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Jump-starting the immune system: prime–boosting comes of age

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A major challenge for immunologists has been the development of vaccines designed to emphasize cellular immune responses. One particularly promising approach is the prime–boost strategy, which has been shown to generate high levels of T-cell memory in animal models. Recently, several papers have highlighted the power of prime–boost strategies in eliciting protective cellular immunity to a variety of pathogens and have demonstrated efficacy in humans. Coupled with recent advances in our understanding of the mechanisms underlying the generation, maintenance and recall of T-cell memory, the field is poised to make tremendous progress. This Review discusses the impact of these recent developments on the future of prime–boost vaccine strategies.

One of mankind's greatest achievements has been the development of vaccines to control infectious disease. Some of the more successful vaccination regimens have either eliminated or completely controlled scourges, such as smallpox and polio. Despite this success, it has become apparent that certain pathogens are not readily controlled by current vaccination approaches. These 'problem' pathogens include HIV, *Mycobacterium tuberculosis* and the malaria parasite, all of which resist the humoral immunity that is characteristically generated by traditional vaccines [1]. Over the past few years, significant effort has been directed toward developing vaccines designed to promote potent cellular immunity to these and related pathogens. The induction of cellular immunity, however, is complex and poses substantial problems for vaccinologists. These include the difficulties in generating cellular immunity that is of sufficient strength, longevity and anatomical distribution.

An obvious approach for establishing strong cellular immunity to specific pathogens is through repeated vaccination. The idea of 'boosting' immune responses has been around as long as vaccines and repeated administrations with the same vaccine (homologous boosting) have proven very effective for boosting humoral responses. However, this approach is relatively inefficient at boosting cellular immunity because prior immunity to the vector tends to impair robust antigen presentation and the generation of appropriate inflammatory signals. One approach to circumvent this problem has been the sequential administration of vaccines (typically given weeks apart) that use different antigen-delivery systems (heterologous boosting). Generically referred to as 'prime–boosting,' this strategy is effective at generating high levels of T-cell memory [2]. Although much of the early work using this strategy was driven by efforts to develop vaccines to control malaria, it was subsequently applied to vaccine development against a variety of pathogens [3]. Given rapidly breaking advances in our understanding of T-cell memory, the field is poised to make substantial progress in the near future.

Prime–boost strategies – recent developments

The basic prime–boost strategy involves priming the immune system to a target antigen delivered by one vector and then selectively boosting this immunity by re-administration of the antigen in the context of a second and distinct vector. The key strength of this strategy is that greater levels of immunity are established by heterologous prime–boost than can be attained by a single vaccine administration or homologous boost strategies. With some of the early prime–boost strategies this effect was merely additive, whereas with some of the newer strategies (usually involving poxvirus or adenovirus boosting) powerful synergistic effects can be achieved. This synergistic enhancement of immunity to the target antigen is reflected in an increased number of antigen-specific T cells, selective enrichment of high avidity T cells and increased efficacy against pathogen challenge [4,5] (Figure 1). In addition, although early studies focused predominantly on $CD8^+$ T-cell responses, it has now become clear that both $CD4^+$ and $CD8^+$ T cells can be strongly induced using appropriate prime–boost strategies.

Recently, several studies have demonstrated the efficacy of prime–boost vaccination strategies in generating cellular immunity to a variety of pathogens. These include, *M. tuberculosis* [6–9], HIV and simian immunodeficiency virus (SIV) [10–18], malaria [19–21], *Listeria monocytogenes* [22], leishmania [23], Ebola virus [24,25], hepatitis C virus [26,27], herpes simplex virus [28,29], human papillomavirus [30] and hepatitis B virus [31]. The tremendous power of prime–boosting was recently further highlighted in a murine model of *M. tuberculosis*. Mice that had been intranasally vaccinated with bacille Calmette–Guerin and then boosted with a recombinant vaccinia virus expressing antigen complex 85A had an ~300-fold reduction in bacterial load in the lungs following aerosol challenge with *M. tuberculosis* (relative to controls) [32]. This level of bacterial control in

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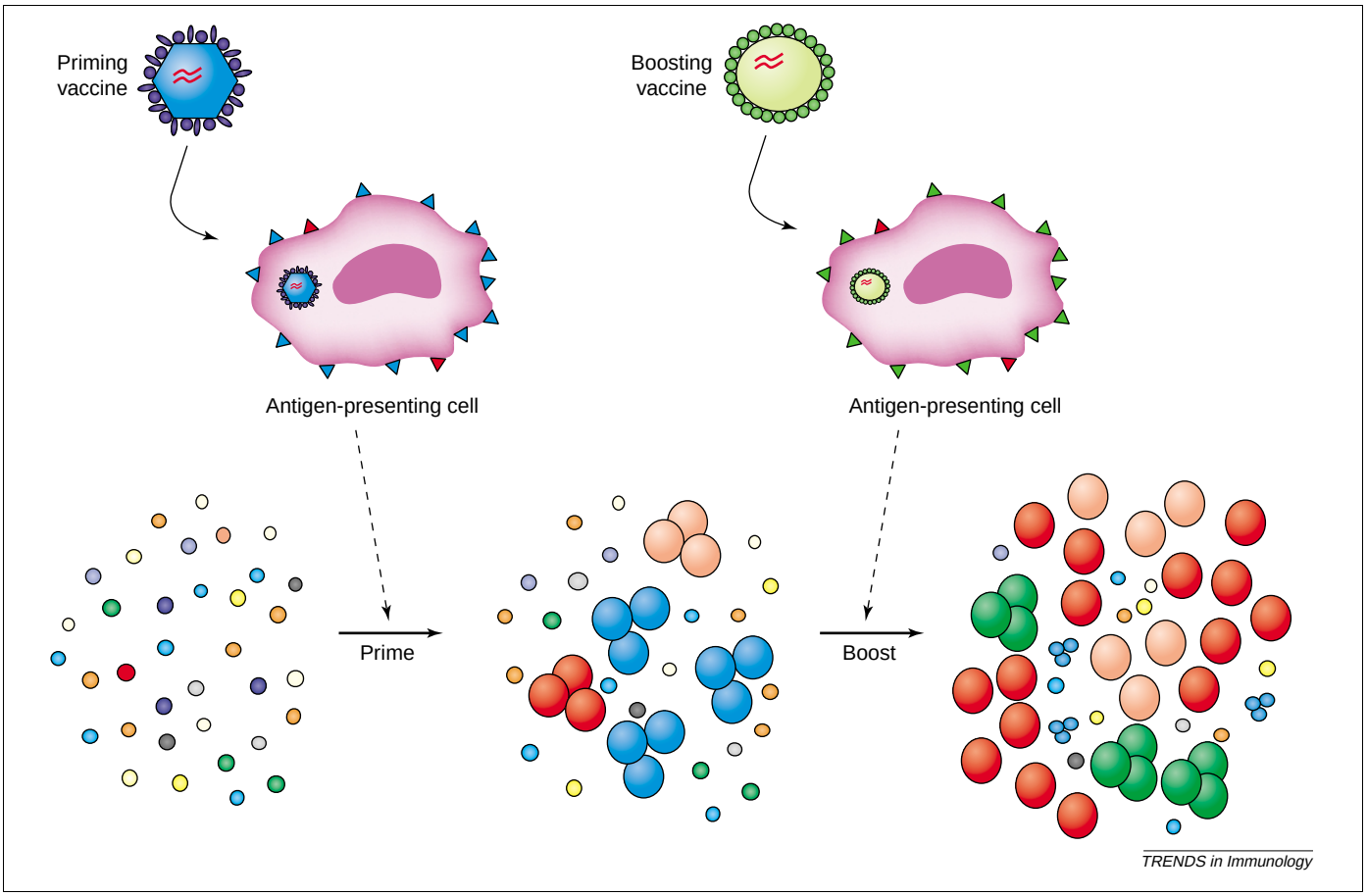

Figure 1. Prime–boost vaccination strategies synergistically amplify T-cell immunity to specific antigens. Priming with the first vaccine results in the presentation of both the target antigen (red triangles) and vector antigens (blue triangles) on antigen-presenting cells (APCs). APCs then stimulate naïve T cells in the lymph nodes and drive the expansion of both target-specific T cells (red cells, high avidity cells are indicated by the darker red) and vector-specific T cells (blue cells). Subsequent boosting with a second vaccine results in the re-presentation of the target antigen (red triangles) and antigens from the second vector (green triangles) on APCs. These APCs then drive the expansion of target-specific memory T cells (red cells) and vector-specific naïve T cells (green cells). This results in both a synergistic expansion of the T cell specific for the target antigen and selection of T cells that have greater avidity for the antigen. The situation with priming and boosting vectors that induce strong T-cell responses to themselves, as well as the target antigens, is shown. However, it should be noted that many vectors, such as DNA and some of the popular replication-defective viral vectors, induce little or no response to the vectors themselves. This is probably a key issue underlying their efficacy.

vaccinated mice is extraordinarily high for experimental vaccination studies in this system. Interestingly, high levels of protection were also seen with homologous boosting, for reasons that are currently unclear. These finding have significant implications for human vaccination against tuberculosis.

Although studies in animal models have been useful, the real challenge for vaccine strategies is to demonstrate efficacy in humans. In this regard, the use of the prime–boost approach for human vaccination was beautifully illustrated in a recent study using DNA- and vaccinia-based vaccines for a pre-erythrocytic malarial antigen [33]. Responses to the prime–boost regimen were five- to ten-fold higher than to either DNA or vaccinia virus vaccines alone. This demonstrated the basic tenet of prime–boosting, namely, a synergistic effect of the two vaccines. In addition, both $CD4^+$ and $CD8^+$ T-cell memory was established and there was evidence for efficacy in a challenge experiment. Of particular interest was the observation that the frequencies of antigen-specific T cells elicited by prime–boosting were greater than those typically found in individuals naturally exposed to malaria. However, the significance of this in terms of protective efficacy is unclear because the natural infection probably induced a broad immunity to many antigens, whereas the vaccine induced a focused response to a single antigen.

The general efficacy of prime–boost vaccination in humans remains to be determined. However, several clinical trials are in progress and some early results are promising [34,35].

What are the mechanisms underlying prime–boosting?

In some respects, prime–boosting can be considered a form of original antigenic sin, a phenomenon that was originally described for antibody responses [36]. The basic observation was that the antibody response generated by a first exposure to influenza virus dominates the response to subsequent infections with influenza virus variants that share only partial homology. In other words, the initial priming events elicited by a first exposure to the virus appear to be imprinted on the immune system. This phenomenon is particularly strong in T cells and is exploited in prime–boost strategies to selectively increase the numbers of memory T cells specific for a shared antigen in the prime and boost vaccines. These increased numbers of T cells 'push' the cellular immune response over certain thresholds that are required to fight specific pathogens

[1,37]. Furthermore, the general avidity of the boosted T-cell response is enhanced, which presumably increases the efficacy of the available T cells [5].

Therefore, what is the mechanism by which prime–boosting synergistically amplifies T-cell memory? One contributing factor is the phenomenon of T-cell immunodominance [38,39]. T-cell responses to different antigens are highly competitive, resulting in a hierarchy of dominant and subdominant epitopes. A T-cell response to a dominant epitope in a pathogen will often suppress the development of a response to a subdominant epitope, which tends to focus the T-cell response on relatively few epitopes in a pathogen. Immunodominance is controlled at two levels [38]. First, intrinsic mechanisms, such as antigen availability and antigen processing, regulate the hierarchy of peptide epitopes presented by MHC complexes on the cell surface. Second, T-cell competition for antigen-presenting cells or other limited resources, such as cytokines, might regulate the level of T-cell priming and expansion. It is this competitive aspect of the response that enables the T-cell response to some epitopes to dominate, whereas others are suppressed. This phenomenon might enable a vaccine boost to greatly amplify T-cell responses to the target antigen by establishing a competition between memory cells specific for the target antigen and naïve T cells specific for the boosting vector (Figure 1). This basic concept has been clearly illustrated in respiratory virus models in which mice vaccinated with a subdominant antigen mount a powerful response to the subdominant epitope (and reduced response to a normally dominant epitope) following subsequent viral challenge [40,41]. Importantly, this competitive aspect of T-cell responses also enables the use of vectors that are highly immunogenic in their own right. For example, vaccinia virus-based vectors are effective in prime–boost strategies, despite the fact that these vectors elicit potent T-cell responses to vaccinia-derived epitopes [42].

Lessons from recent advances in understanding T-cell memory

Any vaccine designed to promote cellular immunity depends on the establishment of potent, long-lived, memory T cells. Although our understanding of the establishment, maintenance and recall of T-cell memory is rudimentary, there have been several recent advances in the field that have significant implications for our understanding of prime–boost vaccination strategies.

The first major advance in the T-cell memory field has been the identification of subsets of memory cells with distinct homing properties, commonly referred to as effector and central memory T cells [43,44]. Central memory T cells express CCR7 and CD62L and persist in the secondary lymphoid organs, whereas effector memory T cells express no, or low levels of, CCR7 and CD62L and persist in various peripheral sites in addition to secondary lymphoid organs. Both populations are able to mediate recall responses but effector memory cells are located in key portals of entry for many pathogens, which enables them to respond immediately to infections in peripheral tissues [45–49]. By contrast, central memory T cells appear to be most effective against systemic infections [50]. These findings are consistent with the observation that the efficacy of the memory T-cell response often correlates with the number of memory T cells in peripheral sites rather than the number in secondary lymphoid organs. For example, in the case of pulmonary infections, there is evidence that vaccines need to elicit mucosal immunity or effector memory T cells pools in the lung itself [48,51–53]. A second major advance is an increasing understanding of the role of $CD4^+$ T cells in the generation of effective $CD8^+$ T-cell memory. Exciting new data point to the role of $CD4^+$ T cells in not only promoting the expansion of primary $CD8^+$ T-cell responses to minor antigens but also in regulating the quality and longevity of $CD8^+$ T-cell memory generated by major antigens [54,55]. For example, the absence of $CD4^+$ T-cell help during a *Listeria monocytogenes* infection does not appear to affect the primary response but results in memory $CD8^+$ T cells that have an impaired ability to clear a secondary challenge [55]. Finally, new concepts are emerging regarding the maintenance of memory T-cell populations. Memory T-cell populations in secondary lymphoid organs undergo continual low-level homeostatic turnover, through a process that is regulated by cytokines, such as interleukin-2 (IL-2), IL-7 and IL-15 [56–59]. This process is independent of persisting antigen and is differentially regulated in $CD4^+$ and $CD8^+$ memory T-cell pools [60–63]. It remains to be established how memory T-cell populations are maintained in non-lymphoid or mucosal tissues, although there is evidence for the continual recruitment of recently activated cells from secondary lymphoid organs [64].

Can we improve prime–boost vaccines?

Although empirical approaches are essential for vaccine development, advances in our understanding of the underlying biology of T-cell memory provide important guidance for this process. Clearly, a better understanding of (i) what type of memory is appropriate for any given pathogen (central versus effector, systemic versus mucosal), (ii) which vaccination protocols most effectively elicit this type of memory (route of administration, number of boosts) and (iii) the relative requirements for various co-factors (co-stimulation, cytokine adjuvants, $CD4^+$ T-cell help), is essential for optimal vaccine development (Box 1).

What are the optimal vectors for delivering antigen?

Of crucial importance for prime–boost strategies is the development of appropriate vectors that are safe, readily delivered, readily manipulated, not affected by prior immunity and are potentially able to elicit either (or both) systemic or mucosal immunity. Crucially, the generation of both $CD4^+$ and $CD8^+$ T-cell immunity requires delivery of the antigen into distinct antigen-processing pathways, which for $CD8^+$ T-cell antigens usually requires local antigen synthesis. A great deal of progress is currently being made in vector design and several vectors have proven to be effective. These include replication-defective adenoviruses, fowlpox viruses, vaccinia virus, influenza virus, Sendai virus and naked DNA [2,4,10,65–67].

Box 1. Key research questions for the development of improved prime–boost vaccination strategies

- What makes a good vector for antigen delivery at the prime and boost phases of vaccination? How do different adjuvant properties of the vector impact the general efficacy?
- What are the optimal combinations, orders and timings of vector delivery for optimal prime–boost vaccination strategies?
- What are the relative benefits of accessory agents in the vaccines, such as genes encoding co-stimulatory molecules, chemokines, cytokines and Toll-like receptor ligands?
- What are the requirements for $CD4^+$ T cells in the generation of different $CD8^+$ T-cell memory pools?
- What are the factors that regulate the distinct types (beneficial or detrimental) of immune responses that can be generated or avoided in prime–boost vaccination strategies
- What are the optimal prime–boost strategies for inducing T-cell immunity at mucosal sites?

One particularly promising vector is a replication-defective vaccinia virus, Ankara, which is both safe and effective at boosting T-cell responses in humans [6,33–35,37,68,69]. Naked DNA is also a tremendously powerful vector, owing to its intrinsic immunogenicity, ease of preparation, manipulation, storage and delivery and low cost [1,70]. In general, DNA appears to be most effective at priming immune responses and is somewhat less effective as a boosting agent. It is not clear why this is the case but it might be a result of the delivery of lower doses of protein antigen (compared to viral vectors) and/or a difference in adjuvant properties. In this regard, it is possible that the efficacy of DNA vaccination can be further improved by increasing the transcriptional efficiency and the longevity of the vaccine *in vivo*. Vaccination strategies in which a DNA prime is boosted with a poxvirus vector are especially effective and have emerged as the predominant approach for eliciting protective $CD8^+$ T-cell immunity. This approach couples the strong priming (but poor boosting) properties of DNA vaccines with the strong boosting properties of vaccines based on viral vectors. It remains to be seen whether this is the optimal approach and several alternatives could offer significant advantages [71].

The underlying mechanism of DNA vaccination is unclear but is thought to depend primarily on the potent adjuvant properties of incorporated cytosine phosphate guanosine (CpG) nucleotide sequences, which operate through Toll-like receptor 9 and scavenger receptors [1,72]. Our understanding of the rules and mechanisms of CpG adjuvant activities is still rudimentary. However, it is probable that progress made over the next few years will have a significant impact on DNA vaccination in general. An interesting new approach that is related to DNA vaccination is the delivery of protein mixed with CpG oligodeoxynucleotides as an adjuvant [73]. When combined with a recombinant adenovirus boost, this strategy biased towards long-lived $CD8^+$ T-cell responses in both systemic and mucosal sites, presumably through a cross-priming mechanism [74,75]. It remains to be seen whether this would be effective in humans.

How can prime–boost strategies be modified to elicit optimal cellular immunity?

Recent advances in understanding T-cell memory have identified several approaches for improving the efficacy of prime–boost strategies. For example, the finding that optimal $CD8^+$ T-cell memory requires appropriate $CD4^+$ T-cell help during both the prime and the boost phases of the response needs to be considered in vaccine design [54,55]. Similarly, vaccine efficacy might be further boosted through the inclusion of specific cytokines or other agents, which enhance the levels or quality of the T-cell memory established [66,76]. Vaccines can also be engineered to generate broader immune responses through the inclusion of multiple antigens [33,77].

Another crucial issue that can affect vaccine efficacy is the timing and order of the prime and boost. In general, it appears that the boost must be delivered at least two weeks after priming, consistent with the idea that resting memory cells are more effectively re-activated than effector cells, which tend to die on re-challenge. The order in which vaccines are delivered will depend on their relative efficacy at priming versus boosting immune responses. As noted earlier, DNA vaccines are often used for priming purposes and viral vectors for boosting. Interestingly, administering a DNA vaccine first by the intramuscular route and followed by an intradermal injection (gene gun) is more efficient than vice versa [78].

How can we ensure that vaccines elicit the appropriate type of immunity?

A crucial factor to be considered in the development of vaccines designed to promote cellular immunity is the type of immunity that is required (CD4 versus CD8, Th1 versus Th2, Tc1 versus Tc2) [71,79]. Clearly, different prime–boost approaches are likely to generate distinct types of immunity and it is essential to ensure that inappropriate immunity is not established. For example, type 2 responses in the lung can be highly detrimental and inadvertent induction of type 2 immunity by a pulmonary vaccine can negatively impact its safety and efficacy [80]. The factors that regulate distinct types of responses are poorly understood and it is difficult to predict in advance what type of response a given vector or route of delivery will favour. Hopefully, the answers to some of these issues will emerge with future research. In addition to eliciting inappropriate types of immunity, it is also possible for vaccines to elicit ineffective responses under some circumstances. For example, exclusive priming of $CD4^+$ T-cell responses can result in the suppression of $CD8^+$ T-cell responses on subsequent pathogen challenge [81]. Similarly, the inadvertent targeting of antigens that might be poorly expressed at the site of infection might reduce vaccine efficacy [82].

How can effective immunity be induced at mucosal sites?

A crucial issue raised by our increasing understanding of T-cell memory is the distinction between mucosal and systemic immunity. The vast majority of pathogens enter through the mucosa and strong immunity at mucosal sites will be of paramount importance for optimal cellular immunity against these pathogens [83]. For example,

there is evidence that optimal cellular immunity to respiratory virus infections depends on pools of effector memory cells resident in the lung airways and interstitium [48,49,84]. It is of interest that the potent cellular immunity against *M. tuberculosis* (discussed earlier) was induced by a prime–boost strategy that included mucosal administration of antigen [32]. In this case, the level of protection observed correlated with the numbers of memory cells in lymph nodes draining the lung, reflecting the establishment of local T-cell immunity. This is consistent with other evidence that systemic versus mucosal administration of vaccines can elicit distinct modes of cellular immunity [29,85]. However, it is important to note that cellular immunity is generally unstable with regard to protective efficacy at mucosal surfaces [49,62,84,86]. This instability remains a difficult problem for vaccines because repeated boosting at a mucosal surface is problematic. One approach to avoid this problem could be to prime systemically and then induce local transient recruitment to mucosal surfaces by non-specific stimuli during a pathogen epidemic. In support of this, studies in respiratory virus systems have shown that certain inflammatory stimuli can be used to recruit large numbers of protective memory cells to the lung [87,88]. The current problem with this approach is the safety of agents used to attract local immunity, although this might change as we learn more about the molecular mechanisms underlying T-cell trafficking.

Conclusions

The development of new vaccines that promote effective cellular immunity is required for the control of pathogens for which classical humoral-based vaccines have been ineffective. Prime–boosting has emerged as a powerful approach for establishing cellular immunity and recent results have demonstrated the efficacy of prime–boost vaccines in generating protective immunity in both animal models and in the clinic. Further development of these vaccine strategies depends on advances in our basic understanding of the mechanisms of how systemic and mucosal T-cell memory is initially established, maintained at different sites and recalled in the context of a subsequent infection.

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