HUMAN VACCINES & IMMUNOTHERAPEUTICS
2019, VOL. 15, NO. 2, 407–411
https://doi.org/10.1080/21645515.2018.1527496

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COMMENTARY



# The effect of antigen dose on T cell-targeting vaccine outcome

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**ABSTRACT**

During the past 3–4 decades, an increasing amount of evidence has pointed to the complex role of the antigen dose or T cell receptor (TCR) stimulation strength on the subsequent type, duration and "flavor" or quality of the response. Antigen dose was initially shown to impact Th1/Th2 bias, and later also shown to differentially affect development and induction of Tregs, Th17, T-follicular helper (Tfh), cells, and others.

In recent years the quality of both CD4/8 T cells during infections, cancer and/or autoimmunity has turned out to be critical for subsequent disease outcome. Importantly, different vaccination strategies also lead to different types of T cell responses, and the role of the antigen dose is emerging as an important factor as well as a tool for investigators to utilize in fine-tuning vaccine efficacy. This commentary will highlight essential background of how antigen dose can impact and affect the quality of T cell responses, and discuss how this translates in different vaccine settings.

**ARTICLE HISTORY**
Received 5 September 2018
Accepted 18 September 2018

**KEYWORDS**
Antigen dose; T cell; stimulation strength; T cell receptor; vaccine; infectious immunity; protection

## Background

Many of the successes in the history of vaccination have been obtained through humoral immunity, or in other words, in diseases in which antibodies where sufficient to provide efficient vaccine-derived protection.[1] Common for many of these vaccines and studies thereof were that relatively high antigen doses were required for efficient immunity and sufficient antibody serum titers.[2] These vaccines have mainly been directed against acute lytic infections such as smallpox, or other viruses that kill the cells they infect, where successful vaccination requires antibodies to prevent virus entry or cell-to-cell transmission rather than kill infected cells, but also vaccinations directed against bacteria like tetanus and diphtheria, which produce toxins that can be neutralized by antibodies[3]

In contrast, immunoprophylaxis against persistent or chronic intracellular infections require priming of T cell immunity in addition to humoral immunity, as B cells/antibodies alone cannot protect against these pathogens. When pathogens persist inside cells for a prolonged period without killing the target cells, T cells are needed to kill infected cells before the virus can spread, as these pathogens are inaccessible to antibodies. This is especially true of chronic infections like HIV and hepatitis C virus, as well as malignant cells in cancer patients. Hence, in the design of today's modern vaccines, there is a lot of focus on T cell responses. Needless to say, the production of antibodies also requires T cell help,[4–6] and the level and quality of T cell help can strongly affect the antibody response[7] as well as the durability of memory CD8 T cells.[8,9]

## Antigen dose and the effect on immune responses

### *Antigen concentration and the priming of antibody responses*

In the context of inducing antibody responses, it is generally believed that at the priming event a high dose of antigen favors increasing plasma cell differentiation and antibody production whereas lower doses favor memory B cell induction. It has also been shown that at the boosting event, a high antigen dose enhances availability of antigen and stimulation of more memory cells.[3] However, in broad terms higher antigen doses cause lower quality of antibodies, particularly in terms of affinity.[10] Importantly, the time lapse between priming and boosting strongly influences the quality of antibody response, where several months between priming and subsequent boosters are recommended in humans.[2]

In order to develop vaccine technologies targeting intracellular infections such as tuberculosis, influenza, HIV, and also tumors, we need to know the factors that determine the ability of vaccines to activate T cells, differentiate them into different subsets, and efficiently establish sustainable T cell memory. These factors include the type of antigen, antigen dose, time of differentiation, MHC-peptide complex and stimulant molecules such as adjuvants,[11,12] and CD4 help. In this commentary, we focus on the effect of the antigen dose on the vaccine-mediated T cell response.

### *Antigen concentration and the priming of T cell responses*

During initial entry of foreign antigen into the body following vaccination, the immune response begins with antigen uptake

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by antigen presenting cells (APCs), especially professional APCs such as dendritic cells (DCs), which carry the antigens to the draining lymph node (dLN) from the site of injection (SOI; or sometimes to local mucosal organized lymphoid tissue) and in turn process and present the antigen to T cells.[13] Transport of antigen to the DLN can occur as free antigen through lymphatics or inside migratory DCs or granulocytes. When the T cell receptor detects a cognate MHC: peptide complex on an APC along with activation signals by co-stimulatory molecules, the T cell enters its' differentiation program and starts proliferating, followed by differentiation to become a distinct T-cell subset[13] of a particular lineage.

TCR-stimulation strength has been linked to the type of response since early studies performed in the 50's by Salvin[14] as well as the famous studies in the 1980's and 90's by Coffman, Bottomly and others.[15–17] These studies all showed that the strength of stimuli could regulate Th1/2 polarization. Later it has been shown that the antigen dose also plays a role in induction of follicular helper T cells,[18,19] regulatory T cells (reviewed in[20]) as well as memory T cells.[21] It has also been demonstrated that the initial T-cell response magnitude and subsequent development and retention of memory T cells are directly related to the antigen dose,[22] and that limiting the antigen dose increased CD4 T cell memory development.[14] Of high interest, a very complex interplay between many of the above-mentioned effects of antigen dosing has been reported to determine the severity of autoimmune disease. Notably, higher antigen doses can lead to deletion of autoreactive T cells and thus improve autoimmune outcome in autoimmune encephalomyelitis,[15] whereas in a dust mite model, oral delivery of low dose antigen was the most effective regimen to prevent autoimmunity.[16] These differences are likely to reflect different auto effector T helper 1 and 2 cell biases in these studies. Furthermore, in the collagen-induced arthritis model, low collagen dose was shown to aggravate disease through increased T cell responses; intermediate collagen doses suppressed both T cell responses and autoimmune disease; while high antigen dose had suppressed autoimmunity albeit to a lesser degree than the intermediate dose. The take home message from these studies was that antigen doses play a crucial role in many diseases, and careful studies of antigen doses are of high priority.

## Fine-tuning vaccine antigen dose and T cell response outcome

Some of the first pivotal studies regarding the role of antigen dose and subsequent quality of responding T cells were performed in our laboratory at the NCI, and showed that culturing CD8 T cells from immunized mice in the presence of high antigen concentrations resulted in effector CD8 T cells of low functional avidity, while CD8 T cells expanded by stimulation with very low antigen concentrations had very high functional avidity. In other words, growing cells with high antigen concentrations led to CD8 T cells that also required high antigen loads to activate effector functions and vice versa. This appeared to be due to two overlapping phenomena: only high avidity T cells were capable of being stimulated by extremely low concentrations of antigen, whereas high concentrations could stimulate both high and low avidity T cells, but induced antigen-induced cell death (AICD) of high avidity CD8 T cells, thereby selecting primarily for low avidity T cells.[23–26] T cells of higher functional avidity were also shown to be more effective in clearing viral infection,[11] and later this was shown also to be the case for tumors,[26–28] and recently expanded to bacterial infections.[29] Later, other groups also reported that the antigen dose played a major role in determining T-cell avidity.[11,22,30] Functional avidity or antigen sensitivity was found to be a key determinant of efficacy of T cell immunity against HIV.[31–34] One mechanism we found to account for the more effective virus clearance was that high avidity T cells could detect infected cells early after infection, when only small amounts of viral proteins had been synthesized and thus before production of functional viral progeny that could spread to other cells if the infected cells were lysed. Low avidity T cells required more antigen and thus were not able to kill infected cells before viral progeny were made later on.[26] To transform a naive T cell into a high avidity cell is the result of a series of collaborations that include engagement of the TCR, structural affinity between TCR and the MHC:peptide-complex on the APC-surface,[35] co-receptors like CD8 density,[36,37] co-stimulators and local cytokine environment,[11] as well as the efficiency of TCR signal transduction.[24] Studies have shown that the magnitude and strength of interaction between the APC and T cell as well as the antigen dose all play an important role in determining the T cell response outcome and ultimately the fate and protective efficacy of the T cell.[11,22] However, when we tried to translate the in vitro selection for high avidity CD8 T cells to in vivo dosing, the lowest doses gave no measurable response. Rather, we had to resort to other methods to elicit preferentially high avidity CD8 T cells with a vaccine, such as increasing costimulation[38] or providing IL-15 at the time of priming.[39]

Other studies have shown that low antigen doses are required to stimulate significant magnitudes of CD4 T-cell responses against *Leishmania major*, as well as tuberculosis and viruses.[11,40] In contrast, the magnitude of CD8 T cell responses seems more directly related to the antigen dose[11,41] compared to their CD4 counterparts, in that higher doses equals higher responses. Elegant studies by the group of Bob Seder at the NIAID showed that increased pathogen burdens resulted in a decreased quality of the responding T cells, primarily shown by a loss of multifunctionality as well as a loss of memory phenotype.[42] This loss of T cell quality was closely related to the protective capacity of the T cells against both *L. major, Mycobacterium tuberculosis* and viral infections. On the other hand, it is important to note that in some instances, a high antigen dose can result in activation of the T cell and subsequent memory T cell formation in the absence of co-stimulatory signals.[43] Our own research found that with a cationic liposomal adjuvant (CAF09) that allowed use of very low in vivo doses of antigen, CD4 T cells were induced at 1–2 logs lower antigen doses than required for CD8 T cells, which were not induced at all at the lowest doses.[11] Moreover, the lower the dose, the higher the functional avidity of the CD4 T cells, but there seemed to be no

direct relation between antigen dose and avidity of CD8 T cells, at least at doses at which they could be induced at all.[11] Protection against a vaccinia infection required an intermediate dose of antigen that induced both high avidity CD4 T cells and sufficient CD8 T cells.[11] We confirmed the importance of CD4 T cell functional avidity on anti-viral protection by adoptive transfers of transgenic virus-specific CD8 T cells along with either high or low avidity virus specific CD4 T cells, that were induced by low/high antigen dose vaccinations. Only the combination of transgenic CD8 T cells along with high avidity CD4 T cells induced by low dose vaccinations resulted in significant protection against virus infection. Interestingly, increasing vaccine antigen dose resulted in higher surface inhibitory receptor (PD-1, CTLA-4, Fas) expression on CD4 T cells, and therefore the high avidity CD4 T cells induced by low dose vaccination were less influenced by negative signals upon stimulation, possibly allowing for a lower activation threshold and hence higher functional avidity.

A risk of promoting vaccines designed to be given with low antigen doses is associated with a potential lack of immune response, and historically this is closely related to the "more-is -better" line of thinking that still dominates especially the wide plethora of infectious diseases for which we do not have reliable immune correlates of vaccine-mediated protection (HIV, TB, HCV, etc.). In most clinical phase I vaccine studies, a dose escalation approach is used to determine the dose resulting in the highest response. While this is logical for diseases with no known immunological correlate of protection (hence, a strong response is presumably better than no response), the highest dose that was tolerated and resulted in significant response has typically been chosen for later phase II/III studies. However, important reasons to curtail this practice are based on several key observations: 1) the use of high-dose antigens can lead to clonal deletion by triggering apoptosis (AICD) especially of high avidity T cells[24,25] 2) high antigen concentrations can induce tolerance in the targeted T cells,[11] 3) high vaccine doses can also lead to terminal differentiation and exhaustion of T cells,[29] 4) adverse events are often more frequent in the higher dose groups, and 5) as we have shown, higher doses may lead to lower avidity of both helper T cells but also potentially antibodies.

### Antigen dose and protection

Within the field of TB vaccine research, antigen dose has been a subject of interest lately. Initially, Claus Aagaard from Statens Serum Institute in Copenhagen demonstrated in mice that the HyVac4 (Ag85B-TB10.4) fusion protein given in the IC31® adjuvant had a quite narrow and low dose optimum (0.01–0.5 μg/mouse) which was orders of magnitude lower than the 1–50 μg that is routinely used by us and others in similar murine studies.[44] This study showed that lower vaccine antigen dose increased both the magnitude and quality as measured by polyfunctionality of the responding CD4 T cells. Importantly, the lower doses improved prophylactic protection against an experimental infection with Mtb. Later, in both mice and human trials, it was shown that the quality of T cells responding to a highly expressed antigen, ESAT-6, was lower (displaying a more exhausted phenotype) than responses against the much less abundant and less highly expressed Ag85B. The lower quality of ESAT-6- compared to Ag85B-specific CD4 T cells has been interpreted as a result of the higher concentration of available ESAT-6 antigen. Importantly, this difference was even more pronounced in antigen experienced Quantiferon+ (QFT+; Mtb-infected) individuals who were vaccinated with the H56 vaccine construct that includes both Ag85B and ESAT-6.[45] Hence, for people who have already been exposed to Ag85B and ESAT-6 through natural infection (and have lower quality ESAT-6 responses due to high antigen availability), the exhaustion of ESAT-6 specific T cells is aggravated after a further antigen "push" by a vaccine containing both the ESAT-6 and Ag85B antigens. This is in line with very recent results in a mouse post-exposure TB model, in which protection from H56 given in the liposomal CAF01 adjuvant was closely related to, and dependent on, low antigen dose (<0.5 ug;[29]). Increased doses of the H56 vaccine in this model resulted in a complete loss of protection, and a more exhausted T cell profile. Similar results with a need for lower doses in post-exposure protection were obtained by the group of Ian Orme.[46]

As previously mentioned, a close relationship between antigen dose and functional avidity of CD8 T cells has been shown for *in vitro* cultures; however, recent data have confirmed this relationship for both CD4 and CD8 T cells after vaccination, although the relationship between dose and avidity is less clear for CD8 T cells, and seems to improve by heterologous prime-boosting.[11,29,47,48] Again, lower doses led to increased avidity and/or protection.

In another example, the degree of protection against *Leishmania major* infection was assessed by the size of lesion and frequency of polyfunctional CD4 + T cells simultaneously producing multiple cytokines. Based on this research, immunization with replication-deficient adenovirus that expresses MML (MML-ADV) and recombinant leishmanial protein plus CpG (MML+CpG) both resulted in protection, but importantly, there was an inverse correlation between vaccine dose and protection.[42]

Another very important parameter to consider in this discussion is vaccine antigen and age of target population: both newborns and also elderly respond less well to vaccinations than individuals with ages in between these two groups. Among different strategies to overcome these problems has been adjusting, especially increasing, the antigen dose. While this is a logical step, it may have negative consequences on the subsequent vaccine responses, which should be carefully monitored.

Therefore, since these issues have not been addressed so far, the collection of these questions raises the big question of how antigen dose can impact and affect the quality of T cell responses, and how this translates into different vaccine scenarios. The answer to this question can be helpful in designing new vaccines technology.

## Perspectives

In this short commentary we have given a number of examples in which lower vaccine antigen doses led to more favorable T cell responses, in terms of quality as measured by a number of effector

functions as well as protective efficacy in both animal and human studies. Our goal with this commentary is to underscore the continued need to carefully monitor and assess the sometimes-overlooked essential role of the vaccine antigen dose on the ultimate vaccine outcome – namely long-term protection. Many lessons have been learned regarding the regulation of downstream immune responses following exposure of the immune system to different levels of antigen concentrations, from the early discoveries of affecting Th1/2 bias to novel findings of regulating T cell lineage fate, phenotype, functional avidity and others after in vivo vaccination or infection. While our results have shown the importance of reducing antigen dose for optimal protection in several models, we do not wish to simply promote that a lower antigen dose will always be better – rather, we urge investigators to use qualitative, and as relevant as possible outcome measurements over mere magnitude of responses in determining the optimum vaccine antigen dose. Still, we urge especially T cell vaccinologists to keep in mind that often, less is, in fact, more.

## Disclosure of potential conflicts of interest

No potential conflict of interest was reported by the authors.

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