Immunol Res (2007) 38:305–318
DOI 10.1007/s12026-007-0005-3

Immunity and protection, the unfolding of a tale

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Published online: 15 May 2007

Abstract Immunological memory is at the core of protective mechanisms against microbial pathogens and possibly of defenses against tumors. Here, a new perspective is offered on the qualitative and quantitative aspects of the T cell response as it relates to protection. Two main points are proposed. First, the conditions of the initial immune response (priming) are critically important in the induction of T cell memory and protection. Second, at the present time, protection against microbial pathogens appears to correlate with the function of central memory T cells. A series of considerations and suggestions are being made for new ways to optimize the induction of protective T cell responses by vaccination both in the immunologically naive and experienced individual; emphasis is placed on: dose of antigen, the availability of T cell help, avoidance of overt inflammatory conditions and efforts to decelerate cellular senescence in responding T cells.

Keywords Immunological memory · T cell responses · Central memory · Effector memory · T cell help · Inflammation · Antigen dose · Cellular senescence · Protection · Bone marrow

Introduction

It was indeed a privilege to be part of the first Symposium of the Robert A. Good Immunology Society. The main topics of the Symposium covered areas of immunology in which I can only claim great interest, and I thought my contribution would be outside the main thrust of the Symposium. However, I am encouraged by the fact that even a topic like

Presented at the First Robert A Good Society Symposium, St. Perersburg, FL 2006.

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mine should find a place in ''Perspectives in Immunology 2006.'' As it will be apparent at the end of my presentation, a simple point will emerge: Protective immunity, as far as T cell immunity is concerned, appears to correlate with a special class of memory T cells (central memory T cells—T_{CM}), that is memory T cells preferentially found in secondary lymphoid organs but also in the bone marrow. Thus, the arguments put forth in my presentation are directly tied to the main thrust of this Robert A. Good Symposium.

To begin, I will start to set the stage for what seems to be the cornerstone not only of my presentation today, but also of the far more important consideration that diseases transmitted to people lacking immunity by invaders with immunity have been a key factor in the history of the world. An example of paramount significance is the conquest of the Incas by the Spaniard Pizarro and the siege of the city of Cajamarca in 1532. Notably, his success was not due to the use of guns but rather to the devastating effect of an epidemic of smallpox in the preceding decades [1]. In retrospect, many would acknowledge that well before the 16th century it was already known that people who had experienced a disease or a poison in one form or another were resistant to a subsequent exposure to the same disease or poison [2, 3]. In other words, immunological memory was effective long before we immunologists began to recognize and study it.

Immunological memory: definition and purpose

Immunological memory is central to life, representing a powerful link between the past and the present of the individual. Remembrance of things past at a purely immunological level signifies a general mechanism of experience organization and function programming. Immunological memory could simply be viewed as a network of recollectable information for the structure and organization of immune responses to come. Many, including myself, believe that the major biological advantage offered by immunological memory, to the individual and the species, is the ''stronghold'' of protection against microbial pathogens spanning across one generation or even across several generations. In broad biological terms, the inability to establish immunological memory is seen as deleterious for the evolution of the species.

In the past decade, a concerted effort has been made to distinguish the phenomenon of immunological memory from its constitutive elements, e.g., the cells, molecules, and genes, and establish a relation with protection (Fig. 1). Progress has been remarkable

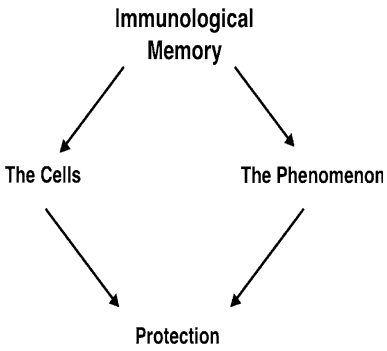

Fig. 1 The relationship between the phenomenon and the cellular components of immunological memory and protection

and while conclusive answers have yet to be provided, new leads have emerged. For this reason, I maintain that immunological memory can be defined as the event that occurs when the immunologically experienced individual re-encounters antigen through infection or vaccination and develops, as a result, a greater and faster response than after the first exposure to the same antigen [4]. Characteristic of the phenomenon is also an increase in the frequency of specifically reactive lymphocytes and heightened sensitivity to antigen. Recent reports prove this to be accurate and applicable to T cell responses [5].

It is commonly accepted as fact that the great majority of vaccines currently in use in humans, from historical models such as smallpox to those recently released, all owe their effectiveness to the induction of antibodies [6, 7]. In this case, neutralizing and opsonizing antibodies work by intercepting the pathogen at the portal of entry, in the blood stream or in the intercellular space. Because exogenous pathogens infect by different pathways, different strategies are required for their interception. Those that initially come in contact with external secretions (e.g., influenza virus), or enter the bloodstream in a cell-free form (e.g., poliomyelitis) can both be intercepted but the purpose will be to prevent infection or disease, respectively. Additional considerations apply to the length of the incubation period. A disease of short incubation period, such as influenza's incubation of less than 3 days, requires that protective levels of serum neutralizing antibody be present at the time of exposure to prevent the establishment of infection [8]. Since the degree of resistance to influenza virus infection is directly proportional to the level of specific hemagglutination-inhibition antibody in the secretion of the respiratory tract [9], it is necessary to maintain antibody titers above levels associated with protection by repeated immunizations. For diseases with a longer incubation period, such as paralytic poliomyelitis, which requires more than 3 days, it is necessary to prime the immune system and induce immunologic memory for durable resistance to paralysis [8].

Analysis of the relation between the development of serum antibodies following single vaccination with non-infectious polio virus vaccine and the establishment of memory shows that immunological memory establishes and persists over time in the presence of

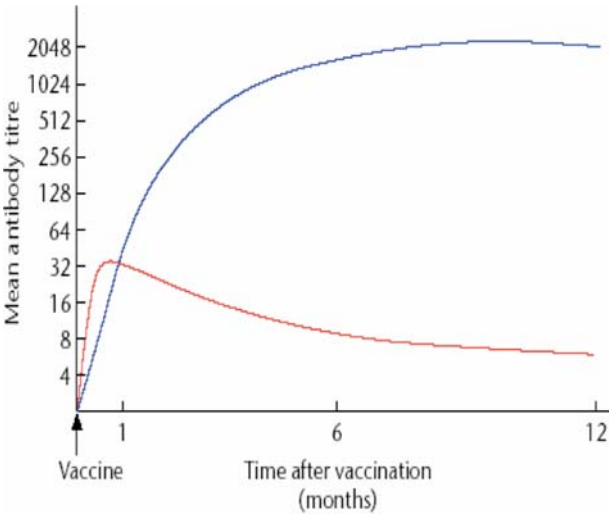

Fig. 2 The relationship between the development of serum antibodies following single vaccination with non-infectious poliovirus vaccine and the establishment of immunological memory. With permission from [10]

low antibody titers (Fig. 2), suggesting that the presence of immunological memory does not need to be equated to the level of immunity detected in the immunized individual prior to antigenic challenge. In other words, immunological memory at the level of antibodies exists in the absence of a demonstrable correlate, once established is durable and based on a dynamic and rapid expansion of specific clonotypes upon antigen recall. Not surprisingly, in the case of many diseases such as measles, mumps, rubella, and smallpox, immunity conferred by infection or vaccination provides lifelong immunity. Anecdotic recounts and scientific observations have measured with some approximation the persistence of immunity against some major infectious agents in terms of 65 years for measles [11], 75 years for yellow fever [12] and 40 years for poliomyelitis [13]. A retrospective analysis of memory antibody responses after smallpox vaccination showed persistence of antibodies after >50 years [14]. This suggests that following appropriate immunization, memory persists for the life of the individual without need for periodic reinforcement by immunization. Thus, it seems that in the course of evolution, the memory antibody response evolved to prevent reinfection of the host by invading extracellular pathogens.

It is commonly accepted that the defense against intracellular pathogens requires T cell immunity. Consequently, memory T cell responses have evolved to provide the individual with specialized mechanisms of defense and protection against intracellular pathogens that would have otherwise caused irreparable damage to the species. Hence, my intention is to draw attention to new facts about the dynamics of the T cell response in order to better understand the origin, maintenance, and dynamic expansion of memory T cells as they relate to protection. The arguments put forward are based on experimental observations made in rodents and non-human primates mainly in the course of vaccination or viral infections.

The dynamics of the T cell response: a starting point

The rules for emergence of a primary response have been given ample attention over the past decades and general principles are understood. The primary response reflects Burnettian clonal selection and expansion [15], and is regulated by an ensemble of factors including the antigen [16], the antigen presenting cell (APC) [17], the milieu of cytokines [18], and costimulatory molecules [19]. In addition, optimal conditions for T cell priming relate to temporal and anatomical factors [20, 21]. It is as if the cardinal principles of the Greek tragedy, i.e., unity of space, time, and action, are recapitulated in the dynamics of a primary T cell response. The immune response occurs in organized lymphoid structures (the site of immune induction) because the conditions for T cell activation are optimally realized within the geometry of the local stroma. Secondary lymphoid organs are also rich in signal 2 (costimulation) [22] so that the conditions for immunogenicity are met. However simple this might appear, the initiation of T cell response is a complex process which depends on the type of immunogen or pathogen and a sufficient display of peptide/MHC complexes (signal 1).

Studies in the past 5 years support a new idea that the conditions and characteristics of the primary T cell response, i.e., the induction of effector T cells, dictate the type of future immunity in quantitative and qualitative terms, including T cell memory (Fig. 3A). The main point is that parameters that control the clonal expansion of the effector phase (antigen dose, inflammation, frequency of precursors, adjuvant etc.) are not independent variables of the priming event but highly connected to the destiny of the immune response persisting once the effector phase wanes. The new postulate predicts that the longevity and

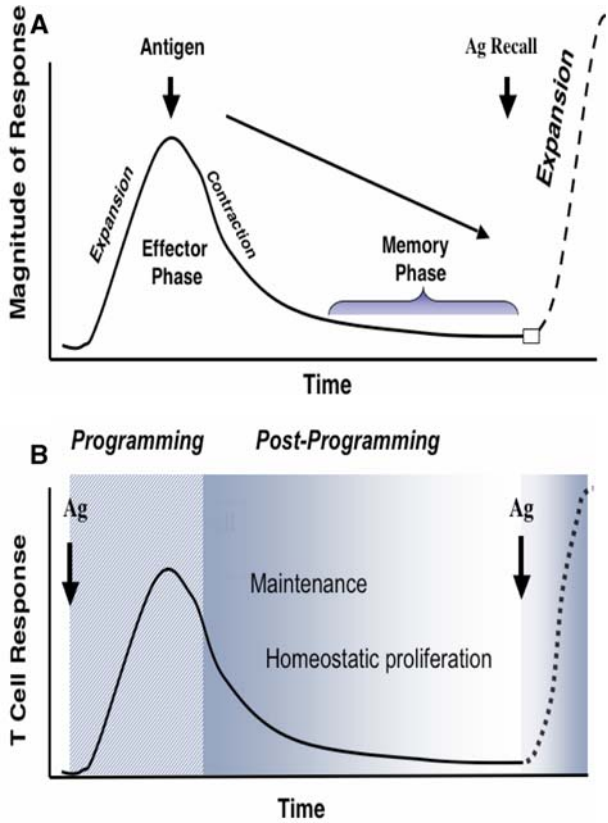

Fig. 3 (**A**) Schematic representation of the course of a primary T cell response. In response to primary infection or vaccination, antigen-specific CD8 T cells undergo a first phase of clonal selection and expansion followed by a contraction phase in which the great majority (~90%) of activated effector T cells undergo apoptosis [23]. During this event the immune response slowly progresses into the emergence of memory CD8 T cells and their maintenance through homeostatic proliferation [24]. This is the origin of a reservoir of antigen specific CD8 T cells expandable upon re-encounter of antigen. There is consensus that this sequence of events takes place within the first 7–10 days from the initial contact with antigen [25, 26]. (**B**) Schematic representation of the two main phases of the primary expansion leading into a long-term response awaiting for the re-encounter with antigen. The programming and post-programming phases and their relationship with maintenance and homeostatic proliferation are shown

functional characteristics of T cell memory are imprinted early on at the time of priming, and that the way priming occurs has, by inference, great relevance on the immune system's ability to confer protection to the individual and control disease.

In the first phase of clonal selection and expansion, naïve T cells are developmentally programmed to divide at least 7 to 10 times during a short period of time and to differentiate into effector CTLs and long-lived functional memory CD8 T cells [27, 28]. This phase is proportionally correlated with the dose of antigen in that the greater the antigen dose the more robust the effector phase will become. The contraction phase that follows is characterized by massive (~90%) apoptosis of the activated effector T cells [23] and is independent of the magnitude of the expansion [29]. After the effect of priming fades away, memory T cells continue to divide, albeit slowly, placing their survival on extrinsic factors, homeostatic proliferation [30, 31] (Fig. 3B). This phase is largely controlled by

cytokines, IL-7 and IL-15 [24, 32–34]. Two cytokines, IL-4 and IL-2, have been shown to enhance functional longevity of T cells [35] or survival of CD8 T cells during the recall response [36]. Surprisingly, both IL-4 and IL-2 exert their effort during the initial encounter with antigen, i.e., during the programming phase (Fig. 3B). Different views exist as to the dependence of the phase of homeostatic proliferation on antigen, MHC and TCR interaction on the one hand [37–40] and costimulatory molecules on the other hand [40, 41]. I will take the view that the long-term survival of memory T cells in vivo depends at least on protracted stimulation by antigen during the initial phase of the primary T cell response.

Requirements for protective memory responses

What are the requirements for the induction and maintenance of purposeful immunity? These have recently been discussed [42] and I will recapitulate herein the main points: the persistence of antigen, the necessity of T cell help, low inflammation, and antigen dose (Table 1).

First, the persistence of antigen in the maintenance of protective responses. Contrary to recent findings that once set in motion a T cell response develops irrespective of antigen [43], protective T cell memory responses against a variety of pathogens [38, 44–46] fade rather quickly when infection is eliminated. Significantly, the presence of antigen even in small quantities appears to be required for the type of memory responses that mediate protection.

Second, the necessity of T cell help. New evidence indicates that T cell help plays an important role in determining the initial activation (programming phase) as well as destiny (post-programming phase) of CD8 T cells, that is the emergence and maintenance of memory T cells [47–52]. Notably, whereas CD8 T cells primed in the absence of T cell help become programmed to undergo TRAIL-mediated apoptosis upon re-encounter with antigen [53], a lack of CD4 T cell help during priming creates unfavorable conditions for the generation of protective responses [49, 52, 54]. Thus, T cell help during priming is essential to the generation of protective CD8 T cell memory responses.

Third, priming in the absence of overt inflammation. Although a degree of inflammation at the time of priming is required to activate the APC, overt inflammatory conditions may play adversely on the induction of T cell memory in that APCs (e.g., dendritic cells) activated in large numbers create increased ratios between APCs and T cell precursors, a condition that may bias lineage commitment (see below). Experiments that have directly tackled this issue show that overt inflammation during priming (e.g., via pathogen or synthetic adjuvant such as CpGs) dramatically diminishes the generation of antigen-specific memory CD8 T cells and severely restricts their expansion upon recall infection [25]. Thus, common practices of taking advantage of an inflammatory umbrella to amplify the expansion phase, and accordingly the generation of effector T cells, may de facto curtail the generation of memory T cells.

Table 1 The conditions of priming that control the memory response

(1) Persistence of antigen
(2) Available T cell help
(3) Low inflammation
(4) Antigen dose

Fourth is the antigen dose. Early observations showed that small doses of antigen favor cell-mediated responses and vice-versa large doses of antigen favor antibody mediated responses [55, 56]. More recently it was appreciated that too much antigen activates a large fraction of available T cell precursors causing their deletion by exhaustion [57]. As mentioned previously, the magnitude of the expansion phase of a T cell response directly correlates with the amount of antigen administered [27]. Finally, hyperactivation of T cells impact the replicative life of somatic cells causing them to enter a state of proliferative arrest, senescence [58]. Replicative senescence is intimately linked with short telomeres and end-stage differentiation of memory T cells [59]. Indeed, in aging, where there is progressive loss of immunological memory there is also a greatly decreased frequency of antigen-specific IFN-γ producing cells [60] and expression of CD57, a hallmark of proliferative capacity [61, 62]. Not surprisingly, chronic HIV infection and excessive antigen stimulation drives cells into a state of replicative senescence with prevalence of $CD57^+$ CD8 T cells [61, 63]. Together these considerations create a conundrum as to which is the most effective method to generate memory T cells.

It is my view that the aphorism ''more is better'' does not apply. Let us examine a few examples in support of this view, focusing on the relationship between antigen dose of the priming event and the magnitude of the expansion phase during the memory recall response. Few reports exist showing an inverse correlation between the priming dose and the magnitude of the recall response [29, 64]. A study performed by Paola Castiglioni in my laboratory [65] using genetically programmed B lymphocytes as APCs to vaccinate against the influenza virus showed that a small priming dose of transgenic B lymphocytes (3×10^2 cells/inoculum) injected intravenously was sufficient to expand tetramer-positive CD8 T lymphocytes after virus challenge at a magnitude comparable to that following priming with higher doses (5×10^3 or 2×10^4 cells/inoculum) or with virus. In non-human primates a DNA vaccine appears to be more effective than a vaccinia vaccine in priming for protective memory response against HIV [66, 67] even though the expression of antigens differs substantially in these two vaccine platforms. The lesson from these observations is simple: the expansion of CD8 T cells after challenge is independent of the number of memory cells present at the time of challenge and there is no advantage in using a high antigen dose at the time of priming if the purpose is to induce memory responses that can be easily expanded upon re-encounter with antigen (Fig. 4).

Memory CD8 T cell subpopulations and protection

In the mouse and in humans, memory T cells are distinguished in two subpopulations: central memory (T_{CM}) and effector memory (T_{EM}) cells based on the expression of L-selectin (CD62L) and the CC-chemokine receptor 7 (CCR7) which determine the ability to home to lymph nodes [68, 69]. Such a distinction has proven relevant to more precisely assign the correlate of protection and also identify the parameters sufficient and necessary to induce protective memory T cells. The characteristics that distinguish T_{EM} and T_{CM} cells are summarized in Table 2. Considerations on how and when memory T cells with characteristics of T_{EM} and T_{CM} cells are generated can be found in [42, 70] and suggest a picture where the two memory T cell subpopulations are established according to a master program of the immune system early during the phase of immune induction, and include $T_{EM} \rightarrow T_{CM}$ conversion as a regulatable element. However, since the raison d'etre of CD8 T cell immunity is protection from intracellular pathogens, there is a more critical question: What is the contribution of T_{EM} and T_{CM} cells to protection?

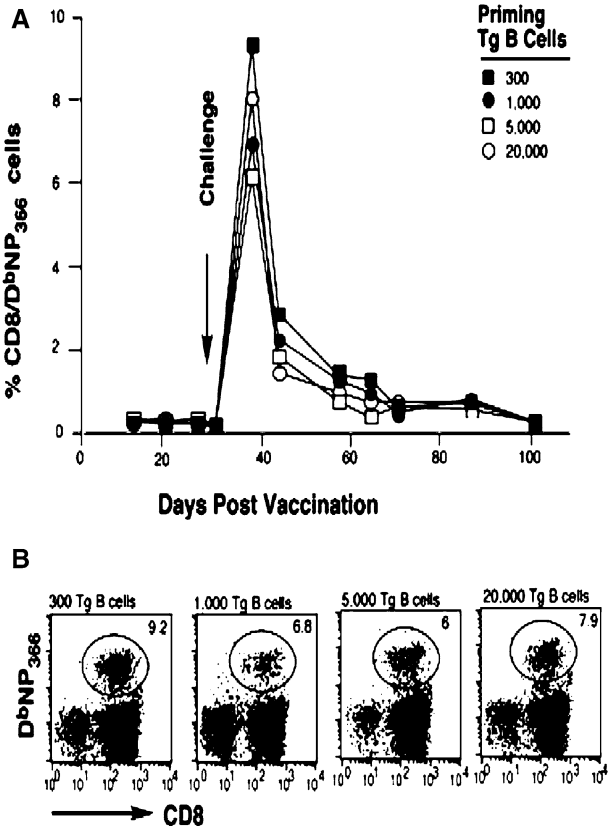

Fig. 4 Analysis of the response in mice primed with different doses of transgenic B lymphocytes shows that a small immunizing dose yields an expansion of memory CD8 T cells comparable to a high dose. (**A**) Mice were primed by single injection of transgenic B lymphocytes ranging from 3×10^2 to 2×10^4 cells/inoculum. Mice were challenged with a sub-lethal dose of A/PR8/34 influenza virus *i.n.* on day 28 after priming and PBLs were analyzed longitudinally as shown in the figure. (**B**) Specificity of the staining with the DbNP366 tetramer. PBLs were collected on day 11 after challenge from groups of mice primed with different doses of transgenic lymphocytes (as indicated in each panel). Peripheral blood was pooled from each group (four mice per group) and cells were then stained with an anti-CD8 monoclonal antibody and DbNP366 tetramer. The percentage of DbNP366 specific CD8 T cells is indicated in each panel. (From [65])

Studies to date exist with respect to protection against viruses and parasites, with only one publication available within the context of tumors. The initial studies by Ahmed and colleagues showed that viral replication *in vivo* is more effectively controlled by CD8 T_{CM} cells [71], consistent with the fact that CD8 T_{CM} cells possess greater *in vivo* cytotoxicity than T_{EM} cells [72]. Protection from disease was addressed in my laboratory using antigen presenting B lymphocytes transgenic for a single CD8 T cell epitope of the A/PR/8/34 influenza virus. In this model system, a single injection of a low dose of (3×10^2 cells/inoculum) vaccine was found to protect from lethal challenge [65] (Table 3). The adoptive transfer of immune $CD62L^{lo}$ and $CD62L^{hi}$ CD8 T cells into naive recipients revealed that resistance to lethal virus challenge in the adoptive recipients was significantly correlated with $CD62L^{hi}$ CD8 (T_{CM}) cells [65]. An experiment in non-human primates (Rhesus macaques) with Genovetta Franchini at the National Cancer Institute confirmed the validity

Table 2 Differential properties of T_{CM} and T_{EM} cells

	T_{CM}	T_{EM}
CD62L/CCR7	+++	±
LN homing	+++	−
Homeostatic proliferation	+++	+
Killing in vivo	++++	++
Ag-driven proliferation	++++	++
IL-2 production	++++	++
Telomere length	++	+

Table 3 Cell dose priming vs. protection in vivo

TLI priming (No. cells)	Lethal challenge (PR8 10^5 PFU)	Survival
γINV^2NP^3		
5,000	+	4/4
1,000	+	6/6
300	+	8/8
100	+	2/7
20	+	0/4
Control DNA		
5,000	+	0/15

From [65]

of this postulate. Using the SIV model, we found an inverse correlation between the frequency of T_{CM} but not T_{EM} cells and virus levels following challenge exposure [73]. Importantly, in SIV-infected animals, in which viral replication was suppressed by anti-retroviral therapy CD8, T_{CM} cells could be expanded by vaccination and again correlated with protection from disease. Others reported that CD8 T_{CM} cells are preferentially associated with protection against *Leishmania major* infection [74], and that the adoptive CD8 T_{CM} cells followed by vaccination confers high protection against experimental tumors [75]. The new emerging paradigm is that T_{CM} cells have a selective advantage for protective responses against disease, a property likely explained by their higher response to antigen recall, higher production of IL-2, higher killing capacity *in vivo*, and propensity to localize to secondary lymphoid organs where APCs reside (Table 2) and where re-activation via direct priming or cross-priming can rapidly occur (e.g., following infection or release of apoptotic tumor cells). Exceptions have been reported and in certain conditions protective advantage has been found to be associated with T_{EM} cells [76, 77].

Précis on protective responses mediated by T cells

The above considerations place new weight on the qualitative rather than quantitative aspects of the immune response. In this context the aphorism ‘‘more is better’’ appears to be of little value and possibly deleterious for vaccine strategies against intracellular

pathogens as these should be tailored to optimally promote the differentiation of naïve precursor CD8 T cells into T_{CM} cells and maintain them through homeostatic proliferation until antigen is re-encountered. I summarize hereunder the set of principles that, at the present time, appear central to the induction of protective T cell responses against pathogens and possibly tumors for which T_{CM} cells offer the best correlate of protection.

(1) A program for protective T cell responses requires adhering to parameters that control the induction of T_{CM} cells (low antigen dose, low inflammation, T cell help, and protracted antigen stimulation during priming). This includes imprinting lineage selection, i.e., the generation and/or the selective expansion of T_{CM} vs. T_{EM} cells.
(2) Successful lineage imprinting will favor the induction and long-term maintenance of memory T cells under conditions that minimize the process of cell senescence after clonal expansion and thereafter. Since there is no way to slow down senescence directly (e.g., controlling the transcriptional activation of telomerase or otherwise reducing telomere attrition), it is important to limit the negative impact of antigen dose and inflammation at the time of priming.
(3) Expansion of T cells upon antigen recall is an intrinsic property of memory T cells ($T_{CM} > T_{EM}$), not a reflection of their number prior to re-encounter with antigen and is an acquired property based on the availability of T cell help at the time of priming.

By controlling priming in qualitative and quantitative terms one can then dictate the quality of the memory T cell response. Because as shown above the antigen dose is an integral part of the programming process it follows that in the context of an existing immune response (e.g., chronic viral infection) it is first necessary to ''reset the clock'' of antigen load, e.g., abating viremia with anti-retroviral therapy. There are several reports on the negative impact of high viral load on the maintenance and effector function of memory T cells [78, 79]. Collectively, these simple principles should enable the design of strategies for the successful vaccination of the *immunologically inexperienced* individual (prophylactic vaccination) as well as the *immunologically experienced* individual (therapeutic vaccination).

The bone marrow. A sanctuary for T_{CM} cells

In the spirit of the Robert A. Good Symposium, a reference to the bone marrow in the context of what has been discussed in the previous sections could not be overlooked. In the immune system, organizational division of labor is one of many facets adding to its complexity. A fact that has emerged in the past few years is that the bone marrow is where T_{CM} cells are preferentially recruited and accumulate [80]. There could be various reasons for this occurrence. One possibility is that the bone marrow provides an environment rich in IL-7 and IL-15, two cytokines that favor homeostatic proliferation, hence providing a shelter where T_{CM} cells can be maintained [81, 82]. Alternatively, it could be that T cells in the bone marrow simply fulfill functions useful to the bones [83]. Resident bone marrow T_{CM} cells have the same characteristics of T_{CM} cells in the spleen or lymph nodes, expanding to antigen rapidly and producing effector cytokines [80]. Apart from cases in which T_{CM} cells could carry their killer function directly in the bone marrow (for instance killing tumor cells resident in the bone marrow [84]), it remains unclear how hibernation in the bone marrow could be interrupted by antigen in the periphery. In other words: How do bone marrow resident T_{CM} cells encounter antigen and what causes them to migrate out of the bone marrow? One can envision many possible scenarios but a provisional solution

may lie in the observation that antigen-laden dendritic cells migrate to the bone marrow even though their number is only a fraction (3–10%) of what would normally exist or recirculate through draining lymph nodes [85]. It is possible that dendritic cells even in such a small number are sufficient to mediate antigen presentation in the bone marrow and reactivate resident T_{CM} cells. Whether or not this event is also sufficient to make T_{CM} cells leave the bone marrow is unclear. Functional studies need to address these issues. In spite of these unknowns, it has already been demonstrated that antigen-reactivated memory T cells from the bone marrow of cancer patients are effective in causing the regression of autologous tumor xenotransplants in NOD/SCID mice [86].

Conclusions

Immunological memory represents possibly the most essential component of the adaptive immune response following natural infection or vaccination. Memory responses that block or curtail infection are mediated by antibodies and are dependent on the pathogen's incubation period [10]. Memory responses that prevent disease are mediated by memory T cell responses for which the dependence on the pathogen's incubation period is less firmly established. Purposeful T cell immunity can be obtained in most cases by vaccination by programming the prevalent induction of T_{CM} cells over T_{EM} cells. This requires controlling the generation, maintenance and expansion upon re-encounter of antigen of this type of memory T cells. The ideas presented here support the view that lineage selection together with other characteristics of memory T cells can be imprinted at the time of priming. It is my view that factors such as (a) low antigen dose, (b) T cell help, and (c) absence of overt inflammatory conditions combined, favor emergence of memory T cells and T_{CM} cell lineage, differentiation and maintenance, including de-acceleration of replicative senescence and telomere attrition. As to post-infection vaccination, similar principles apply but require that antigen levels be abated by anti-retroviral therapy or other measures first. Ultimately, since after their induction T_{CM} cells lodge in great number and proportion in the bone marrow, it is almost certain that the future success of vaccination will involve the bone marrow. The pioneering work of Robert A. Good whose memory we convened to honor cannot be better rewarded.

Acknowledgments This work was supported by National Institutes of Health (NIH) grants AI062894 and CA092119.

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