Trained innate immunity as underlying mechanism for the long-term, nonspecific effects of vaccines

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ABSTRACT
An increasing body of evidence shows that the innate immune system has adaptive characteristics that involve a heterologous memory of past insults. Both experimental models and proof-of-principle clinical trials show that innate immune cells, such as monocytes, macrophages, and NK cells, can provide protection against certain infections in vaccination models independently of lymphocytes. This process is regulated through epigenetic reprogramming of innate immune cells and has been termed “trained immunity.” It has been hypothesized that induction of trained immunity is responsible for the protective, nonspecific effects induced by vaccines, such as BCG, measles vaccination, and other whole-microorganism vaccines. In this review, we will present the mechanisms of trained immunity responsible for the long-lasting effects of vaccines on the innate immune system. J. Leukoc. Biol. 98: 347–356; 2015.

Introduction
Traditionally, the host defense against invading pathogens is divided into innate and adaptive immunity. The innate immune system, relying on monocytes, macrophages, neutrophils, dendritic cells, and NK cells as its cellular effectors, recognizes pathogens by use of pattern recognition receptors (e.g., TLRs, NOD-like receptors, C-type lectin-like receptors, RIG-I helicases) that bind pathogen-associated molecular patterns on pathogens. This results in a rapid activation of host defense: cytokine and chemokine production, phagocytosis, ROS/NOS production, and other antimicrobial killing mechanisms, as well as antigen presentation to the adaptive immune system [1, 2].

As the innate immune system is considered not to possess immunologic memory, its actions are seen as nonspecific and to induce an identical effect upon every encounter with a pathogen. The adaptive immune system, which consists of T- and B-lymphocytes, acts through specific recognition of antigens by lymphocytes, which induces memory cells that will greatly enhance the response to a later infection with a similar pathogen. Classic vaccination is based on this adaptive immune mechanism. As the activation of lymphocytes and generation of memory cells are slow processes (days to weeks), vaccination is used to induce a "safe/controlled" first activation of the immune system that will result in a specific memory and will protect the vaccinated host from secondary infection with the pathogen against which it was vaccinated [3].

However, recent data challenge the paradigm that innate immunity is completely devoid of adaptive characteristics. Nonspecific beneficial effects of vaccines on mortality and morbidity, as a result of infections, have been increasingly reported, which could not be explained solely by adaptive immunity [4]. In both observational studies and randomized controlled trials, it was shown that BCG reduced overall mortality, mainly by a decrease in lower respiratory infections and sepsis (further discussed below). As this effect seems to be established within days, this is more consistent with an effect on innate immunity than on adaptive immunity. Moreover, studies in plants and nonvertebrates that totally lack adaptive immunity have also shown protective host immune responses in models of vaccination or after certain infections (reviewed in ref. [5]). These observations led to the hypothesis that certain infections or vaccines are able to induce reprogramming of the innate immune responses, leading to nonspecific protection to reinfection, a process named trained immunity [5, 6] (Fig. 1).

Abbreviations: BCG = bacillus Calmette-Guérin, DTP = vaccine against diphtheria, tetanus, and polo, ERIC = European Research Council, H3K4me1/3 = mono-/trimethylation of histone 3 lysine 4, HSPC = hematopoietic stem and progenitor cell, MDP = muramyl dipeptide, mTOR = mammalian target of rapamycin, NOD2 = nucleotide-binding oligomerization domain 2, PPD = purified protein derivative, RIG-I = retinoic acid-inducible gene 1, ROS = reactive oxygen species, TCA cycle = tricarboxylic acid cycle or citric acid/Krebs cycle, Treg = regulatory T cell, TT = tetanus toxoid, YFV = yellow fever vaccine

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An important model to study the mechanisms mediating trained immunity (TCR-interacting molecule) is the protection to re-infection induced by *Candida albicans* or its cell-wall component, β-glucan. It was shown that mice infected with a nonlethal *C. albicans* infection had a lower mortality from a lethal *C. albicans* infection, 7 d later. This protective effect appeared to be T/B cell independent, as recombination-activating gene 1-deficient mice were still partially protected [7], just as SCID mice in a BCG-induced trained immunity model (discussed below) [8]. In contrast, CCR2-deficient mice, in which myeloid cells are trapped in the bone marrow, lost all protection: this demonstrates that only the loss of innate immune effects (trained immunity) and adaptive memory (through loss of APC function) leads to a complete inactivation of the protective effects induced by BCG. In an in vitro experimental model, monocytes primed with β-glucan displayed a reprogramming of their function, among which, an increased proinflammatory cytokine response to secondary stimuli. This appeared to be mediated by a dectin-1-Raf1 pathway and led to increased methylation of histone activation markers, such as H3K4me3 and transcription of the *TNFA* and *IL6* genes upon stimulation with (non)related pathogens [7]. Subsequently, inhibition of histone methyltransferases thwarted these effects, demonstrating a role of this epigenetic-based mechanism for induction of innate immune memory, a conclusion supported by genome-wide changes in H3K4me3, H3K4me1, and H3K27ac (histone 3 lysine 27 acetylation) [7, 9]. Other microbial components were also shown to induce long-term reprogramming of innate immune responses. It should be noted, however, that the functional reprogramming of innate immune cells can result in an enhanced response after restimulation, as seen for *Candida*, but can also result in a reduced response, indicative of tolerance, as seen for LPS (Fig. 1) [10]. On the other hand, one could also argue that it would be more appropriate to depart from the view that 1 particular training stimulus would induce an increase or decrease of cell function: in fact, it can be observed that each of these stimuli induces at the same time up-regulation of certain genes, whereas down-regulating others [9].

Pathway analysis of the transcriptome and epigenome profiles of trained monocytes also demonstrated a central role for the mTOR pathway and glycosylation for induction of innate immunity. Similar to activated lymphocytes [11] or cancer cells [12], a shift in glucose cellular metabolism from oxidative phosphorylation toward aerobic glycosylation (known as the Warburg effect) was necessary for the function of trained cells [13]. Hence, trained immunity is induced through epigenetic reprogramming of the transcriptional programs, as well as through a switch in the cellular metabolism of glucose that insures the energy sources for the cells.

The capacity to induce trained immunity has been also reported for BCG vaccination [8, 14]. Furthermore, other vaccines have also been shown to have long-lasting effects on all-cause mortality, and it has been hypothesized that they induce innate immune training effects [15]. In the present review, we will focus on the nonspecific effects of vaccines and the evaluation of their capacity to induce trained immunity and heterologous immune effects.

**Figure 1. Long-term effects of vaccines on innate immune function.** Upon vaccination, the innate immune system becomes activated by the vaccine. The stimulation of innate immunity can lead to long-term effects leading to an increase response to a (nonrelated) infection. For example, BCG vaccination leads to training of innate immunity and a more effective host response, accompanied by a reduced mortality, as a result of nonrelated infections. On the contrary, a previous infection with a Gram-negative microorganism and endotoxemia can lead to immunotolerance and a decreased response to an infection.

**BCG**

The BCG vaccine is a live-attenuated vaccine against tuberculosis, *Mycobacterium bovis*. Its protective effect against tuberculosis differs greatly between different populations and different forms of tuberculosis [16]. This is mostly attributed to different rates of priming with environmental mycobacteria, which would nullify the additional protective effect of BCG vaccination (reviewed in ref. [17]).

**Nonspecific effects on mortality and morbidity**

In addition to its specific protective effect against tuberculosis, BCG has been shown to possess several nonspecific effects. BCG induced potent, nonspecific effects during therapy for bladder cancer [18], topical therapy for common and genital warts [19–21], diffuse cutaneous leishmaniasis [22], and even as treatment for asthma or type 1 diabetes [23, 24]. In addition, reports from the 1950s and 1960s showed that BCG vaccination or stimulation with other mycobacterial proteins [25] protects against infection with *Staphylococcus aureus*, *Salmonella enteritidis*, *Mycobacterium fortuitum* [26, 27], *Yersinia pestis* [28], *Klebsiella pneumoniae* [29], *Schistosoma mansoni* [30], HSV and vaccinia virus [31], *Leishmania major* [32], and tumors [33] in mice, with effects that lasted up to 11 mo after vaccination. A small number of studies has also reported increased susceptibility to *Salmonella typhi* or *Eberthella typhosa* after BCG vaccination [34–36].

Early human observational data are also available to support the hypothesis that BCG induces important nonspecific effects. After the introduction of BCG vaccination in the province of Norrbotten in Sweden in 1931, mortality in the BCG-vaccinated children in the first year of life was 6.6% compared with 22.2% in the nonvaccinated, an effect that was too big to be only a result of the protection to tuberculosis [37]. One year later, in 1932, the...
Pasteur Institute reviewed its safety data on BCG vaccination and showed a mortality of 7.0% among vaccinated compared with 13.3% among nonvaccinated children [38]. Several trials performed in the 1940s and 1950s in the United States and the United Kingdom show decreased overall mortality in children vaccinated with BCG (reviewed in ref. [39]). More recent observational studies also showed similar nonspecific, protective effects by BCG [40]. Several observational studies performed in West Africa showed that BCG vaccination (as determined by vaccination card, tuberculin test, or scar formation) was associated with a decreased overall mortality, with mortality ratios ranging from 0.41 to 0.55 [41–43], and similar protective effects of BCG were observed in several Asian countries [44, 45]. Apart from these observational data, 2 randomized controlled trials have been performed to study the nonspecific effects of BCG. In a first trial, 2320 low birth-weight children (who normally will not receive the BCG vaccine until they have gained enough weight) were randomized to receive BCG at birth or a delayed BCG vaccination. A mortality rate ratio of 0.49 (0.21–1.15) was shown after 3 d and 0.55 (0.34–0.89) after 4 wk in favor of the BCG-vaccinated neonates. The effect was most prominent in children with the lowest birth weight (<1.5 kg) and was mainly a result of fewer cases of neonatal sepsis, respiratory infections, and fever [46]. A similar effect was observed in a smaller trial [47]. A case-control study performed in this population found beneficial effects of BCG vaccination on the incidence of acute lower respiratory-tract infections and infection with respiratory syncytial virus. This effect was more marked in girls compared with boys [48]. Revaccination of children aged 19 mo to 5 yr with low or no reactivity to a tuberculin skin test did not have effects on overall mortality. Although this may have been a result of the fact that many children received DTP afterward, in children who had received all of their DTP vaccines before enrollment, revaccination with BCG was associated with a mortality rate ratio of 0.56 (0.31–0.99) [49].

Nonspecific immunologic effects of BCG

The beneficial, nonspecific effects of BCG vaccination have been proposed to be mediated by 2 types of mechanisms: lymphocyte-mediated heterologous effects or by innate trained immunity. Cross-reactivity of T-lymphocytes, a process termed "heterologous immunity" [50], has been long proposed to mediate some of the nonspecific effects of vaccines. Heterologous immunity has been especially well described in virus infections, and it has been shown in mice that BCG can protect against infection with vaccinia virus through a mechanism dependent on TCR signaling and IFN-γ production in CD4 cells [51]. In an experimental model in hamsters, BCG vaccination increased the lytic activity of peritoneal cells against herpes simplex-infected cells, an effect dependent on nonadherent Thy1.2 cells [52]. Furthermore, it has been shown that CD4 and CD8 memory cells can be activated in an antigen-independent manner, and therefore, they can boost Th1 and Th17 responses upon secondary nonrelated infections [53–58]. Interestingly, BCG revaccination of 2-yr-old children in Guinea-Bissau, West Africa, resulted in lower TNF and higher IL-10 responses to LPS, PHA, and PPD [59], an effect that might be dependent on Treg induction by the second BCG vaccination, as it was shown that mice that were presensitized with mycobacteria developed more Treg induction and higher IL-10 response upon subsequent vaccination with BCG [60]. Furthermore, BCG vaccination has been shown to enhance the antibody titer and T cell responses to other vaccines, such as hepatitis B, polio, and pneumococcal conjugate vaccination [61, 62].

In addition to heterologous immunity, experimental and epidemiologic data point to long-term effects of BCG on the innate immune responses. A mice study showed that BCG/PPD-activated macrophages were able to produce more H2O2 and were better capable in killing C. albicans [63]. Also SCID mice (which lack functional lymphocytes) showed an increased survival from Candida sepsis and less fungal outgrowth after BCG vaccination, with spleen monocytes producing more TNF upon ex vivo restimulation [8]. BCG-vaccinated, B cell-deficient mice were as effectively protected from S. mansoni infection as the wild-type controls [64]. Administration of MDP, which is a constituent of mycobacterial cell walls, confers protection against infection with vaccinia virus and HSV in mice, and this effect was mediated by peritoneal macrophages and not dependent on IFN-γ [31]. Mice vaccinated with BCG showed increased resistance against malaria infection, which was associated with increased transcription of antimicrobial proteins compared with nonvaccinated mice [65].

In humans, BCG vaccination was shown to induce potentiation of the activity of NK cells [22, 66], and the protective effect of BCG vaccination on disseminated candidiasis was partially dependent on NK cells [66]. Compared with cord blood monocytes from unvaccinated babies, cells from vaccinated infants showed increased expression of granulysin and perforin upon stimulation [67]. In a trial in Guinea-Bissau with 467 low birth-weight newborns, ex vivo cytokine production was up-regulated upon stimulation with several TLR agonists [68], and an association of the number of M1 versus M2 macrophages for the risk of recurrence after intravesical BCG therapy supports an important role of innate immune cells for the nonspecific effects of BCG [69]. An increase in antimicrobial peptide production in infants vaccinated with oral polio vaccine and BCG was also observed in a human observational study [70].

Molecular mechanisms behind the nonspecific effects of BCG on trained immunity

Little is known regarding the molecular mechanisms mediating trained immunity induced by BCG vaccination. In a human vaccination trial, BCG resulted in an increased proinflammatory cytokine response of monocytes for up until 3 mo after vaccination, upon ex vivo stimulation with Mycobacterium tuberculosis, S. aureus, or C. albicans. This was accompanied by a small increase of the activation markers CD11b, TLR4, and CD14. The increased cytokine production was a result of higher mRNA expression, which was accompanied by an increased HIF1α/miR-570 on IL-6 and TNFα promoters [8]. However, these effects mostly reversed 12 mo after vaccination, arguing that trained immunity is shorter lived than classic adaptive immune memory. In addition, in an in vitro model, in which monocytes are trained for 24 h with BCG and restimulated 1 wk later, it was shown that trained immunity induced by BCG was dependent on the recognition by the NOD2 receptor, as monocytes of NOD2-deficient patients could not be trained. Moreover, BCG induction of trained immunity in vitro was inhibited by the addition of the histone methyltransferase 5′-deoxy-5′-(methylthio)-adenosine [8], suggesting that epigenetic changes at the level of histone

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methylolation play an important role. These effects of trained immunity by BCG in vivo were shown to wane after 1 yr, whereas heterologous effects on Th1 and Th17 responses were still visible at this time-point [14]. Finally, the induction of trained immunity by BCG was shown to be dependent on autophagy [71]. Moreover, single-nucleotide polymorphisms in autophagy genes were correlated with clinical outcome of bladder-cancer patients who were treated with BCG instillations, hinting to the fact that the effect of BCG on bladder carcinoma might also be mediated by the innate immune system. An overview of the molecular mechanisms responsible for the induction of trained immunity by BCG is presented in Fig. 2.

**YELLOW FEVER VACCINE**

The YFV is a live-attenuated vaccine that in developed countries is only administered to travelers going to yellow fever-endemic countries. In contrast, in many countries in Africa and South and Middle America, the YFV is part of the routine vaccination program [72]. The YFV results in viremia, 3–7 d after vaccination, which results in a lifelong immunity against yellow fever, making the YFV one of the most effective vaccines in use [73]. Although no prospective clinical trials have been performed, observational studies may suggest that YFV exerts potent, nonspeciﬁc effects. In an observational study (2007–2011), where the effect on overall mortality of live and live plus inactivated vaccines was compared, subjects (9–23 mo old) were vaccinated with the measles vaccine, with or without a pentavalent vaccine containing DTP, *Haemophilus influenzae* type B, and hepatitis B. In the last few years of the study, the YFV was added to the vaccination program. Although confounding factors between the periods with and without YFV may have inﬂuenced the results, mortality declined after introduction of the YFV from 15.2 to 7.0 for the 6 mo follow-up and from 18.2 to 9.2 for the 12 mo follow-up [74].

**Nonspecific immunologic effects of YFV**

Interestingly, nonspeciﬁc immunologic effects of YFV have also been reported. When human volunteers were vaccinated with the YFV, and 7, 15, and 30 d after vaccination, isolated neutrophils, monocytes, and NK cells were restimulated ex vivo with the YFV, a higher TNF-α signal in the nonstimulated cells and a higher IL-10 production in nonstimulated and stimulated cells were observed up until 30 d after vaccination. NK cells did not show any difference for IFN-γ, but IL-4 production was decreased signiﬁcantly in the nonstimulated cells, still 30 d after vaccination [75]. In a similar vaccination study, an increased amount of proinﬂammatory monocytes (deﬁned as CD14+CD16+HLA-DR++) was observed after YFV, which ﬁts with the up-regulated cytokine staining [76]. Changes induced by YFV in NK cells were determined in a vaccination study in 8 volunteers, showing a signiﬁcant up-regulation of TLR3 and TLR9 expression on NK cells until 10 and 7 d after vaccination, respectively. Interestingly, minor changes in TLR3 and TLR9 were seen up to 60 d postvaccination. Activation markers, such as CD69 and HLA-DR, -PQ, and -DR, were expressed signiﬁcantly higher until 10 d postvaccination, and especially the latter remained, on average, expressed higher until 60 d [77, 78]. In conclusion, mounting evidence suggests that YFV induces long-term activation of monocytes and NK cells.

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Figure 2. Molecular mechanisms of trained immunity induced by BCG. During the uptake and digestion of BCG by the monocytes and macrophages, autophagy plays an important role. In the autophagolysosome compartment, BCG is digested, with the release of MDP that activates NOD2. Through a signaling cascade that remains to be deciphered, NOD2-dependent signals induce epigenetic reprogramming, such as H3K4 trimethylation, which is responsible for relaxing the chromatin and increasing gene transcription. Rip2, Receptor-interacting protein kinase 2.
minority monocyte and NK cell populations [83, 84], and also sex-differential effects were not taken into account in these analyses, possibly masking potential effect [85].

INFLUENZA VACCINE

Influenza vaccination is done with live or inactivated vaccines that consist of 3 or 4 influenza strains, which may differ each new season, depending on the strains that are predicted to be most common during the influenza season. In many high-income countries, the inactivated vaccine is given annually to people with a higher risk for complications of influenza (e.g., chronically ill, elderly, weakened immune system) or to health care workers [86], whereas the live-attenuated vaccine is also recommended to children in the United States.

Nonspecific immunologic effects of influenza vaccine

Fourteen and 28 d after vaccination with an inactivated influenza vaccine, PBMCs were isolated, and ex vivo stimulation with LPS, M. tuberculosis, C. albicans, and S. aureus was performed. This showed a tendency for increased TNF-α and IL-6 production, whereas IL-1β, IFN-γ, and IL-10 production was down-regulated [87]. A similar trend was observed in a trial in which older (>65 yr) and younger (21–30 yr) individuals were vaccinated with inactivated influenza vaccine. Blood was drawn before and 2, 7, and 28 d after vaccination, showing an increase in IL-6 and TNF-α staining of CD14+CD16− and CD14+CD16+ monocytes in the younger volunteer group. In contrast, in the older individuals, an increase in IL-10 intracellular staining was observed in both types of monocytes [88]. In another trial, healthy volunteers were vaccinated with a live-attenuated influenza vaccine. PBMCs were stimulated ex vivo with the vaccine and cytokine production was assessed at 4, 7, and 11 wk after vaccination. A decrease in IL-10 production was observed, whereas the production of proinflammatory cytokines was decreased at 4 and 11 wk postvaccination, with a slight increase at 7 wk. No explanation for the difference between the results at 7 and 11 wk was proposed by the authors [89].

Influenza vaccine is known to be recognized by TLR7 [90], but not much is known regarding the mechanisms through which influenza vaccine modulates innate immunity. However, potentiation of NK cell function is probably one of the most relevant. In this respect, it has been shown that influenza is a potent stimulator of NK activity and that it increases the number of CD69+ spleen NK cells [78]. In a model of spontaneous metastasis of 4T1 breast tumor, when resection of the primary tumor was combined with an inactivated influenza vaccination, a reduced amount of metastases was observed. This effect could be reversed by depleting NK cells [78]. In humans, who were vaccinated with the inactivated influenza vaccine, an up-regulation of CD69 expression, an increase of NK cytotoxicity [78], and an up-regulation of Nkp44 and 2B4+ NK cells were shown, which lasted until 14 d after vaccination with the live-attenuated vaccine [91].

VACCINIA

Vaccinia virus belongs to the family of Poxviridae, genus Orthopoxvirus, and has been used extensively to vaccinate against smallpox, the very severe disease caused by the related variola virus. Vaccination campaigns with vaccinia were very successful, leading to the eradication of smallpox in 1977. Vaccinia-induced protection against smallpox is thought to rely on the induction of neutralizing antibodies and the generation of memory T cells [92]. Two observational studies on the nonspecific effect of vaccinia on overall mortality have been performed in Guinea-Bissau. Adults were screened for the presence of a vaccinia scar, and this was associated with a decrease in overall mortality, ranging from 40 to 80% [93, 94]. Furthermore, vaccinia vaccination has been linked with protection against melanoma and non-Hodgkin lymphoma [95–97].

Nonspecific immunologic effects of vaccinia

The epidemiologic observation is complemented by a number of experimental studies in animals. Peritoneal cells and spleen cells from hamsters vaccinated with vaccinia showed an increased ex vivo cytotoxic activity toward cells infected with HSV [52, 98]. For peritoneal cells, this effect was mediated by a Thy1.2-positive fraction and not by adherent macrophages, pointing to the fact that lymphoid cells are most likely involved [52]. In the spleen cells, however, this effect was shown to be dependent on a Thy1.2-negative cell population, consistent with vaccinia activation of NK cells [98].

Vaccinia has also been shown to modulate the cytokine production capacity. An increase of IFN-γ, IL-6, and TNF production was found when spleen cells of vaccinia-challenged mice were cultured with vaccinia-infected cells compared with spleen cells from naive mice. The IL-6 and TNF production was shown to be dependent on the adherent cells, compatible with monocytes and macrophages, whereas IFN-γ production was dependent on CD4 and adherent cells. Interestingly, when spleen cells from vaccinia-challenged mice were stimulated with HSV-infected cells, a strong increase in IL-6 and TNF, but not IFN-γ, production was noted, indicative of nonspecific stimulation [99]. The up-regulation of proinflammatory cytokine release to unrelated stimuli seems to be present after in vivo but not in vitro infection with vaccinia virus. Dendritic cells show a decreased production of IL-6 and TNF-α upon LPS and polyinosinic:polycytidylic acid stimulation when infected with vaccinia in vitro compared with mock infection but increased production of these cytokines to LPS stimulation when cells are isolated from mice infected in vivo [100, 101]. Other effector mechanisms of innate immune activation are also influenced by vaccinia infection, as murine peritoneal cells harvested 2 wk after vaccinia challenge showed increased production of ROS when stimulated with zymosan and whole heat-killed C. albicans; this effect lasted for 3 wk after vaccinia infection [102].

As several studies point to a role of TLR2 in the innate immune recognition of vaccinia, and engagement of TLR2 by palmitoyl-3-cysteine-serine-hyline-4 has been shown to prime ROS production in murine and human macrophages, this effect could potentially be mediated by TLR2 activation by vaccinia [103, 104]. However, TLR2 stimulation in this study led to decreased cytokine responses, and therefore, it is unlikely to account fully for the effects of vaccinia. Interestingly, stimulation of TLR2 in HSPCs leads to alteration in the functional phenotype of progenitor monocytes and macrophages [104]. One could speculate that TLR2 recognition of vaccinia in HSPCs could lead to phenotypic changes that
account for the observed epidemiologic long-term, nonspecific effects.

Molecular mechanisms behind the nonspecific effects of vaccinia

The in vivo effects of vaccinia challenge in humans have been characterized in a gene-expression study in which PBMCs were isolated from 24 healthy volunteers before and 3, 6, and 55 d after vaccination. Most of the genes that were differentially expressed showed the greatest modulation 6 d after vaccination. However, a large number of genes were still differentially expressed 55 d after vaccination, including several genes related to innate immune function. On the early time-points after vaccination, a striking up-regulation of monocyte/macrophage-specific markers was observed, such as CD63 and CD16, as well as NK-specific genes, such as killer cell lectin-like receptor subfamily B, member 1, and ZAP70. Interestingly, expression of IL-8 and its receptor CXCR2 were both up-regulated 55 d after vaccination, as well as IL-18 and AT-rich interactive domain 5A, which stabilize IL-6 and lead to increased IL-6 production in vivo. Several genes involved in cell metabolism, which have been shown to be up-regulated in models of trained immunity (e.g., hypoxia-inducible factor 1-α transcription factor), were also increased 55 d after vaccinia challenge [83].

MEASLES VACCINE

Measles vaccination is part of worldwide vaccination programs, sometimes incorporated in the measles-mumps-rubella vaccine. Protection against measles infection after vaccination is thought to rely on humoral- and cellular-adaptive responses [105]. Apart from its specific effect against measles infection, numerous observational studies from low-income countries and a randomized trial from West Africa have shown that measles vaccination is associated with decreased overall mortality and morbidity [106–108]. Furthermore, a study from Gambia, West Africa, has shown that nasopharyngeal carriage of Streptococcus pneumoniae and H. influenzae is decreased after measles vaccination and YFV compared with before vaccination [109].

Nonspecific immunologic effects of measles vaccine

Natural measles infection is well known to induce immunosuppression, which is mainly thought to be dependent on lymphocyte depletion and silencing [110, 111]. Inhibitory effects of measles vaccination on immune responses have been reported as well [112, 113]. Interestingly, wild-type measles virus and vaccine strains are recognized differentially by monocytes, with wild-type measles virus recognized by TLR2 [114], whereas for recognition of vaccine strains, TLR3 and RIG-I seem to be of importance [115–117]. This difference in innate immune recognition could point to the fact that natural measles infection and measles vaccination might have differential effects on nonspecific immunologic responses. Several studies have assessed nonspecific responses ex vivo before and after measles vaccination. Ovsyannikova et al. [118] reported that after measles vaccination, the ex vivo production of innate (IL-6, TNF-α) and adaptive (IFN-γ, IL-2) cytokines, upon PHA stimulation, is decreased during 2 wk after vaccination. However, 30 d postvaccination, levels of IL-6, IFN-γ and IL-2 are increased compared with baseline. After a second measles vaccination, a different pattern was seen, with increased IL-6, TNF-α, and IFN-γ responses lasting until 2 wk postvaccination. Pabst et al. [119] compared cellular recall responses with TT and Candida antigen and cytokine production of PBMCs upon stimulation with PHA before and after (14, 22, 30, and 38 d) primary measles vaccination in infants. Two weeks after measles vaccination, a transient drop in cellular recall responses to TT and Candida antigen was seen, but the IFN-γ response to PHA was increased significantly, 14 and 38 d after measles vaccination. Likewise, Hussey et al. [120] found decreased lymphoproliferative responses to PHA, 2 wk and 3 mo after primary and secondary vaccination with the Schwarz strain of measles vaccine but an increased concentration of the macrophage activation marker neopterin, 2 wk after vaccination. In contrast, Jensen et al. [121] compared ex vivo cytokine responses with a panel of nonspecific stimuli, before and 6 wk after measles vaccination, and found no influence on innate (TNF-α, IL-10) or adaptive (IFN-γ, IL-13, IL-5, IL-17) cytokine production. Overall, vaccination with measles seems to induce a transient suppression of lymphoproliferative responses but a slight increase in innate immune responses, as measured by nonspecific cytokine production.

OTHER VACCINES

In addition to the vaccines detailed above, for which a relatively abundant amount of information is available with regard to their nonspecific immunologic effects, similar data, although less detailed, are known for other vaccines. For human papilloma virus vaccination, it has been shown that 2 wk after primary and secondary vaccination, an increased expression of NK activation markers NK group 2, member D, Nkp30, and Nkp46 is observed, as well as increased expression of Ig-like transcript 2 (negative regulatory receptor) on monocytes [122].

One of the most controversial discussions in the literature concerns DTP vaccination, for which deleterious effects on mortality have been reported by some, albeit not all, researchers. One could speculate that these effects may, at least partly, be explained by an effect on the innate immune system. Several observational studies performed in environments with high pediatric mortality have shown increased overall mortality in the period in which DTP vaccine is the most recently administered vaccine. This effect was more pronounced in girls compared with boys [43, 123, 124]. Likewise, an increase in the incidence of intestinal infection with rotavirus and Cryptosporidium parvum was observed in girls who had received DTP as most recent vaccination [125, 126]. Furthermore, in a murine study, vaccination with DTP resulted in increased pathology and mortality when mice were challenged with RSV, 1 wk after immunization. This effect was more pronounced with purified pertussis toxin and in an experiment with an adoptive transfer of spleen cells, not dependent on CD4 or CD8 T cells [127].

In light of the beneficial, nonspecific effects of the BCG vaccine, which may be exerted through trained immunity, it
could be speculated that DTP might have an opposite effect on the innate immune system compared with BCG, inducing functional reprogramming of the immune system predisposing for increased susceptibility to infections. However, whether this is true and how DTP—or its adjuvant (alum)—exerts this effect remain to be demonstrated.

**FUTURE PERSPECTIVES**

The potential of vaccines to have nonspecific effects on diseases other than the primary target infection is increasingly recognized as a very important phenomenon, as seen in the recommendation of the World Health Organization Strategic Advisory Group of Experts Working Group, which argues for the need for more studies to investigate the nonspecific effects of vaccines [128]. Given the evidence accumulated so far, it is likely that many of the nonspecific effects of vaccines are, at least in part, mediated by reprogramming of the innate immune system.

This conclusion has several implications that warrant further research into the effects of vaccination on the innate immune system.

First, by taking into account these beneficial (and sometimes maybe deleterious), nonspecific effects of vaccines, there is potential for a more rational design of vaccination policies, by delivering vaccines in an order that confers the maximum beneficial effects and protection against off-target diseases during childhood [40].

Second, if currently used vaccines with strong beneficial, nonspecific effects but moderate, specific protective effects, such as BCG, are to be replaced by newer vaccines that confer a greater specific protective effect, then these new vaccines should also be evaluated for their nonspecific effects. The old vaccine should not be withdrawn and substituted by a new vaccine without evaluation of the effect of this change on overall mortality.

Third, the nonspecific effects of vaccines that have been phased out of vaccination policies as a result of the eradication of their target disease, such as vaccinia (worldwide), BCG (developed world), or perhaps, in the future, polio vaccine and measles vaccine, could still have beneficial effects without protecting against their target diseases. These potential beneficial effects should be investigated, as these vaccines might still confer benefits even if their respective target diseases have been eliminated.

Fourth, many adjuvants that are used to enhance the adaptive immunologic response to vaccination against infections, and more recently cancer, act through activation of the innate immune system. Further research is warranted to identify innovative approaches for the design of novel classes of vaccines that combine effective induction of adaptive immune responses with elicitation of innate immune memory (trained immunity).

Fifth, with increasing lifespan, immunosenescence is a growing problem, which may be alleviated by use of vaccines that induce innate immune training.

Finally, the study of the beneficial, nonspecific effects of vaccines might lead to the development of new strategies to use them in patient groups suffering from immunodeficiencies, who cannot be vaccinated with live vaccines.

**REFERENCES**


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