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Understanding the immunology of the Zostavax shingles vaccine

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Zostavax is a live-attenuated varicella zoster virus (VZV) vaccine recommended for use in adults >50 years of age to prevent shingles. The main risk factor for the development of shingles is age, which correlates with decreasing cell-mediated immunity. These data suggest a predominant role of T cell immunity in controlling VZV latency. However, other components of the immune system may also contribute. In this review, we will discuss how the immune system responds to Zostavax, focusing on recent studies examining innate immunity, transcriptomics, metabolomics, cellular, and humoral immunity.

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Introduction

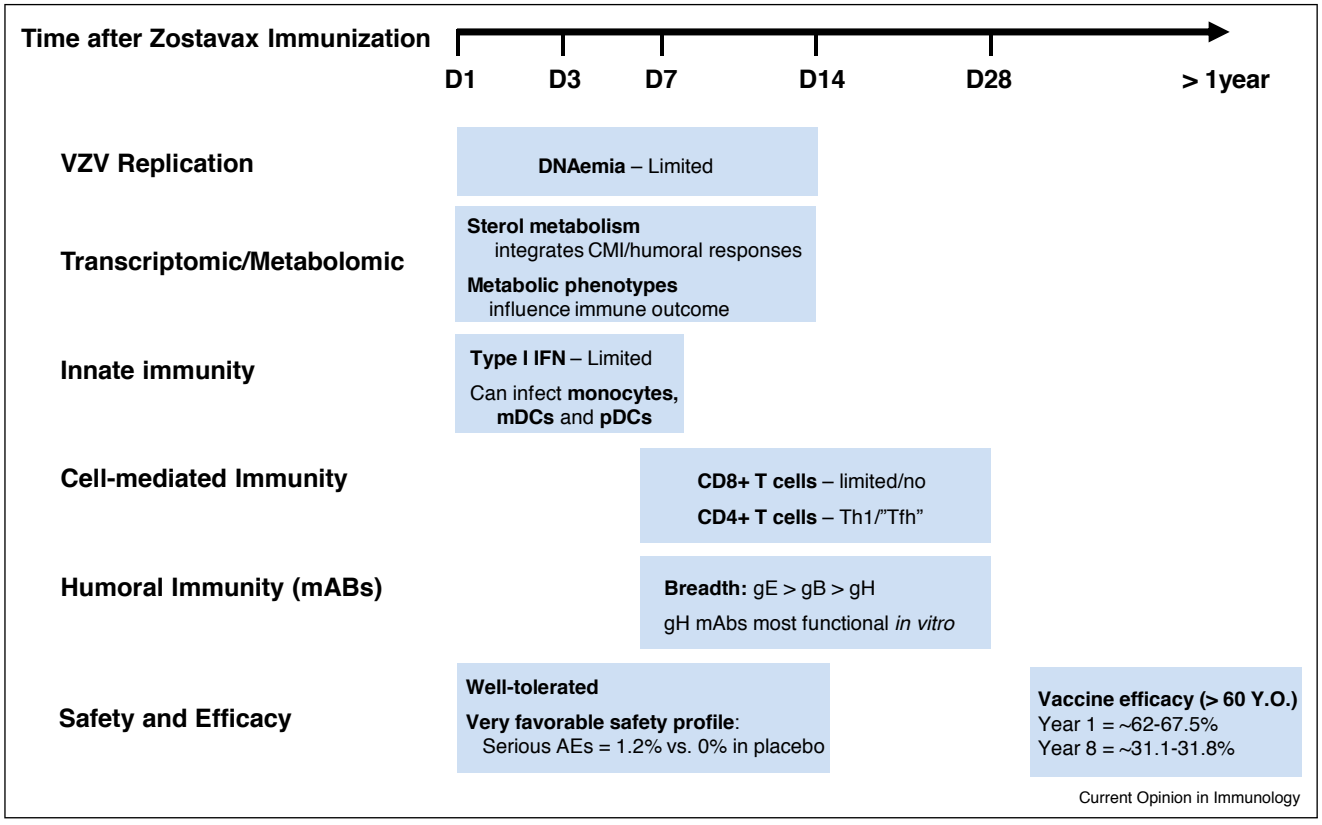
Herpes zoster (HZ/shingles) is a disease caused by reactivation of varicella zoster virus (VZV), a DNA alphaherpesvirus. Primary VZV infection, chickenpox, is followed by the virus establishing asymptomatic and latency in sensory ganglia. The major risk factor for VZV reactivation and thus HZ is age [1], which is correlated with reduced VZV-specific cellular immunity [2–8]. Clinically, herpes zoster is characterized by neural pain and a vesicular skin rash in the dermatome that is innerved by the affected sensory ganglion. A frequent complication is persistent pain after healing of skin lesions, also called postherpetic neuralgia (PHN). Pain can be debilitating, is difficult to manage and decreases patients' quality of life [1]. Therefore, immunization of the elderly with Zostavax is recommended in the US since 2006 for over 60 years-old [9] and since 2011 as of 50 years of age [10]. Zostavax, a live-attenuated vaccine, uses the same Oka/Merck virus strain as the childhood chickenpox vaccine Varivax. The latter is routinely employed in the US since 1995 [11]. Zostavax contains at least 14-times the potency of Varivax to induce robust immune responses in the elderly [14] and is given as single dose [9,10]. It is very well tolerated, has a very favorable safety profile (Figure 1 [12,13] and mainly mild adverse events are reported [15–17]. In the initial trial, patients were followed over a median of three years and Zostavax reduced HZ incidence by 51% and PHN by 66% [14]. Subsequent long-term studies in subjects older than 60 years showed a vaccine efficacy and effectiveness in preventing HZ at 62–67.5% one year after immunization and around 31% after eight years (Figure 1) [18,19].

In 1965, Hope-Simpson hypothesized that VZV-specific immunity can play an important role in the prevention of HZ disease [20]. This hypothesis has been well supported since showing that cell-mediated immunity is a major determining factor in the level of risk and severity to HZ [2–8]. The relative importance of humoral immunity in protection against HZ has remained unclear. There are data to suggest that humoral immunity may not play a critical role in preventing shingles, as with age, overall VZV antibodies do not decrease [8]. In this review, we will summarize the most recent data on the innate and adaptive immune responses elicited after Zostavax immunization.

Innate immune response, transcriptome, and metabolomics analysis

During primary infection with natural VZV, the innate immune system responds early and quickly [21,22]. IFN-α is produced after natural VZV infection and can inhibit VZV replication *in vitro* [21]. Treatment *in vivo* with exogenous IFN-α has been shown to reduce the severity of chickenpox in immunocompromised children [22]. Additionally, in a humanized SCID model of VZV pathogenesis, VZV replication in the skin was associated with extensive production of IFN-α by neighboring epidermal cells. Blocking the IFN-α response in these mice resulted in a dramatic increase in VZV in the skin [23].

Figure 1



Immune response, safety profile and durability after Zostavax immunization.
Schematic showing the time course of the peak responses in DNAemia, metabolites, innate immunity, cell-mediated immunity, humoral immunity (data obtained from monoclonal antibodies isolated from human subjects that received Zostavax) after immunization with Zostavax. Additionally, overall safety and durability after immunization are listed. 'Tfh' = Tfh-like cells measured in the peripheral blood express CXCR5, CXCR3 and ICOS.

In 2017, an integrative analysis of the transcriptomic, metabolomic, cell frequencies and immune response after Zostavax immunization was performed [24••]. Zostavax immunization resulted in the upregulation of interferon-induced antiviral genes such as *MX1* and *IFI44L* and genes involved in antibody production such as *TNFRSF17* and *MZB1*. According to a set of previously described blood transcription modules (BTMs), the transcription signatures seen after Zostavax immunization were similar to those after seasonal influenza and meningococcal vaccines [25]. However, compared to the Yellow Fever Virus (YFV-17D) vaccine, which is another live-attenuated viral vaccine, several BTMs related to innate immunity were weakly induced including BTMs related to antigen presentation, dendritic cells, antiviral innate immune signaling and type I IFN response [24••,25]. These data suggest that Zostavax is a weak stimulator of innate responses and dendritic cell activation. In line with these observations, Zostavax/VZV was able to efficiently infected monocytes, monocyte derived DCs (mDCs) and plasmacytoid DCs (pDCs) *in vitro* without substantial activation of these cells as measured by CD80 expression and secretion of TNF and IL-6 [24••]. Furthermore, pDCs produced IFN-α after TLR9 agonist treatment (CpG-A) but not after infection with Zostavax/VZV. However, several chemokines such as CXCL8 (IL-8), CCL2 (MCP-1), CXCR9 (MIG), and CXCL10 (IP-10) were detected in the supernatant of infected PBMCs. Thus, although Zostavax/VZV can infect monocytes and DCs *in vitro*, it does not induce significant innate immune activation of these cells (Figure 1).

Using an untargeted high-resolution metabolomic approach, the largest number of differential metabolites were detected at days 1 and 14 post Zostavax immunization (Figure 1). At day three, several metabolic pathways were strongly associated with gene expression for MHC-TLR7-TLR8 cluster, antigen presentation, myeloid DC activation and B cell signatures [24••]. Furthermore, early gene expression for calcium signaling and steroid metabolism correlated with later CD4+ T cell activation and B cell signature genes on day three correlated with the number of CXCR3+ Tfh-like cells on day seven. Finally, sterol metabolism was shown to integrate cellular (Tfh-

like) and humoral (IgG) immune responses [24••]. Overall, there were significant correlations between transcriptomics, metabolomics and cell frequencies in subjects after immunization with Zostavax. However, Zostavax did not induce the same level of innate immune activation as seen with YFV-17D, influenza, or meningococcal immunization [24••,25,26].

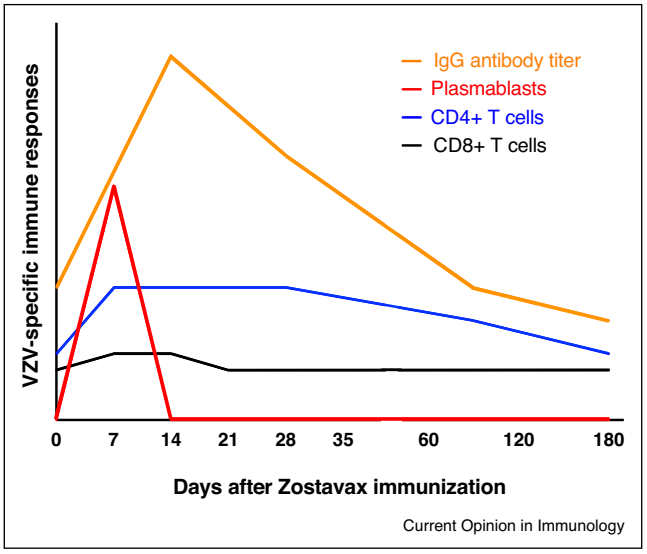
Cell-mediated immunity

Cell-mediated immunity (CMI) seems to be primarily responsible for preventing the development of shingles. HZ typically develops as people age and is primarily thought to be due to a decrease in CMI [7,8]. CD4+ T cells play a major role in primary VZV infection as deficiency in CD4+ T cells, but not CD8+ T cells, leads to severe primary VZV disease such that patients with idiopathic CD4+ deficiency are at increased risk for HZ [27]. VZV-specific memory CD4+ T cells are detectable during latency and increase during HZ [28] and upon exogenous re-exposure [29].

Zostavax immunization leads to an increase in VZV-specific CD4+ T cells [24••,30–32] that peak in the peripheral blood after 1–4 weeks (Figures 1, 2) ([8,24••] and unpublished data). CD4+ T cells, which proliferated after immunization (Ki-67+), expressed phenotypical markers of Th1 cells (T-bet, CXCR-3) and blood 'Tfh'-like cells (CXCR5, CXCR3, ICOS) (Figure 1) [24••,32,33] and downregulated the Th17-defining transcription factor RORγt and the homing marker CCR7 [24••]. These CD4+ T cells were broadly reactive to several functionally important VZV proteins, including structural glycoproteins (gB, gE, gI, gH, gM) and tegument proteins such as the major viral transactivator IE62, latency virus protein IE63 and ORF9, which is indispensable for viral replication ([31,32] and unpublished data). The majority of VZV-specific CD4+ T cells were poly-functional (CD154+/IFN-γ+/IL-2+/TNF-α+), increased after immunization and exhibited both an effector (CCR7−/CD45RA−/CD45RO+) and central (CCR7+/CD45RA−/CD45RO+) memory phenotype [32]. In the skin, however, Zostavax immunization does not appear to alter the number of tissue resident memory CD4+ T cells (Trm) [34•]. Overall, these results indicate that immunization with Zostavax results in an increase in the number of poly-functional memory CD4+ T cells (Figure 2).

In general, there have been little to no CD8+ T cell responses detected after immunization with Zostavax [24••,32,35] (Figures 1, 2). We previously showed in seven human leukocyte antigen (HLA)-A2+ subjects CD8+ T cells-specific for HLA-A2-restricted peptides at baseline. Upon immunization, there was only a boost in the CD8+ T cell response in two of these donors, and both had concomitantly VZV DNA detectable in the blood, indicating active viral replication [24••,35–37]. Interestingly, one subject that showed prolonged DNAemia at day 14 also exhibited the highest VZV-specific CD8+ T cell response. Of note, VZV DNA is mainly detectable at low levels in the blood of a subset of vaccinees, predominantly during the first days after immunization (56% and 48% at day one and day three, respectively) and only in 17% at day 14 (Figure 1) [38•]. Although CD8+ T cell boost responses to Zostavax are low/scarce, two main targets of VZV- specific CD8+ T cells have been identified so far. First, ORF9 (Tegument VP22)-specific polyfunctional CD8+ T cells after immunization were shown to increase in few subjects, but no CD8+ T cells-specific for gB, gE, IE62, and IE63 were detectable [32]. Second, we identified the HLA-A2-restricted VZV CD8+ T cell epitope ILIEGIFFV from the Ribonucleotide reductase subunit 2 (ORF18), which is conserved between the herpesviridae VZV, HSV-1, HSV-2 and EBV [35]. ILI-specific CD8+ T cells are boosted upon VZV reactivation [39•], but their frequency rarely increases after Zostavax immunization [35]. In summary, Zostavax immunization induces predominantly CD4+ T cell responses and weakly boosts CD8+ T cell responses (Figure 2).

Figure 2



Kinetics of adaptive immune responses to Zostavax immunization. Schematic illustrating the kinetics of VZV-specific CD4+ T cells (blue), CD8+ T cells (black), plasmablasts (red) and anti-VZV IgG antibody titers (orange) after immunization with Zostavax.

Humoral immunity

Although CMI is thought to be the predominant mechanism for the prevention of shingles, the relative importance of humoral immunity in protection against HZ has remained unclear. The overall antibody titers to VZV do not wane with age [8], and antibodies seem not to mediate protection in stem cell recipients [40]. However, longitudinal analyses of neutralizing antibodies, antibodies

that inhibit cell-to-cell spread or antibodies directed at specific glycoproteins, have not been performed. Previous studies have shown that serum with high levels of VZV-specific antibodies (VariZIG; IV immunoglobulin) administered to high-risk populations, for example, immunocompromised children, newborns and pregnant women, after exposure to VZV provides some protection against VZV [41–44]. Zostavax immunization induces a plasmablast response (IgG and IgA), which peaks at day seven post immunization (Figures 1, 2) [24••,45•]. VZV total glycoprotein-specific IgG and IgA titers significantly increase in both young and elderly adults, peak between 2–4 weeks after immunization and wane to near baseline levels by day 180 (Figure 2) [24••]. However, the increase of VZV-specific IgG titers was significantly greater in young adults. Overall, there was a negative correlation between the fold-increase in VZV-specific IgG titers and baseline levels. These data suggest that high pre-existing antibody titers to VZV may limit the ability of the Zostavax vaccine to induce higher levels of antibody post-immunization, possibly through limiting viral replication. Following natural VZV infection, antibody titers to glycoprotein E are highest, followed by glycoprotein B and then glycoprotein H [46,47]. We have recently demonstrated that after immunization with Zostavax, this hierarchy of gE > gB > gH antibody titers is also observed on the single cell level by generating monoclonal antibodies from plasmablasts seven days after immunization (Figure 1) [45•]. We hypothesize that the observed plasmablast response originates from the VZV-specific memory B cell compartment due to the high number of somatic hypermutations [45•]. Although gE-specific antibodies predominate the humoral immune response after natural infection and Zostavax immunization, they do not neutralize in the absence of added complement or inhibit cell-to-cell spread, unlike gH-specific antibodies *in vitro* (Figure 1) [45•,46,48–57] or *in vivo* in a SCIDhu model of VZV infection [58]. Overall, these data show that further studies should be conducted to evaluate the role of antibodies in limiting HZ disease.

Conclusions

In this review, we have discussed the innate and adaptive immune responses elicited by immunization with Zostavax, a well-tolerated live-attenuated virus vaccine with a very favorable safety profile for prevention of shingles. There is detectable but low innate immune activation after immunization and in the few instances where VZV replication has been identified, CD8+ T cell responses were evident. The predominant immune responses to this vaccine consist of CD4+ T cell and humoral responses.

Conflict of interest statement

N.L.S. and K.A.V. are employees of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA, and receive company stock as part of their compensation.

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