signaling pathways. Although the differentiation of memory B cells following BCR-independent activation has been demonstrated in vitro [10,53,72,73] the degree to which this occurs in vivo is currently unknown.

4.3. Multiple mechanisms maintain serum antibody

Given the experimental evidence available to date it is likely that multiple mechanisms come into play to maintain serum antibody levels (Fig. 2). There are no available data directly examining the longevity of plasma cells in humans. Based on murine studies some long-lived plasma cells (~10%) can survive for the lifespan of a mouse [51]. It is not inconceivable that a plasma cell given the right environment could survive and secrete antibody for extended periods in humans. Neuronal cells, for example, can survive and function lifelong. The bone marrow provides a niche for long-lived plasma cells where factors that promote survival exist. Even so, given the finite space and potential competition from new plasma cells for the niche some long-lived plasma cells may eventually die. Also, in mice, in the absence of memory B cells the numbers of long-lived plasma cells do decline over time [51]. This suggests that the turnover of memory B cells in an antigen-independent (or dependant) fashion into plasma cells may supplement the long-lived plasma cell pool thereby maintaining stable numbers of antigen-specific plasma cells for life. In the case of chronic or latent infections it is likely that memory B cells will differentiate into plasma cells due to the presence of specific antigen. Memory B cells also rapidly differentiate into plasma cells upon re-infection or antigenic challenge.

It is hard to conceive that one mechanism would have evolved to maintain serum antibody levels given the fact that antibody is the first line of defense and the only mechanism by which to recognize free pathogens. It is more likely and

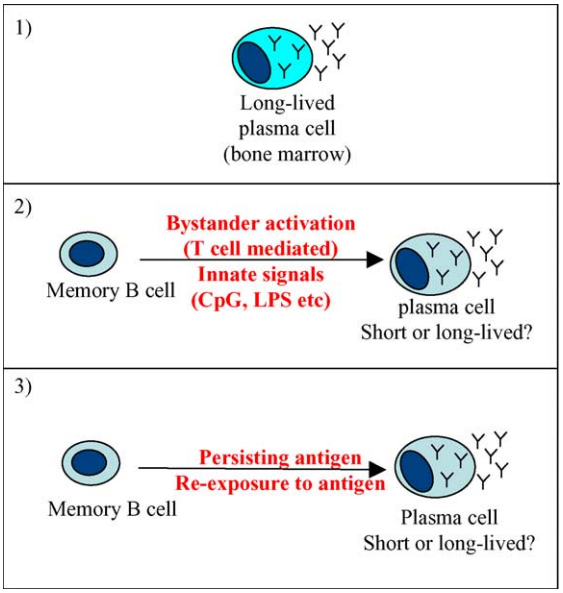

Fig. 2. Multiple mechanisms to generate long-term persisting antibody. (1) The presence of long-lived plasma cells in the bone marrow generated during the primary infection or vaccination produce antibody for extended periods, potentially lifelong. (2) The turnover of memory B cells into short-lived or long-lived plasma cells due antigen independent polyclonal activation. Polyclonal activation could include non-specific CD4 T cell help or activation by innate signals such as CpG or LPS. (3) The differentiation of memory B cells into short-lived plasma cells due to the presence of persistent antigen, for example, during a chronic infection or due to re-exposure to antigen.

experimental evidence suggests that multiple mechanisms as outlined above come into play. Many issues still need to be addressed. For example, do memory B cells differentiate into long- or short-lived plasma cells when stimulated independent of the BCR? What are the relative contributions to the antibody pool from long-lived plasma cells versus differentiation of memory B cells? The definitive question, which is very difficult to address experimentally, is how long can a plasma cell survive in a human bone marrow? Further understanding of the mechanisms that regulate the development and the survival of long-lived plasma cells and memory B cells would aid in vaccine development in particular where antibody is important for protection.

5. CD8 T cell memory: generation and maintenance

In recent years we have begun to understand how memory CD8 T cells are generated, what mechanisms maintain memory T cells once generated, and the relationship between memory T cells and protective immunity. The CD8 T cell response can be divided into three distinct phases [74]. The first is the expansion phase where the initial activation and clonal expansion of CD8 T cells occurs. The second phase is the contraction or death phase where 90–95% of the activated effector CD8 T cells die via apoptosis. The final phase is the establishment and maintenance of CD8 T cell memory. Recent studies have suggested that all three phases

may be programmed shortly following antigenic stimulation [75–79].

5.1. Programming of memory CD8 T cell differentiation

The initial activation of naïve CD8 T cells leads to a rapid expansion of cells and commitment to the acquisition of effector functions [74]. Effector functions in most instances include the ability to produce IFN-γ and TNF-α, chemokines such as RANTES, acquisition of cytotoxic activity via the granzyme/perforin granule exocytosis pathway, and gaining the ability to migrate to non-lymphoid tissues [74,80–85]. The priming of naïve CD8 T cells in vivo is influenced by multiple factors including TCR affinity, innate immunity, cytokine levels, levels of antigen, extent of co-stimulation, and antigen presenting cells (APCs). Each of these factors impact on the subsequent development of effector and memory CD8 T cells by imprinting a developmental program associated with changes in gene expression that is passed on from daughter cell to daughter cell [83,85–87]. Several studies have demonstrated that a 24-h stimulation with antigen is sufficient to set a developmental program and for clonal expansion. The central finding of these studies is that this program is passed on to daughter cells instructing them to undergo at least 7–10 rounds of division without further antigenic stimulation [75–78]. There is evidence that CD8 T cell contraction may also be programmed upon activation [79]. These studies suggest that all phases of the CD8 T cell response may be programmed shortly after antigen stimulation.

The CD8 T-cell program can vary depending on the quality of stimulation received during activation resulting in heterogeneity within the effector and memory populations. In addition, extrinsic factors such as the length of time antigen is available (for example, chronic versus an acute infection) can influence the development and quality of memory CD8 T cells (Fig. 3) [88–91]. For the optimal activation, expansion, and differentiation of CD8 T cells into functional memory cells in addition to receiving signals via the T cell receptor (TCR) (signal 1) and the co-stimulatory molecule CD28 (signal 2) other co-stimulatory molecules or signals may also play a role. The development of effector functions and survival can be promoted by a third signal such as IL-12 and/or adjuvants [92,93]. Signaling through CD27, CD40, 4-1BB and ICOS may promote continued expansion, survival or memory differentiation of CD8 T cells [94–98]. On the other hand, negative regulators, including cytotoxic T lymphocyte antigen 4 (CTLA-4), B and T lymphocyte attenuator (BTLA) and programmed death 1 (PD-1) may inhibit clonal expansion [99,100]. Recent studies have demonstrated that help from CD4 T cells during activation also plays a role in the formation of competent CD8 T cell memory [13–17].

The majority of effector CD8 T cells die via apoptosis during the contraction phase [74,101,102]. What is unique about the 5–10% of CD8 T cell effectors that survive, differentiate and then enter the long-lived memory CD8 T cell pool? Recent studies have shown that IL-7 and expression of the IL-7 receptor (IL-7R) may provide important signals for survival during effector to memory cell differentiation [103]. Precursors of memory CD8 T cells can be identified within the effector population based on the high expression of IL-7Rα chain (CD127) [103]. These cells that make up 5–10% of the effector population and preferentially populate the memory pool whereas the IL-7Rα low effector cells are predominantly lost during the effector to memory transition. Another study by Madakamutil et al. [104] demonstrated that expression of the homotypic form of CD8 which uses the α chain of the CD8 molecule (CD8αα) promoted the survival of CD8 memory T cell precursors. CD8αα was expressed on a subset of CD8αβ T cells during LCMV infection but was lost after viral clearance. Consistent with the studies of Kaech et al.

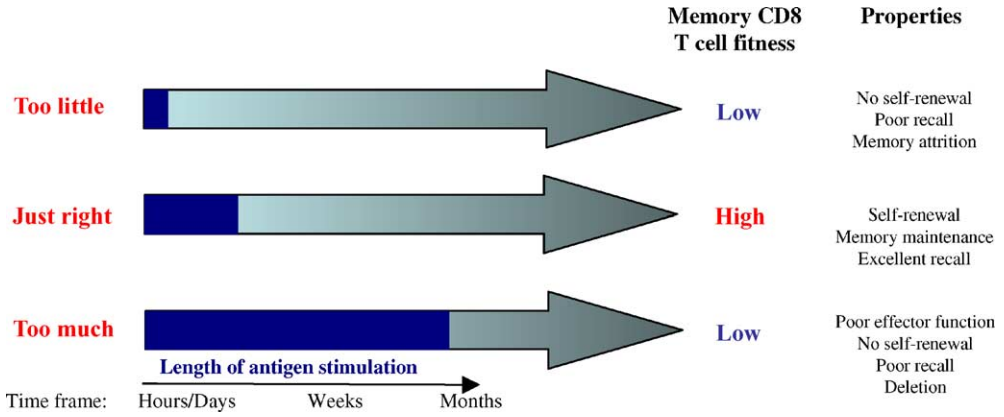

Fig. 3. Different outcomes of memory CD8 T cell development: The Goldilocks model. A program of differentiation and clonal expansion is set within the first 24 h of antigenic stimulation. 'Too little' stimulation results in limited CD8 expansion and poor memory development. 'Too much' stimulation, for example, during chronic infections where antigen can persist for weeks or indefinitely, leads to functionally incompetent "exhausted" memory CD8 T cells, and eventual clonal deletion. 'Just right' stimulation results in memory CD8 T cells that acquire memory traits, including the ability to undergo homeostatic proliferation, rapid proliferation upon secondary challenge, and the ability to produce cytokine.

[103] the expression of CD8αα correlated with increased expression of IL-7Rα. CD8αα expression also correlated with increased expression of IL-15Rβ, and the anti-apoptotic factor Bcl-x_L. Mice that were deficient in the enhancer element that is required for CD8αα expression but have no defect in CD8αβ expression had a severe defect in CD8 T cell memory development supporting a role for CD8αα in the survival of CD8 T cell memory precursors. These studies provide further evidence that memory CD8 T cells are direct descendants of effector CD8 T cells. Combined these findings confirm other groups studies supporting a linear model of memory T cell differentiation [105].

A number of studies in mice using gene expression analysis and functional characterization have shown that memory CD8 T cell development is a gradual process which continues on for several weeks after infection is resolved [83,85–87]. Microarray analysis of LCMV-specific CD8 T cells at various time points after primary infection demonstrated that changes in gene expression were still occurring weeks after virus was cleared [83]. The changes in gene expression over time correlated with an acquisition of memory cell traits including, gaining the ability to produce IL-2, the ability to proliferate vigorously to antigen, and to undergo homeostatic proliferation in response to IL-7 and IL-15. A key finding from these studies is that surviving effector cells are not "optimal" memory cells until they have completed a program of differentiation gradually acquiring memory cell properties over time. While we have come a long way in understanding memory CD8 T cell differentiation many questions still remain. What dictates a CD8 T cell fate determining who does re-express IL-7α and CD8αα and who does not? Is the expression of IL-7α and CD8αα determined extrinsically or intrinsically or both? Is memory CD8 T cell differentiation instructional, are 5–10% of CD8 T cells predestined to become memory cells even before the initial activation event, or is it a stochastic process which programs cells after the initial activation event to become memory cells?

5.2. CD8 memory T cell subsets

The memory CD8 T cell population can be further subdivided based on the expression of the cell surface molecules CD62L and CCR7 which are involved in homing to lymph nodes [106–113]. The $CD62L^{Hi}/CCR7^{Hi}$ memory subset is present in the lymph nodes, spleen, and blood. The $CD62L^{Lo}/CCR7^{Lo}$ subset is present in the spleen and blood but not in the lymph nodes and is enriched in non-lymphoid tissues [113,114]. Based on initial experiments suggesting that the $CD62L^{Lo}/CCR7^{Lo}$ subset were better producers of IFN-γ and TNF-α after stimulation, the $CD62L^{Lo}/CCR7^{Lo}$ memory phenotype cells were coined the "effector" memory T cells (T_{EM}) [110]. The $CD62L^{Hi}/CCR7^{Hi}$ memory phenotype cells that could produce IL-2 and were predominantly located in lymphoid tissues were termed the "central" memory T cells (T_{CM}). It was proposed that the T_{EM} being located in peripheral tissues were the first line of defense against invading pathogens where as the T_{CM} located in the lymph nodes generated another round of effector T cells [110]. These original experiments defining the T_{EM} and T_{CM} subsets were carried out in vitro with polyclonal human T cell populations [110]. Recent studies in both humans [115] and mice [86,116] have found that when stimulated with cognate antigen both subsets produced IFN-γ and TNF-α equivalently. However, as demonstrated in the earlier study [110] only the T_{CM} cells produced IL-2. In addition, the $CD62L^{Hi}/CCR7^{Hi}$ (T_{CM}) memory CD8 T cells have a greater proliferative capacity compared to the $CD62L^{Lo}/CCR7^{Lo}$ (T_{EM}) memory CD8 T cells [85]. When compared on a cell-to-cell basis the $CD62L^{Hi}/CCR7^{Hi}$ subset provided better protective immunity after both a systemic or peripheral LCMV challenge. This was due to the generation of a larger effector population as a consequence of the greater proliferative capacity of the $CD62L^{Hi}/CCR7^{Hi}$ memory CD8 T cell subset [85]. However, for localized infections it is possible that the tissue resident memory CD8 T cells may play a more important role. Using a Sendai viral lung infection model the $CD62L^{Lo}/CCR7^{Lo}$ memory CD8 T cells mounted a recall response equivalent to the $CD62L^{Hi}/CCR7^{Hi}$ memory CD8 T cell subset [117].

There has been a lot of interest in determining the lineage relationship between the $CD62L^{Lo}/CCR7^{Lo}$ and the $CD62L^{Hi}/CCR7^{Hi}$ CD8 T cell memory subsets [105,110,118–120]. In acute infections after the antigen is cleared, there is a gradual conversion from $CD62L^{Lo}CCR7^{Lo}$ to $CD62L^{Hi}CCR7^{Hi}$ within the memory CD8 T cell pool [85,121,122] that correlates with increased protective immunity, IL-2 production, and homeostatic turnover [85]. In the LCMV system it has been possible to directly track CD8 T cell the lineage of $CD62L^{Lo}CCR7^{Lo}$ and $CD62L^{Hi}CCR7^{Hi}$ CD8 T cells in the memory compartment [85]. These experiments revealed that in the absence of antigen the $CD62L^{Lo}CCR7^{Lo}$ subset of memory CD8 T cells differentiated directly into $CD62L^{Hi}CCR7^{Hi}$ memory CD8 T cells and this conversion corresponded to the acquisition of the ability to undergo homeostatic turnover. These experiments demonstrated that these two memory CD8 T cell subsets are not independent lineages, but rather are related along a common differentiation pathway. Thus, these observations together with the gene expression profiling mentioned above support a linear and progressive model of memory CD8 T cell differentiation in which antigen stimulation drives naïve CD8 T cells to become activated and differentiate into effector CD8 T cells [83,85]. If antigen is cleared, as occurs following an acute infection or vaccination, these effector CD8 T cells will first differentiate into $CD62L^{Lo}CCR7^{Lo}$ memory T cells. In the continued absence of antigen these $CD62L^{Lo}CCR7^{Lo}$ memory CD8 T cells will undergo further differentiation into $CD62L^{Hi}CCR7^{Hi}$ memory CD8 T cells and also acquire the defining properties of memory T cells including IL-2 production following antigen stimulation, robust proliferative responses to antigen, and long-term antigen-independent maintenance via homeostatic turnover.

5.3. Maintenance of CD8 T cell memory

Memory CD8 T cells can be present at relatively constant numbers for many years after infection or vaccination. One defining characteristic of the memory CD8 T cell pool is that it can be maintained long-term at constant numbers. Homeostasis of the memory CD8 T cell pool is achieved by the slow turnover of memory CD8 T cells. This slow homeostatic proliferation of memory CD8 T cells is balanced with both survival and death, resulting in no net increase in memory CD8 T cell numbers. Analogous to the maintenance of memory B cells memory CD8 T cells do not require antigen for survival or homeostasis [47,123]. In addition, memory CD8 T cells do not require the presence of MHC class I molecules. Murali-Krishna et al. [124] demonstrated that LCMV-specific memory CD8 T cells can persist, undergo homeostatic proliferation, and remain functional in the absence of MHC class I molecules. Recent studies have identified roles for the cytokines IL-7 and IL-15 in the maintenance of memory CD8 T cells [125–130].

Zhang et al. [131] demonstrated that memory phenotype CD8 T cells ($CD44^{hi}$) selectively expressed high levels of CD122 (IL-2/IL-15Rβ), a receptor molecule shared by both the IL-2 and IL-15 receptors. In addition they showed that IL-15 caused selective proliferation of $CD44^{hi}$ CD8 T cells. Another group demonstrated that the in vivo blockade of CD122 but not IL-2 inhibited the proliferation of memory CD8 T cells implicating a role for IL-15 in memory CD8 turnover [132]. Evidence that IL-15 was involved in the maintenance of memory CD8 T cells came from the generation of mice deficient in either IL-15 [133] or the IL-15 receptor alpha chain (IL-15Rα) [134] which both had decreased numbers of memory phenotype CD8 T cells. Upon closer analysis using antigen-specific systems it was shown that memory CD8 T cells could be generated in these mice but declined in numbers over time [125,126,129]. This decline in antigen-specific memory CD8 T cells was due to a lack of homeostatic proliferation. LCMV-specific memory CD8 T cells generated in IL-15 cytokine deficient mice when transferred into wild type mice regained the ability to undergo proliferation [125]. Interestingly, the expression of the high affinity IL-15 receptor, IL-15Rα is not required on memory CD8 T cells for maintenance. Instead the expression of IL-15Rα is required on other bone marrow-derived cells that trans-present IL-15 to the low affinity IL-15Rβ and γ on memory CD8 T cells [135–137].

In contrast to IL-15, IL-7 appears to be more important for the survival rather than the proliferation of memory CD8 T cells [134,138,139]. Transgenic mice expressing IL-7 driven from the MHC class II promoter had increased numbers of memory CD8 T cells however underwent proliferation at the same rate as normal cells [138]. As mentioned the expression of the IL-7Rα is down regulated on the majority of effector CD8 T cells generated during viral infection. The subset of effector CD8 T cells that do survive and enter the memory CD8 T cell pool are those that express high levels of IL7Rα [103]. Part of the survival effects of IL-7 on memory CD8 T cells may in part be due to the sustained expression of BCL-2 induced by IL-7 [139,140]. In summary, memory CD8 T cell maintenance does not require antigen or interactions with MHC class I but is regulated by the combined actions of IL-15 and IL-7 that promote proliferation and survival, respectively.

6. Concluding comments

Understanding the mechanisms that generate and maintain humoral and cellular immune memory has important implications for the design of both preventative and therapeutic vaccines. The ability to manipulate the number of effector CD8 T cells that will ultimately differentiate into memory cells or increase long-lived plasma cell numbers would be powerful tools for vaccine development.

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