

# Immunological memory in humans

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## Abstract

Specificity and memory are the defining characteristics of adaptive immune responses. Vaccines are predicated on the existence of immune memory, and the robustness of immune memory is a primary determinant of vaccine efficacy. How is immune memory maintained? Much progress has been made in this area over the past several years, and new human studies have added key insights into the longevity of B and T cell immune memory in the absence of antigen.

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## 1. Introduction

The primary function of the immune system is to defend against microbial pathogens. It accomplishes this task at two levels: first by generating a specific immune response against the invading pathogen to control the infection; and second by remembering the pathogen. Immune memory can take the form of a constant vigilance (circulating antibody) and/or the ability to mount an accelerated immune response upon re-exposure to the same pathogen (memory T cells, memory B cells). This rapid recall response can either completely prevent disease or greatly lessen the severity of clinical symptoms. The first documentation of immune memory dates back to the time of the Greek historian Thucydides who recorded that the “same man was never attacked twice” while describing the plague of Athens in 430 B.C. [1]. It is remarkable that nothing was known about the immune system or about microbes when Thucydides made his astute observations on immune memory; it would be more than 2000 years before we gained an appreciation of the immune system and learned that microbes cause infectious diseases.

However, in the 50 years since Burnet’s seminal theory of clonal selection [2], it has now become well established that memory and specificity constitute the two defining features of adaptive immune responses. In this review we will summarize the state of knowledge of immune memory in animal

models—where a great deal of work has been done—and then will cover some recent studies focused on translating that animal model knowledge into an understanding of immune memory in humans.

Immunological memory is the basis for vaccination. The discipline of vaccinology originated from the observation that previous exposure to a disease conferred resistance to a subsequent episode. The earliest attempts to put this into practice were made in China and India around 900 A.D. [3]. Those ancient techniques involved inoculating pustular material from smallpox patients into healthy people. This process, called “variola”, resulted in disease but it was generally a milder disease than a natural smallpox infection. Importantly, “variolated” individuals developed immunological memory and were then protected against naturally acquired smallpox. Though crude and inconsistent, this process gradually became common practice throughout China, India, the Middle East, Africa, and (later) Europe and was the precursor of modern smallpox vaccination—indeed, variolation was the precursor of all forms of modern vaccination.

How long does immune memory last? The mechanisms involved in sustaining immunological memory can be divided into two categories: antigen-dependent and antigen-independent. Periodic re-exposure to the pathogen is an effective way to maintain high levels of immunity, as such re-infections (or reactivation of latent infections) are usually asymptomatic or produce only mild clinical symptoms and serve as a natural “booster” to the immune system. Having said this, it is equally important to understand immune memory in the absence of anti-

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Table 1  
Long-term human immunity in the absence of re-exposure to the pathogen

Infection	Duration of immunity (years)
Measles on the Faroe Islands [4]	65
Yellow fever virus in Norfolk, VA [6]	75
Polio in remote Eskimo villages [5]	40

gen, to know how long immunity lasts to a non-endemic pathogen.

There are several classic examples that have clearly documented long term protective immunity lasting up to 75 years in humans in the absence of re-exposure to the pathogen (Table 1) [4–6]. These observations have been crucial in shaping our ideas about immunological memory because they showed that the immune system could remember an encounter that occurred many years ago. But those observations were generally crude, and modern molecular immunology techniques must be brought to bear on these issues to develop a mechanistic understanding of the multiple components of immune memory in humans.

## 2. Basic studies in immunological memory

During the past decade, major advances have been made in identifying antigen-independent mechanisms of maintaining immunological memory [7–10]. Using a combination of approaches to analyze a variety of immune responses in mice (using both infectious and non-infectious model systems), it is now clear that memory CD4<sup>+</sup> T cells, memory CD8<sup>+</sup> T cells, and memory B cells can persist in the absence of antigen [7,8,10,11].

### 2.1. Memory B cells

The longevity of memory B cells in mice in the absence of antigen has been controversial. Gray and Skarvall originally reported cell adoptive transfer studies of B cells into irradiated animals indicating that memory B cells have an extremely short lifespan in the absence of antigen (~3 days) [12]. However, experiments done shortly thereafter by Rajewsky and coworkers in intact animals suggested the memory B cell numbers were stable, and they observed a slow rate of memory B cell proliferation (<1 division per month), indicating that the proliferation was homeostatic and not antigen driven [13]. Experiments done by others have suggested that the early observations by Gray and coworkers may be an unexpected artifact due to the use of irradiated host animals [14]. Bachmann and coworkers performed memory B cell adoptive transfers into unirradiated animals, and those cells were maintained [14]. More recently, a transgenic approach utilized by Rajewsky and coworkers has largely settled the matter [11]. Rajewsky engineered mice

with a genetic switch, such that memory B cells expressing an NP-specific B cell receptor (BCR) could be changed in vivo to instead express a PE-specific BCR. By doing this they could track the PE-specific B cells (which have never seen antigen (PE)), and they observed that the cells persisted in an antigen-independent manner just as well as other memory B cells [11]. It should be noted that Rajewsky and coworkers have also shown that B cells must constantly maintain BCR expression for survival [15], and therefore it is possible that constant low-affinity (or very low affinity) cross-reactive binding is involved in B cell survival (naïve and memory).

### 2.2. Long-lived plasma cells

There are two populations of plasma cells: long-lived and short-lived. Long-lived plasma cells are a central part of immune memory, as these cells are largely responsible for the long term continuous secretion of antibody. In contrast to memory B cells, plasma cells are terminally differentiated and cannot be stimulated by antigen to either divide or increase their rate of antibody production. Since pre-existing antibody provides the first line of defense against infection by microbial pathogens, the importance of plasma cells in protective immunity cannot be overstated.

The traditional view was that all plasma cells (antibody secreting cells) are short-lived cells-half-life estimates ranging from 3 to 14 days and therefore continuous antigenic stimulation of memory B cells was necessary to replenish the pool of rapidly dying plasma cells and thereby maintain antibody production. This view has been replaced by a new model describing two populations of plasma cells: short-lived plasma cells that produce antibody shortly after antigen exposure, and long-lived plasma cells that survive for extended periods (half-life = 3–4 months in mice) (Fig. 1) [16–20]. This finding may provide an explanation for the remarkable longevity of antibody responses seen in humans after certain acute infections and vaccinations. Teleologically, the current model of a B cell response generating short-lived plasma cells first and long-lived plasma cells and memory B cells later is appealing because, in an infection, it is valuable to get anti-pathogen antibodies produced as rapidly as possible (which is done by those antigen-specific B cells that differentiate into plasma cells early in extrafollicular foci), but it is also important to evolve high affinity antibodies that are much more efficient at controlling and eliminating the pathogen (which is done by the antigen-specific B cells that initiate germinal centers). The plasma cell response in secondary lymphoid tissues peaks during the first 2 weeks and then declines within 2–4 weeks after infection [19,21]. As splenic plasma cell populations decline, antigen-specific plasma cells begin to migrate to and/or accumulate in the bone marrow compartment [22,23]. After the germinal center reaction subsides, the bone marrow becomes the predominant site of antibody production, with

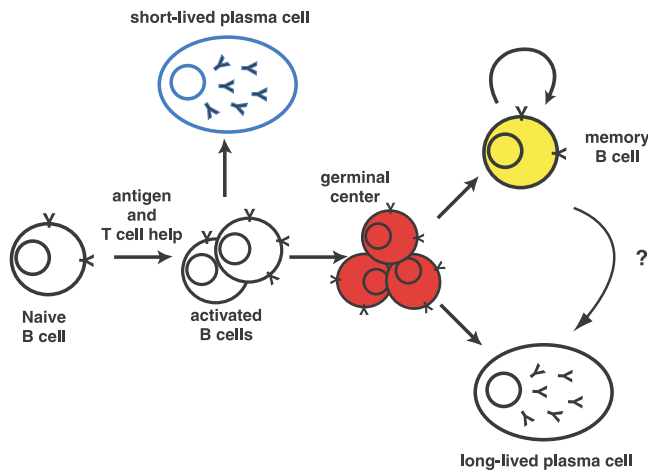


Fig. 1. Memory B cell and plasma cell differentiation. Following antigenic stimulation, naïve B cells undergo clonal expansion and form clusters of activated B cells known as extrafollicular foci. These activated B cells can either differentiate into short-lived plasma cells, or they can migrate back into the follicle and initiate a germinal center reaction. After proliferation and affinity maturation, germinal center B cells produce both long-lived plasma cells that produce high affinity antibodies and memory B cells that have high affinity B cell receptors. Memory B cells presumably self-renew by homeostatic proliferation. Memory B cells may also periodically differentiate, in an antigen-dependent or antigen-independent manner, into long-lived plasma cells to maintain long-term antibody production.

80–90% of the host's plasma cells located in this compartment [19,21].

### 2.3. Memory T cells

From a number of studies, it is now clear that memory CD4<sup>+</sup> T cells and memory CD8<sup>+</sup> T cells can persist in the absence of antigen (reviewed in detail elsewhere [7,10,24]) (Fig. 2). It has also been shown that memory T cells are not static; they regularly undergo homeostatic proliferation to replenish their numbers and that this proliferative renewal does not require stimulation with antigen or MHC I [25–30]. Much recent work has been focused on the role of cytokines in establishing and maintaining T cell memory. IL-15 and

IL-7 are critical for the maintenance of memory CD8<sup>+</sup> T cells [31–34], but only IL-7 is essential for maintenance of memory CD4<sup>+</sup> T cells [31,35]. It has now been shown that IL-7 is also a critical factor in the generation of CD4<sup>+</sup> and CD8<sup>+</sup> T cell memory [35–38]. Cytokines such as IL-15 and IL-7 may play key roles in competition between naïve and memory cells [39,40], and between memory T cells of different specificities [41–43]. In another area of work, several recent studies have shown that CD4<sup>+</sup> T cells are generally not essential to generate effector CD8<sup>+</sup> T cell responses but are essential to generate quality memory CD8<sup>+</sup> T cells [44–46], though this may vary in different situations [47]. In keeping with the truism, the advances over the past few years in understanding T cell memory have opened up as many questions as they have answered. The next big questions are: what is the nature of CD4<sup>+</sup> T cell help to CD8<sup>+</sup> T cells and how pervasive or varied are the issues of CD8<sup>+</sup> T cell memory quality [48,49]? And how is the birth and death of memory T cells regulated to maintain constant numbers (or relatively constant numbers [43,50]) over the life of the animal?

In summary, experimental studies done in mice have unequivocally shown that memory CD8<sup>+</sup> T cells, memory CD4<sup>+</sup> T cells, memory B cells, and long-lived plasma cells can often persist for the life of the mouse ( $\geq 2$  years) in the absence of antigen. What about in humans?

### 3. Immune memory in humans

There are classic reports of long term immunity in humans in the absence of re-exposure to a pathogen, as mentioned earlier (Table 1) [4–6]. However, those studies have left many important questions unanswered. How stable is long term immune memory in humans? And is the stability similar or different in the multiple compartments of the adaptive immune system (circulating antibodies, memory CD8<sup>+</sup> T cells, memory CD4<sup>+</sup> T cells, and memory B cells)? If immune memory is not stable, what are the kinetics of decline? What are the cellular and molecular processes involved in

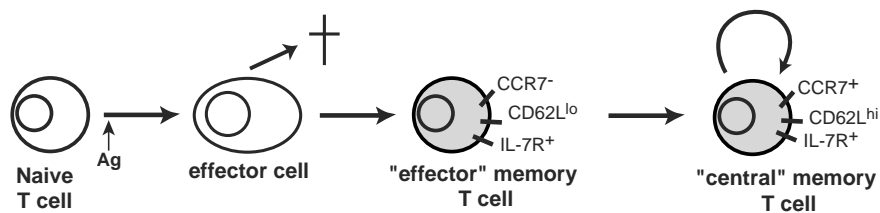


Fig. 2. Memory T cell differentiation. Naïve T cells proliferate and differentiate into effector cells in the presence of antigen and costimulation. It appears that then an IL-7R<sup>hi</sup> subset of effector cells differentiate to become memory T cells [36,38]. Memory T cells have been divided into two categories: “effector memory” and “central memory” [9]. Recent work suggests that these are two stages of a linear differentiation of memory T cells, with full maturation resulting in “central memory”-type cells (CD62L<sup>hi</sup>CCR7<sup>+</sup>) [72]. Memory T cells undergo self-renewal via homeostatic proliferation. Memory T cell homeostatic proliferation predominantly occurs in the central memory subset. There are several other models of T cell differentiation, which are discussed in detail elsewhere [7,9,10,24].

Table 2  
Kinetics of long-term immune memory in humans in the absence of antigen

Vaccination	Duration of immune memory	Reference
Inactivated poliovirus vaccine in Sweden	Serum antibody = 30+ years	[51]
Smallpox vaccine (vaccinia virus), USA	Memory B cells = 60+ years	[52]
	Serum antibody = 60+ years Memory CD4 <sup>+</sup> T cells = 14 years $t_{1/2}$	[54]
	Serum antibody = 75+ years Memory CD4 <sup>+</sup> T cells = 8–12 years $t_{1/2}$ Memory CD8 <sup>+</sup> T cells = 50%, 8–15 years $t_{1/2}$ ; 50%, short $t_{1/2}$ or sudden loss?	

memory cell maintenance? These questions are generally difficult to answer in humans, both because the timeframe of interest is decades and it is difficult to find situations where re-exposure to antigen/vaccine/pathogen can be excluded as a source of intermittent “booster immunizations” maintaining the immune memory. Three recent human studies have found ways around these issues and have made key contributions to our understanding of human immune memory (Table 2) [51–53]. These studies build on pivotal earlier T cell and serological studies in humans [54–62].

Many vaccines induce serum antibody responses that persist for decades [63], but most reports of durable antibody responses in humans are plagued by nagging questions about the potential of intermittent re-exposure to the antigen (live measles/mumps/poliovirus vaccines given to nearby children, tetanus in the soil, etc.). An impressive study of antibody levels that avoided this issue was the large cross-sectional study of poliovirus immunity done in Sweden [51].

The Swedish population is almost ideal for such a study, for several reasons: (1) poliomyelitis has been eliminated in Sweden since 1962; (2) only inactivated poliovirus vaccine (IPV, the Salk vaccine) provided by a single supplier has ever been used in the Swedish population; (3) the final booster immunization is given at the young age of 5 years old; (4) the enterovirus infection burden in Sweden is extremely low, and there are very few opportunities for introduction of poliovirus from foreign sources; and (5) Sweden has excellent health care records and public health surveillance. Given those factors, it was striking to see that when the Swedish population was surveyed for poliovirus immunity in 1991, substantial anti-poliovirus antibody titers were detected in all age groups [51]. Interestingly, there was virtually no difference in serum antibody titers among the different age groups (all at more than 10 years post-vaccination), indicating that anti-poliovirus antibody titers are stably maintained

in the absence of additional immunizations or exposure to live virus [51]. Interestingly, declining levels of anti-tetanus and anti-diphtheria antibody titers were observed in that same study, suggesting that not all immune memory is created equal [51].

Though the Swedish study described above is a valuable study, it was designed as an epidemiological study, and was only focused on antibody titers. Two cross-sectional studies published this year looked at antigen-specific immune memory in humans in much greater detail, using the smallpox vaccine as their model system [52,53]. Immune memory after smallpox vaccination (DryVax, vaccinia virus (VV)) is a valuable benchmark for understanding the kinetics and longevity of B and T cell memory in the absence of re-exposure to antigen, since immunization against smallpox was standard but was stopped in 1972 (in the USA) and smallpox disease was declared eradicated worldwide in 1980 [3]. Since the smallpox vaccine is a live virus vaccine, it is also an excellent model of a well-defined acute infection. In addition, there is currently great public health interest in smallpox immunity due to the possible threat of bioterrorism [64].

### 3.1. Long-term humoral immunity in humans

Crotty et al. showed that smallpox vaccine-specific memory B cells can be detected for 60 years or greater after vaccination [52]. Importantly, memory B cell levels appeared to be stable from 10 to 60 years post-vaccination, indicating that antigen-specific memory B cells are maintained by robust homeostatic mechanisms. Of interest, there was a 10-fold drop from peak memory B cell responses that occurred sometime before 10 years post-vaccination/infection (the exact time is currently unclear and needs to be determined by additional studies) and then the memory B cell frequency stabilized. This biphasic kinetic was unexpected. The two phases observed may be due to: (1) migration of memory cells out of circulation over several years (perhaps due to a differentiation program designed to keep cells in circulation in the near term, to be most available for protection against diseases that are endemic or cause frequent epidemics); (2) cellular competition (or programmed cell death) making space for new memory B cells; or (3) short-term (1–5 years) retention of antigen by follicular dendritic cells [65–67] before exhaustion of the antigen depot and subsequent decline in memory B cell numbers to antigen-independent homeostatic levels.

Crotty et al. [52] and Hammarlund et al. [53] both demonstrated that antibody responses after smallpox immunization were maintained for more than 60–75 years. Impressively, the serum levels of anti-VV antibodies were stable from 1 to 60+ years post-immunization. Similar anti-VV antibody data has been published in other studies [68,69]. Total anti-VV antibody levels correlated reasonably well with neutralizing anti-VV antibody levels, providing some indication that antibody responses of different specificities are

maintained equally well. In addition, Crotty et al. showed that there was a positive correlation between anti-VV memory B cell frequency and anti-VV serum antibody levels [52]. Bernasconi et al. recently showed a similar positive correlation for other antigens using a related technique [70]. In both cases the correlation was moderate, indicating that there may be multiple factors controlling the relationship between circulating memory B cell levels and circulating antibody levels. A third study observed no correlation, again indicating that multiple factors may control the relationship between memory B cells and serum antibody levels [58].

How is the antibody production maintained for 60+ years? Long-lived plasma cells are crucial for the maintenance of antibody levels. But with a half-life of 3–4 months (as determined in mice [18]), long-lived plasma cells must still be replenished to maintain antibody levels for years/decades. One proposal is that long-lived plasma cells in a human may, in fact, live for decades. A second proposal is that intermittent antigen-independent differentiation of memory B cells to long-lived plasma cells occurs, possibly linked to homeostatic proliferation of memory B cells (Fig. 1). Lanzavecchia and coworkers have recently explored this issue in humans and have proposed an alternative model: bystander activation of memory B cells (during an irrelevant infection) results in polyclonal stimulation of memory B cells, driving their differentiation into long-lived plasma cells [70]. A separate study did not observe bystander activation [59]. Resolution of these disparate models awaits further study. Also, the physiology of long-lived plasma cells has not been studied in humans, and this is a key area for future research that may help illuminate the relationship between memory B cells, long-lived plasma cells, and the maintenance of humoral immunity.

### 3.2. Long-term T cell memory in humans

These new studies of anti-smallpox immune memory also showed that memory T cell responses were long-lived in the absence of antigen [52,53], following up on the earlier work of Ennis and coworkers [54]. Both recent studies observed that memory T cell levels were long-lived but declined with a half-life of 8–15 years [52,53]. Memory CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells were both generated after smallpox vaccination [53,71]. Slifka and coworkers performed a detailed analysis of the memory T cell populations and showed that IFN $\gamma$ <sup>+</sup>TNF $\alpha$ <sup>+</sup> memory CD4<sup>+</sup> T cells declined with a half-life of 8–12 years [53]. But, surprisingly, the IFN $\gamma$ <sup>+</sup>TNF $\alpha$ <sup>+</sup> memory CD8<sup>+</sup> T cells did not follow the same pattern. In 50% of individuals CD8<sup>+</sup> T cell memory slowly declined with a half-life of 8–15 years, but in the other 50% of individuals CD8<sup>+</sup> T cell memory was rapidly lost by some unknown point between 3 and 20 years post-immunization [53]. This was a very puzzling finding. Why would CD8<sup>+</sup> memory behave so differently in half of the people? This is an intriguing issue that warrants further investigation.

## 4. Conclusions

Overall, these studies have now made an important step forward in our understanding of human immune memory by studying the kinetics and longevity of memory B cells, antibodies, memory CD4<sup>+</sup> T cells, and memory CD8<sup>+</sup> T cells in the same individual, all to the same pathogen, where the maintenance of immune memory could be observed in a well-defined antigen-independent situation. The studies raise interesting questions and pave the way for a variety of future studies into the mechanisms of human immune memory.

Why would memory B cells be able to survive for longer than memory T cells? It is difficult to think of molecular mechanisms that would only manifest differences over a timeframe of decades, when it is expected that memory lymphocytes undergo homeostatic proliferation approximately once every few months (though these studies have yet to be done in humans). One possibility is that memory B cells possess more robust DNA repair systems such that, over time, they are more competent to repair damage from environmental insults and thereby maintain their proliferative potential. This may be somehow tied to a clear developmental difference between mature B and T lymphocytes: mature B cells maintain the ability to undergo recombination and somatic hypermutation, which are dependent on DNA repair systems.

Many questions remain about these different compartments of immune memory. Are the patterns of long-term maintenance of memory B cells, memory T cells, and antibody levels observed in these new studies going to hold true for situations other than smallpox immunization? Or do different immunizations/infections result in memory responses that behave differently [56,57]? That will almost certainly be the case for chronic and latent infections (due to the presence of antigen), but it may also be true for different types of vaccines and acute infections. And do these patterns hold true for all memory T cell subsets? These and other questions await future studies.

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