Review

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A small jab – a big effect: nonspecific immunomodulation by vaccines

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Recent epidemiological studies have shown that, in addition to disease-specific effects, vaccines against infectious diseases have nonspecific effects on the ability of the immune system to handle other pathogens. For instance, in randomized trials tuberculosis and measles vaccines are associated with a substantial reduction in overall child mortality, which cannot be explained by prevention of the target disease. New research suggests that the nonspecific effects of vaccines are related to cross-reactivity of the adaptive immune system with unrelated pathogens, and to training of the innate immune system through epigenetic reprogramming. Hence, epidemiological findings are backed by immunological data. This generates a new understanding of the immune system and about how it can be modulated by vaccines to impact the general resistance to disease.

Vaccines against infectious diseases

By definition, 'A vaccine is a biological preparation that improves immunity to a particular disease. A vaccine typically contains an agent that resembles a disease-causing microorganism, and is often made from weakened or killed forms of the microbe, its toxins or one of its surface proteins. The agent stimulates the body's immune system to recognize the agent as foreign, destroy it, and 'remember' it, so that the immune system can more easily recognize and destroy any of these microorganisms that it later encounters' (http://en.wikipedia.org/wiki/Vaccine). Emerging evidence suggests that this definition needs to be rewritten. Vaccines against infectious disease undoubtedly have specific disease-protective effects but there is also increasing evidence that they affect the resistance to other infectious diseases, so-called nonspecific effects, and these effects may be strongly beneficial but also sometimes detrimental.

Historically there are many examples suggesting that vaccines may have not only disease-specific effects but also effects on other diseases. When *Vaccinia*, the first human vaccine, was introduced in the early 19th century it was noticed that recipients were protected not only against smallpox but also against conditions as diverse as atopic diseases, measles, scarlet fever, and syphilis [1]. When the bacille Calmette–Guérin (BCG) vaccine was introduced in Sweden more than 80 years ago mortality generally was documented to be nearly 3-fold lower among BCG-vaccinated children. Because the main mortality reduction occurred in infancy it could not be explained solely by the prevention of tuberculosis, which mainly killed older children. The author therefore suggested that, 'BCG vaccine provokes a non-specific immunity' [2].

During the past few decades, research in areas with high infectious disease pressure, such as in West Africa, has revived the issue of nonspecific effects of vaccines. It began in the early 1990s during randomized trials of a new high-titer measles vaccine in Guinea-Bissau and Senegal. This vaccine was fully protective against measles and could be given as early as 4–5 months of age. Nevertheless, it was associated with a surprising 2-fold increased mortality for females compared with the standard measles vaccine given at 9 months of age [3]. This unexpected negative effect of a routine vaccine led to a systematic investigation of all routine vaccines for their potential nonspecific and sex-differential effects. A series of observational studies found nonspecific effects for all the routine childhood vaccines, and randomized trials were initiated. These trials confirmed that the standard measles vaccine [4,5] and BCG vaccine [6,7] actually have beneficial nonspecific effects. There are also recent observational data available that suggest *Vaccinia* may likewise have beneficial nonspecific effects [8–10]. Worryingly, diphtheria-tetanus-pertussis (DTP) vaccine, although protective against the three target diseases, increases female mortality from other infectious diseases [11], and it turned out that DTP vaccine administered after the measles vaccine was the explanation for the increased female mortality observed in the high-titer measles vaccine trials, as explained below.

Epidemiological findings showing nonspecific effects of vaccines have been dismissed or minimized as being biologically implausible, and they may indeed seem to contradict the definition of a vaccine. However, this definition is based on the factors investigated – namely specific protection. There has been little work conducted to examine nonspecific effects on other infections. Now, emerging

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immunological data indicate that specific T and B cell classical immunological memory and nonspecific memory traits such as macrophage- and NK-cell-primed immune responses are built complementarily in the normal functioning of the immune system [12–14].

Here, we examine the epidemiological evidence supporting nonspecific effects of vaccines and describe how evidence of T-cell-mediated cross-reactivity and trained innate immunity may provide a mechanistic explanation for these effects. Hence, it may be time to consider a new definition of vaccines that could read, 'a vaccine is a biological preparation that improves immunity to a particular disease and, at the same time, may alter the general level of resistance towards unrelated pathogens in the recipient'.

Epidemiological evidence for nonspecific effects of vaccines

Measles vaccine

Standard titer measles vaccine is recommended at 9 months of age in low-income countries where measles infection is endemic and often fatal. Many observational studies have shown that measles-vaccinated children have substantially lower mortality than can be explained by the prevention of measles-related deaths, for reviews, see [5,15]. Observational studies are obviously prone to selection bias, and beneficial effects are to be expected if it is the healthiest children who are vaccinated. However, many of these observational studies were 'natural experiments', such as studies comparing the mortality before and after the introduction of measles vaccine and other studies where it was logistical factors rather than maternal choice that determined whether a child was vaccinated or not. The evidence became sufficient to justify randomized trials and from 2003 to 2009 a randomized trial was conducted in Guinea-Bissau, testing the effect of providing an additional standard titer measles vaccine at 4.5 months [4]. All children had received their three DTP vaccines before enrolment, and all children received measles vaccine at 9 months of age. The results confirmed the *a priori* expectations: compared with children who received the recommended standard measles vaccine at 9 months, children who received measles vaccine at 4.5 months and at 9 months of age had a 30% (95% CI = 6–48%) reduction in all-cause mortality from 4.5–36.0 months of age; only 4% could be explained by the prevention of measles-related deaths [4]. No study contradicts these observations by showing that measles vaccine only protects against measles infection and measles death. A World Health Organization (WHO)-commissioned review concluded that the effect of measles vaccine found in community studies from low-income countries was compatible with the expected effect of measles infection on overall mortality [16]. However, this was based on only two studies out of the more than ten studies that had found a beneficial nonspecific effect of measles vaccine, and the analysis and the conclusion of the WHO study was later challenged [17]. Hence, there is considerable evidence that measles vaccine is associated with more survival benefits than expected based on the specific protection. To date, these observations have not been pursued, partly due to the absence of a mechanistic understanding [18].

BCG

BCG should be given at birth in low-income countries but is often delayed. Firstly, vaccination of low-birth-weight (LBW) infants is postponed in many countries; secondly, many normal-birth-weight infants are vaccinated late for logistical reasons. Observational studies have indicated that early BCG vaccination is associated with reduced mortality, for a review, see [19]. Again, the observational studies may be prone to selection bias and randomized trials are warranted. The lack of vaccination of LBW neonates provides an opportunity to conduct a trial, randomly allocating LBW neonates to BCG at birth or the usual postponed BCG. Now two trials in LBW neonates show that BCG at birth almost halves neonatal mortality, the combined estimate being a 48% (95% CI = 18–67%) reduction [6,7]. This effect is clearly not attributed to tuberculosis prevention because tuberculosis is a rare cause of death in neonates. Hence, as is the case for measles vaccine, the beneficial nonspecific effects on mortality seen in the observational studies were confirmed in subsequent randomized trials. There are no studies to contradict the finding that BCG increases overall survival far beyond that expected by the protection against severe tuberculosis. In fact, a reinterpretation of trials conducted in the 1940s and 1950s in Europe and the USA suggests that BCG reduced nonaccidental and non-tuberculosis deaths by 25% (6–41%) [20]. Of interest, a recent Dutch randomized trial showed that by 18 months of age BCG-vaccinated children had a tendency for a lower incidence of eczema and therefore significantly reduced use of medication for eczema compared with BCG-unvaccinated children [21]. Hence, all available epidemiological evidence suggests that BCG may have strong nonspecific beneficial effects on the immune system. However, there are still skeptics that dismiss the beneficial effects of BCG as biologically implausible [22].

Vaccinia

During the second half of the 20th century the potential for positive side effects from *Vaccinia* was reviewed; and new evidence on 'para-immune effects' was included [1]. More recent studies have focused on the phasing out of the smallpox vaccine in the 1970s and compared vaccinated and unvaccinated cohorts. In low-income countries a *Vaccinia* scar has been associated with reductions of more than 40% in overall mortality among adults [9,10]; in high-income countries smallpox vaccination has been associated with a tendency for reduced risk of asthma [23], and significantly reduced risk of malignant melanoma [24] and infectious disease hospitalizations [8]. There are no studies that contradict these observations. However, it should be noted that no randomized trials testing the effect of *Vaccinia* on overall mortality and morbidity have been conducted.

DTP vaccine

DTP vaccine against diphtheria, tetanus, and pertussis does not seem to have the same beneficial effects as BCG, measles vaccine, and *Vaccinia* and in fact opposing effects are observed [11]. In areas with herd immunity to pertussis females have higher mortality if they have received DTP

than if they have not, and they have higher mortality than males who received DTP [11,25]. The negative effects of DTP are observed when it is the most recent vaccination; BCG or measles vaccine given after DTP reverses the negative effects of DTP [11]. This suggests that the sequence of vaccination is important. This phenomenon also explains the unexpected 2-fold increase in female mortality during the high-titer measles vaccine trial [3]: the high-titer vaccine was given to children at 4–5 months of age irrespective of DTP vaccination status and many children received DTP afterwards, with a negative effect for females [26]. After the initial report of negative effects of DTP vaccine [27], WHO commissioned nine sites to study the effect of DTP on overall mortality. The six published studies concluded that there were no negative effects of DTP vaccine; in fact all these studies found strong beneficial effects of DTP on overall mortality [28–33]. However, controversy ensued regarding the design of these WHO-commissioned studies that had important methodological shortcomings [34,35]. For instance, the studies had counted 'no information about vaccination' as 'unvaccinated'. Because it is only possible to obtain information from surviving children in most low-income settings, many dead children were erroneously classified as 'unvaccinated', creating a so-called 'survival bias', which will always produce highly beneficial effect estimates for the most recent vaccine [36]. Because all the studies on the overall mortality effect of DTP vaccine are observational studies they are probably prone to selection bias, but this selection bias would tend to work in favor of vaccinated children, and therefore the consistent observation of negative effects in studies without survival bias is worrying.

Immunological evidence for nonspecific effects of vaccines

As reviewed above, although epidemiological evidence for nonspecific effects of vaccines is accumulating, more recently from randomized trials, the perceived lack of biological plausibility has been a major obstacle in recognizing and further investigating these effects. Hence, it is important to consider immunological mechanisms that may mediate such effects.

Below, we describe how novel insights in understanding the adaptive immune system and innate immunity provide arguments that state exposure to a pathogen leads not only to specific immunological memory (represented by memory T and B cells) but also to Tcell-mediated cross-reactivity, as well as generally altered innate immune response (Figure 1).

T-cell-mediated cross-reactivity: 'heterologous immunity'

Each individual has a unique life-long history of infections and vaccinations, and each exposure leaves an imprint on the immune system that can affect future innate and adaptive immune responses to new pathogens. This concept of 'heterologous immunity' [14,37] could explain the observation that vaccines may have nonspecific effects because the vaccines encode antigens that cross-react with other pathogens (Figure 1).T cell responses are impacted by previous infections with unrelated viruses, and basic principles of T cell cross-reactivity and heterologous immunity that impact disease outcome in adult mice and humans have been identified (Box 1). Cross-reactive T-cell-mediated heterologous immunity is probably a common

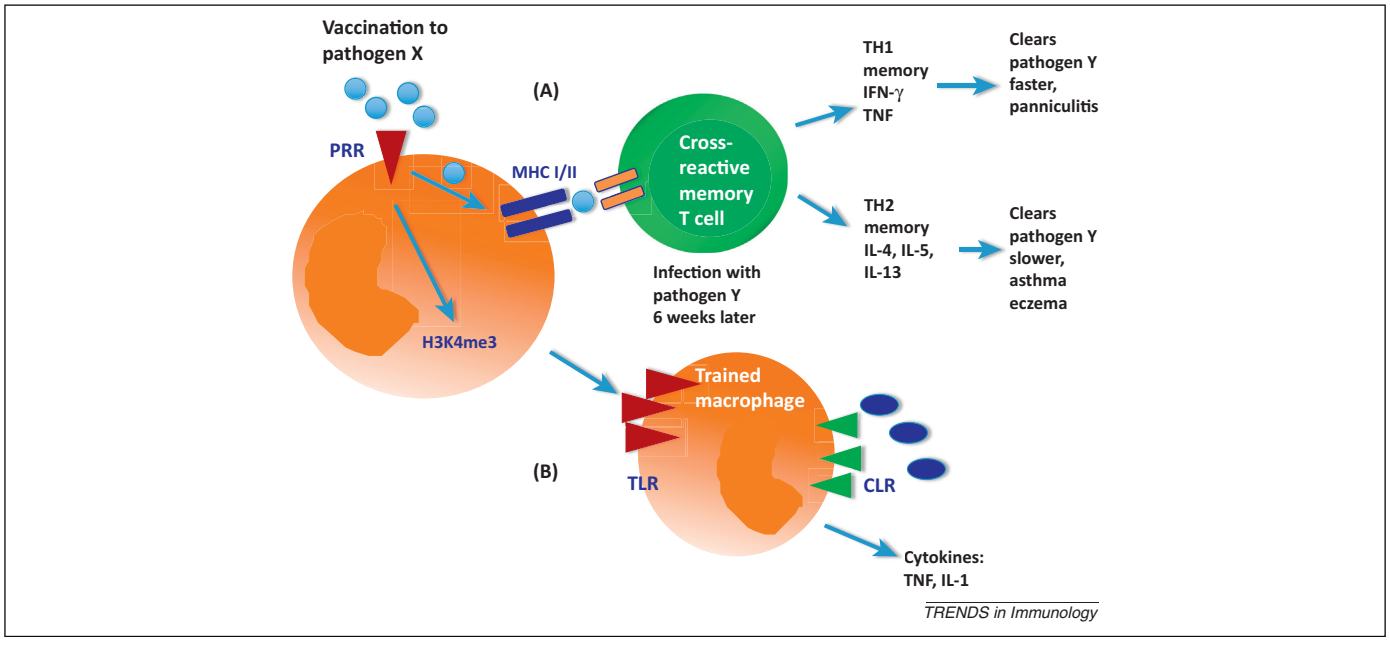

Figure 1. Possible immunological mechanisms explaining nonspecific effects of vaccination. After vaccination for pathogen X [e.g., bacille Calmette–Guérin (BCG)] two possible immunological mechanisms may explain nonspecific effects. **(A)** T-cell-mediated cross-reactivity: memory CD4 and CD8 T cells are generated that are cross-reactive with pathogen Y. Upon exposure to pathogen Y there is rapid activation of the cross-reactive memory T cells. If these cells are Th1-like then this would result in rapid production of cytokines, which clears the pathogen quickly, but if the response is overactivated there may be some immunopathology such as panniculitis. If the memory cells were Th2-like this could impede the clearance of pathogen Y, leading to death and maybe also contribute to the induction of asthma or eczema. **(B)** Epigenetic reprogramming (HeK4 trimethylation)-induced training of monocytes/macrophages. Upon exposure to pathogen Y there is a more rapid activation of the altered innate antigen-presenting cells, and an increased release of proinflammatory cytokines with more rapid clearance of pathogen Y. Abbreviations: PRR, pattern recognition receptors; MHC, major histocompatibility complex; TLR, Toll-like receptors; CLR, C-type lectin receptors.

Box 1. Principles of T cell cross-reactivity and 'heterologous immunity'

- T cell cross-reactivity is common between unrelated pathogens and alters T cell immunodominance in sequential or simultaneous infections [14,38,44,49,50].
- Networks of cross-reactive T cells can alter the efficiency of the effector response and thus influence protective immunity or immunopathology, resulting in either beneficial or detrimental heterologous immunity [37–47].
- T cell cross-reactivity can lead to narrowing of the T cell repertoire, giving rise to viral escape mutants [43].
- Cross-reactive T cell receptor (TCR) private specificity determines the disease outcome of an individual resulting in repertoire narrowing, protective immunity, or immunopathology [43–47].
- The size of the T cell cross-reactive response can directly correlate with the severity of immunopathology and can be tolerized or inhibited with anti-interferon (IFN)-γ [47].
- Mutation of vital pathogenic cross-reactive epitopes prevents immunopathology [47,89].
- T cell cross-reactivity increases with age [48].

and an important determinant in the pathogenesis of animal and human infections [38–50]. In some scenarios, beneficial heterologous immunity can provide partial protective immunity and be the difference between life and death [37,40,51]. For example, lymphocytic choriomeningitis virus (LCMV)-immune mice develop 10–100-fold lower *Vaccinia* virus (VV) titers than naive mice and are protected from lethal VV infections [37,39–41]. Interestingly, prior immunity to BCG also led to protective immunity to subsequent VV challenge [39].

In the setting of human disease it is more difficult to test if heterologous immunity plays a role in protective immunity, because people do not notice when they do not become sick. However, recently abundant memory-phenotype CD4 T cells specific to viral antigens were found in the peripheral blood of adults who had never been infected [52]. For instance, in HIV-seronegative individuals there was recognition of influenza A virus (IAV) and HIV antigens by some of these cross-reactive CD4 T cells. In another study middle-aged HLA-$A2^+$ Epstein–Barr virus (EBV)-seronegative adults constantly exposed to EBV in their environment were found to have strong IAV-$M1_{58}$/EBV-$BMLF1_{280}$ cross-reactive CD8 responses. These cross-reactive T cells had unique T cell receptor (TCR) repertoires, produced interferon (IFN)-γ to EBV epitopes, and lysed EBV-infected targets (L. Watkin et al., unpublished).

In other scenarios detrimental heterologous immunity can lead to severe immunopathology. For instance, when LCMV-immune mice are infected with VV intraperitoneal (i.p.) some of these mice develop severe panniculitis, in the form of inflammation and necrosis of visceral fat tissue [14,37,45]. This type of abdominal fat pathology is seen in human syndromes of unknown etiology, such as Weber–Christian disease or lupus erythematosis, whereas erythema nodosum, a more benign and more common form of panniculitis, involves inflammation of subcutaneous fat tissue [53] sometimes seen after vaccinations with VV, human papillomavirus (HPV), and hepatitis B virus (HBV) [37,54–57]. Under conditions of heterologous immunity, there is variation in pathogenesis among genetically identical mice, due to the private specificity of the T cell repertoires [45]. T cell responses to the K^b-restricted VV-a11r epitope [44,58] can cross-react with LCMV-encoded NP_{205}, GP_{34}, or GP_{118} peptides, resulting in a network of cross-reactive epitopes encoded by VV, LCMV, and Pichinde virus (PV) [44,46]. Upon adoptive transfer of LCMV-immune splenocytes into naive mice VV infection sometimes expands LCMV NP_{205}-specific T cells, but other times LCMV GP_{34}- or GP_{118}-specific T cells [46]. Adoptive transfer of memory cells from one donor into two to three congenic hosts resulted in similar levels of panniculitis and usage of the same cross-reactive epitope in each host after VV challenge, demonstrating that this variability in response reflects the private specificity of the LCMV-immune T cell repertoire unique to the individual host [45]. Thus, the epitope specificity of a T cell response in genetically identical individuals with the same histories of infection is influenced by private specificity of the individual, helping explain the great variability in disease outcome when people are infected with the same virus. In another mouse model, IAV-immune mice were infected with LCMV, the severity of lung pathology directly correlated with and was predicted by the frequency of IAV-$PB1_{703}$- and -PA_{224}-specific memory responses, which cross-reacted with LCMV-$GP_{33/34}$ and -GP_{276}, respectively [47]. Eradication or functional ablation of these pathogenic memory T cell populations, using mutant viral strains, peptide-based tolerization strategies, or short-term anti-IFN-γ treatment, inhibited severe lesions such as bronchiolization from occurring. These studies suggest that if cross-reactive T cell epitopes that are detrimental can be identified in human vaccines they could be removed or modified.

In recent years advances have been made in documenting detrimental heterologous immunity during human viral infections. Severe dengue virus infections occur when a host immune to one serotype contracts an infection with another serotype. The resultant pathology, in the form of dengue hemorrhagic fever and shock syndrome, has been proposed to involve cross-reactive memory T cells that may be of high affinity to the first virus but cross-reactive at low affinity to the second virus infection [59,60]. A highly focused response to a CD8 cross-reactive response between IAV and hepatitis C virus (HCV) [61] in acutely infected HCV patients was associated with severe fulminant hepatitis [62]. Extensive studies examining CD8 cross-reactivity between several epitopes of IAV and EBV identified a network of cross-reactive responses [42,44] that have been associated with the induction of infectious mononucleosis. Investigation into the increased rate of pertussis outbreaks in vaccinated children recently showed that children who received the new acellular DTaP vaccine had higher rates of pertussis. The highest rates, however, were in the groups that received DTaP first and were boosted with DTP based on whole-cell pertussis (DTwP). DTaP only possesses some of the pertussis antigens, suggesting that this locks the immune response to certain epitopes and inhibits the development of more protective responses when boosted with DTwP [63]. This argues that prior vaccination can change the immune system of individuals leading to a different response to infections.

To conclude, T-cell-mediated heterologous immunity provides a plausible biological mechanism by which vaccines may affect the immune response to a subsequent

unrelated infection, and also explains how, in certain situations, a vaccine could have detrimental effects on the outcome of secondary infections.

Training the innate immune system

Activation of cross-reactive T cell responses, as seen in heterologous immunity, might explain some of the nonspecific effects of vaccination. However, there is also evidence suggesting that the altered resistance to subsequent infections after vaccination or infection with an unrelated pathogen cannot be attributed to adaptive immune responses alone, and that innate immune responses are in a heightened state of activation. Several classes of pattern recognition receptors (PRRs) including Toll-like receptors (TLRs), C-type lectin receptors (CLRs), NOD-like receptors (NLRs), and RIG-I-like receptors (RLRs) recognize specific pathogen-associated molecular patterns (PAMPs) [64], and an increased expression of PRRs has been suggested to be at least in part responsible for the primed innate immunity [65]. Moreover, historical and recent studies demonstrate that innate immune responses have adaptive characteristics that can contribute to protection against subsequent unrelated infections, a process that has been termed 'trained immunity' [13] (Box 2; Figure 1).

The fact that innate immune responses exhibit memory characteristics after the first encounter with a pathogen (the 'training effect') is accepted in plant immunology and is widely studied in invertebrates (Table 1) [66–69]. Systemic acquired resistance (SAR) is the central process providing protection against reinfection in plants. Epigenetic processes are crucial for the innate immune memory that contributes to SAR in plants [70,71]. Moreover, epigenetic changes can provide transgenerational transmission of resistance in plants, with the acetylation of H3K9 being central for this process [72], and epigenetic-based mechanisms may represent a general mechanism for long-term priming of innate immune responses.

Although older studies suggest resistance to infection in nude mice [73], only recently has innate immune memory in mammals been studied in depth. The main cellular effectors of innate immunity are the neutrophils, NK cells, and monocytes/macrophages. Neutrophils are terminally differentiated and short-lived, and their capacity to participate in long-term immunological memory is small. By contrast, recent studies reveal memory characteristics in monocytes and NK cells. In a recent study in mice, memory NK cells offered the host protection against viral infections in a T- and B-cell-independent manner [74]. During cytomegalovirus (CMV) reactivation in humans an expansion of NKG2C^{+} NK cells is observed, which represents the human counterpart of the memory NK cells in mice [75]. A central role for the NK-memory-induced protection has been shown to be played by CXCR6^{+} memory NK cells from the liver [76].

How monocyte function is modulated by microbial stimuli is an important aspect in determining host defense. Certain TLR ligands, such as lipopolysaccharide (LPS), induce a state of tolerance in monocytes/macrophages that can be maintained for several days and even weeks [77,78]. Epigenetic mechanisms are central to the process of LPS tolerance [79], demonstrating that infections can lead to long-term reprogramming of innate immune cells. In addition to tolerance, training of innate immunity can be induced after infection or vaccination. Infection models in mice show that BCG vaccination protects against secondary infections with *Candida albicans* or *Schistosoma mansoni*, and protection appears to be mediated at least partially through T-cell-independent mechanisms [80], and involves activated tissue macrophages [81]. Moreover, peptidoglycans from the microbiota enhance systemic innate immunity through NOD1-mediated signaling [82].

These data showing nonspecific protective effects by vaccines (such as BCG) or microbial ligands are complemented by studies showing that when the attenuated PCA-2 strain of *C. albicans* was injected in the mice it induced protection not only towards the virulent CA-6 strain of *C. albicans* but also towards the bacteria *Staphylococcus aureus* [73]. Importantly, protection was also induced in athymic mice, demonstrating a T-cell-independent mechanism [83]. In these murine models protection was dependent on macrophages [73] and proinflammatory cytokine production [84], suggesting a role for innate immune mechanisms. A possible mechanism underlying trained immunity was suggested in recent studies demonstrating that *C. albicans* infection led to epigenetic reprogramming of monocytes through H3K4 trimethylation, resulting in protection of T- and B-cell-deficient *Scid* and *Rag1*$^{-/-}$ mice against lethal systemic candidiasis [12,85]. In addition to epigenetic reprogramming, the functional modulation of monocytes and macrophages during trained immunity has been linked to an increased expression of PRRs that are crucial for pathogen recognition such as the lectin receptors macrophage receptor with collagenous structure (MARCO), dectin-1, and pentraxin-3 [65]. The increased expression of PRRs will determine an improved recognition of every pathogen being recognized by that particular receptor and, through that, induce an improved activation of host defense mechanisms.

Can humans also develop trained immunity and, if so, does this occur through similar immunological mechanisms to those described above? An increasing body of evidence suggests that this may be the case. Herpes virus latency was shown to confer protection against bacterial infection through systemic activation of macrophages and production of IFN-γ, and it was suggested that herpes viruses are symbionts (rather than pathogens) in humans

Box 2. Characteristics of 'trained innate immunity'

- Induced after a primary infection or vaccination, and confers protection against a secondary infection through mechanisms independent of T and B cell adaptive responses.
- Increases nonspecific resistance of the host to reinfection, and thus provides cross-protection to other infections.
- The cellular mechanisms that mediate trained immunity involve innate immune cells such as macrophages and natural killer (NK) cells, and entail improved pathogen recognition by pattern recognition receptors (PRRs) and an enhanced protective inflammatory response.
- Molecular mechanisms that induce trained immunity involve epigenetic reprogramming (DNA and histone modifications, miRNA), rather than gene recombination that characterizes adaptive immune memory.

Table 1. Examples innate immunity memory in plants, invertebrates, and mammals

Organism	Experimental model	Biologic effect	Specificity	Mechanism
Plants – 'systemic acquired resistance'				
Large variety of plants [70–72,90,91]	Viruses, bacteria, fungi	Protection against reinfection	Variable	Salicylic acid Epigenetic mechanisms
Non-vertebrates				
Beetle [92]	LPS or bacterial prechallenge	Protection against reinfection	–	Transgenerational priming
Drosophila [66]	*Streptococcus pneumoniae* *Beauveria bassiana* *Serratia marcescens*	Protection	+	Serine protease CG33462
Anopheles gambiae [67]	Midgut flora	Protection against *Plasmodium*	+	Toll-dependent hematocyte-differentiation factor
Mammals 'trained immunity'				
Mice [73,83,85]	*Candida albicans* BCG	Protection against candidiasis	–	Monocyte epigenetic reprogramming
Mice [74,76]	Murine CMV Hypersensitization	NK-cell-dependent	+	$Ly49^+$ NK cells Hepatic $CXCR6^+$ NK cells
Humans [12]	BCG vaccination	Protection against nonrelated infections	–	Monocyte reprogramming

Abbreviations: LPS, lipopolysaccharide; BCG, bacille Calmette–Guérin; CMV, cytomegalovirus; NK, natural killer.

[86]. Furthermore, vaccination of volunteers with BCG showed that, in addition to induction of specific T cell responses, nonspecific innate immune responses to unrelated pathogens were also increased for at least 3 months after the vaccination [12]. This 'trained immunity' was associated in humans with epigenetic reprogramming of monocytes at the level of H3K4 trimethylation [12]. Hence, these long-lasting effects may explain the protective action against a multitude of infections observed in BCG-vaccinated children.

To conclude, these data suggest a picture in which the innate immune system is characterized by adaptive features, and can be trained to provide partial protection against infection independent of the classical T and B cell adaptive immunity. NK cells and monocytes have emerged as the main mediators of trained immunity in mammals, with functional reprogramming (e.g., through epigenetically mediated mechanisms) mediating these effects. Other cell types such as dendritic cells may also be phenotypically influenced during trained immunity, but future studies need to assess their involvement. However, studies on trained immunity are still in their infancy and more investigations, both on immunological and molecular mechanisms, as well as physiological characteristics of these effects, are needed in the future to characterize the nonspecific effects of vaccines mechanistically.

A new paradigm: vaccines modulate general resistance

The epidemiological data indicate that vaccines have nonspecific effects that may be just as important or even more important for childhood survival than their specific effects [4]. Existing studies suggest a general pattern, namely that the live vaccines: BCG, measles vaccine, and *Vaccinia* are associated with beneficial nonspecific effects, leading to reduced all-cause mortality, whereas the inactivated, alum-adjuvated DTP vaccine is associated with increased susceptibility to other unrelated infections, particularly in females.

Although there is no direct evidence that T-cell-mediated cross-reactivity and trained innate immunity play important roles in generating the nonspecific effects of these vaccines, these two novel mechanisms do support the biological plausibility by demonstrating that the encounter with one pathogen may alter the immune response to subsequent completely unrelated pathogen challenges, and this may result in improved outcomes, but could also at times be detrimental.

We still need to find out exactly how the vaccines induce nonspecific effects and why the live vaccines: BCG, measles vaccine, and *Vaccinia* are associated with benefits, whereas the opposite is seen for DTP. It is also imperative to understand why these effects are only seen as long as a vaccine is the most recent vaccine; the effect can be reversed with a new vaccine. Also, we need to understand why these effects seem more pronounced in females. The most striking observation so far is the parallel between the epidemiological observation that BCG given to LBW neonates reduces neonatal mortality from all causes by 48% [7]. The immunological studies show that BCG induces epigenetic modulations of human monocytes leading to increased proinflammatory cytokine production, and in a murine model this translates into increased protection from a lethal unrelated infection [12].

Paradigms and dogmas: why have these effects been overlooked?

If vaccines can modulate the immune system in a more general way, as suggested by epidemiological and immunological data, it opens an avenue to a new understanding of the immune system as a learning system. Just like the brain, the immune system seems to extend what has been learned in one context to new contexts. In the brain it is known that inference takes place, because of the obvious mismatch between the sparse information provided by our senses and the strong generalizations and powerful abstractions we make [87]. However, our current perception of the immune system is more simplistic. It was, to a large extent, shaped in the 1950s with the formulation of the clonal selection hypothesis. This line of thinking has emphasized the adaptive immune system and the specific antigen recognition and specific memory, which have been crucial in vaccine development, perhaps at the expense of

Box 3. Future questions about nonspecific effects of vaccines

Future epidemiological studies

Randomized trials

Randomized trials measuring the overall effect on health of vaccines should be pursued for old and new vaccines but are hampered by a catch 22 situation: it is considered unethical to conduct randomized trials with already recommended vaccines to measure their overall effect on morbidity and mortality even though these effects were never measured. However, it is often possible to alter the timing of vaccines, as has been done for bacille Calmette–Guérin (BCG) and measles vaccine in Guinea-Bissau. Also, it is often possible to take advantage of 'natural experiments', when a new vaccine is introduced or its sequence in the vaccination program is altered, to compare vaccinated and unvaccinated same-age children without a lot of potential confounding factors.

Studies in high-income settings

Most studies of nonspecific effects have been done in low-income countries with high infectious disease pressure. However, the few studies that have looked for nonspecific effects in high-income countries have also found them; not for mortality, because few children die, but for morbidity and hospital admissions [8].

Effect of other immune-modulators

The effects of vaccines on the immune system may be modulated by other immune-modulating factors. Interactions are found between vaccines and high-dose vitamin A supplementation [93] and two vaccines may have completely different effects when administered simultaneously [94]. We need to explore systematically what is likely to happen when our effective interventions are administered with other vaccines, drugs, or micronutrients and in different sequences.

Future immunological studies

T-cell-mediated cross-reactivity

We need to be able to identify when heterologous immunity is useful or detrimental when designing vaccines, and identify those individuals who are at risk for pathology. Severe pathology induced by heterologous immunity could be circumvented by developing new therapeutic interventions such as using cytokine blockers or peptide tolerization or using vaccines lacking cross-reactive epitopes involved in induction of immunopathology [37,40,51]. To accomplish this T cell epitopes need to be identified for common human viral and bacterial pathogens.

Trained innate immunity

We need to know for how long trained immunity induces an enhanced innate cytokine response upon stimulation, what the correlates of protection to be assessed are in situations in which nonspecific protective effects are suspected, and whether live vaccines are more effective in inducing trained immunity. Moreover, additional studies need to be performed to investigate the most efficient route and schedule of vaccination for inducing trained immunity. Last but not least, more needs to be learned regarding the intimate molecular and immunological mechanisms leading to trained immunity. Although epigenetic mechanisms have been shown to mediate monocyte reprogramming during trained immunity, the extent of these modifications remains to be assessed, starting with the relative contribution of histone and DNA modifications (methylation, acetylation, etc.) to the role of miRNAs, and to identifying the pharmacological modulators that could further enhance the efficiency of the innate immunological memory.

examining cross-reactive features of the immune system as well as the memory capacity of the innate immune system. Although tens of thousands of studies assessing disease-specific, antibody-inducing effects of vaccines have been conducted, most people have not examined whether vaccines have nonspecific effects because current perception excludes such effects. It is noteworthy that there are no data that contradict the claim that vaccines have nonspecific effects, and the few researchers who have looked for them have found them.

Why do nonspecific effects of vaccines continue to be overlooked in spite of increasing evidence? The concept that vaccines have nonspecific effects – sometimes detrimental – is a major issue with important and to some extent unpredictable consequences for public health. However, this should not be an argument for ignoring very important biological phenomena. Further epidemiological and immunological studies are clearly warranted (Box 3).

Concluding remarks

Nonspecific effects of vaccines have been dismissed or ignored because they are difficult to explain biologically. However, the nonspecific effects of vaccines are reproducible in randomized controlled trials [88] and the potential implications merit further investigations. The new evidence that vaccines induces cross-reactivity and train the innate immune system, and that these effects can be beneficial and detrimental, provides biological support for the epidemiological findings. In our opinion it is now urgent that we explore the effects of vaccines in a much more systematic and open-minded manner. We need to understand how the immune system learns and how the training can be optimized to increase general resistance and decrease morbidity. This may have far-reaching consequences for health and health expenditure; a well-planned and appropriate vaccination schedule based on scientific studies may be of great benefit and could reduce morbidity and mortality in low- and high-income countries.

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References

1 Mayr, A. (2004) Taking advantage of the positive side-effects of smallpox vaccination. *J. Vet. Med. B: Infect. Dis. Vet. Public Health* 51, 199–201

2 Naeslund, C. (1932) Résultats des expérience de vaccination par le BCG poursuivies dans le Norrbotten (Suède) Septembre 1927–Décembre 1931. In *Vaccination Préventative de la Tuberculose de l Homme et des Animaux par le BCG: Rapports et Documents Provenant des Divers Pays* (la France exceptée), pp. 274–281 (in French)

3 Aaby, P. *et al.* (1994) Sex-specific differences in mortality after high-titre measles immunization in rural Senegal. *Bull. World Health Organ.* 72, 761–770

4 Aaby, P. *et al.* (2010) Non-specific effects of standard measles vaccine at 4.5 and 9 months of age on childhood mortality: randomised controlled trial. *BMJ* 341, c6495

5 Aaby, P. *et al.* (2012) The optimal age of measles immunisation in low-income countries: a secondary analysis of the assumptions underlying the current policy. *BMJ Open* 2, 4

6 Aaby, P. *et al.* (2011) Randomized trial of BCG vaccination at birth to low-birth-weight children: beneficial nonspecific effects in the neonatal period? *J. Infect. Dis.* 204, 245–252

7 Biering-Sorensen, S. *et al.* (2012) Small randomized trial among low-birth-weight children receiving bacillus Calmette–Guerin vaccination at first health center contact. *Pediatr. Infect. Dis. J.* 31, 306–308

8 Sorup, S. *et al.* (2011) Smallpox vaccination and all-cause infectious disease hospitalization: a Danish register-based cohort study. *Int. J. Epidemiol.* 40, 955–963
9 Jensen, M.L. *et al.* (2006) Vaccinia scars associated with improved survival among adults in rural Guinea-Bissau. *PLoS ONE* 1, e101
10 Aaby, P. *et al.* (2006) Vaccinia scars associated with better survival for adults. An observational study from Guinea-Bissau. *Vaccine* 24, 5718–5725
11 Aaby, P. *et al.* (2012) Testing the hypothesis that diphtheria-tetanus-pertussis vaccine has negative non-specific and sex-differential effects on child survival in high-mortality countries. *BMJ Open* 2, 3
12 Kleinnijenhuis, J. *et al.* (2012) Bacille Calmette–Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. *Proc. Natl. Acad. Sci. U.S.A.* 109, 17537–17542
13 Netea, M.G. *et al.* (2011) Trained immunity: a memory for innate host defense. *Cell Host Microbe* 9, 355–361
14 Welsh, R.M. and Selin, L.K. (2002) No one is naive: the significance of heterologous T-cell immunity. *Nat. Rev. Immunol.* 2, 417–426
15 Aaby, P. *et al.* (1995) Non-specific beneficial effect of measles immunisation: analysis of mortality studies from developing countries. *BMJ* 311, 481–485
16 Cooper, W.O. *et al.* (2003) Do childhood vaccines have non-specific effects on mortality? *Bull. World Health Organ.* 81, 821–826
17 Aaby, P. and Jensen, H. (2005) Do measles vaccines have non-specific effects on mortality? *Bull. World Health Organ.* 83, 238
18 Moxon, R. *et al.* (2012) The new decade of vaccines – Authors' reply. *Lancet* 379, 27
19 Roth, A. *et al.* (2006) Bacillus Calmette–Guerin vaccination and infant mortality. *Expert Rev. Vaccines* 5, 277–293
20 Shann, F. (2010) The non-specific effects of vaccines. *Arch. Dis. Child.* 95, 662–667
21 Steenhuis, T.J. *et al.* (2008) Bacille–Calmette–Guerin vaccination and the development of allergic disease in children: a randomized, prospective, single-blind study. *Clin. Exp. Allergy* 38, 79–85
22 Fine, P.E. *et al.* (2012) Non-specific effects of BCG? *J. Infect. Dis.* 205, 515 author reply 517–518
23 Bager, P. *et al.* (2003) Smallpox vaccination and risk of allergy and asthma. *J. Allergy Clin. Immunol.* 111, 1227–1231
24 Pfahlberg, A. *et al.* (2002) Inverse association between melanoma and previous vaccinations against tuberculosis and smallpox: results of the FEBIM study. *J. Invest. Dermatol.* 119, 570–575
25 Aaby, P. *et al.* (2004) The introduction of diphtheria-tetanus-pertussis vaccine and child mortality in rural Guinea-Bissau: an observational study. *Int. J. Epidemiol.* 33, 374–380
26 Aaby, P. *et al.* (2003) Differences in female-male mortality after high-titre measles vaccine and association with subsequent vaccination with diphtheria-tetanus-pertussis and inactivated poliovirus: reanalysis of West African studies. *Lancet* 361, 2183–2188
27 Kristensen, I. *et al.* (2000) Routine vaccinations and child survival: follow up study in Guinea-Bissau, West Africa. *BMJ* 321, 1435–1438
28 Breiman, R.F. *et al.* (2004) Effect of infant immunisation on childhood mortality in rural Bangladesh: analysis of health and demographic surveillance data. *Lancet* 364, 2204–2211
29 Moulton, L.H. *et al.* (2005) Evaluation of non-specific effects of infant immunizations on early infant mortality in a southern Indian population. *Trop. Med. Int. Health* 10, 947–955
30 Vaugelade, J. *et al.* (2004) Non-specific effects of vaccination on child survival: prospective cohort study in Burkina Faso. *BMJ* 329, 1309
31 Elguero, E. *et al.* (2005) Non-specific effects of vaccination on child survival? A prospective study in Senegal. *Trop. Med. Int. Health* 10, 956–960
32 Lehmann, D. *et al.* (2005) Benefits of routine immunizations on childhood survival in Tari, Southern Highlands Province, Papua New Guinea. *Int. J. Epidemiol.* 34, 138–148
33 Nyarko, P. *et al.* (2001) Immunization status and child survival in rural Ghana. *Population Council Working Papers* no. 147
34 Fine, P.E.M. and Smith, P.G. (2007) Editorial: 'Non-specific effects of vaccines'- an important analytical insight, and call for a workshop. *Trop. Med. Int. Health* 12, 1–4
35 Aaby, P. *et al.* (2007) DTP vaccination and child survival in observational studies with incomplete vaccination data. *Trop. Med. Int. Health* 12, 15–24
36 Jensen, H. *et al.* (2007) Survival bias in observational studies of the impact of routine immunizations on childhood survival. *Trop. Med. Int. Health* 12, 5–14
37 Selin, L.K. *et al.* (1998) Protective heterologous antiviral immunity and enhanced immunopathogenesis mediated by memory T cell populations. *J. Exp. Med.* 188, 1705–1715
38 Selin, L.K. *et al.* (1994) Cross-reactivities in memory cytotoxic T lymphocyte recognition of heterologous viruses. *J. Exp. Med.* 179, 1933–1943
39 Mathurin, K.S. *et al.* (2009) CD4 T-cell-mediated heterologous immunity between mycobacteria and poxviruses. *J. Virol.* 83, 3528–3539
40 Chen, H.D. *et al.* (2001) Memory $CD8^+$ T cells in heterologous antiviral immunity and immunopathology in the lung. *Nat. Immunol.* 2, 1067–1076
41 Chen, H.D. *et al.* (2003) Specific history of heterologous virus infections determines anti-viral immunity and immunopathology in the lung. *Am. J. Pathol.* 163, 1341–1355
42 Clute, S.C. *et al.* (2005) Cross-reactive influenza virus-specific $CD8^+$ T cells contribute to lymphoproliferation in Epstein–Barr virus-associated infectious mononucleosis. *J. Clin. Invest.* 115, 3602–3612
43 Cornberg, M. *et al.* (2006) Narrowed TCR repertoire and viral escape as a consequence of heterologous immunity. *J. Clin. Invest.* 116, 1443–1456
44 Cornberg, M. *et al.* (2010) CD8 T cell cross-reactivity networks mediate heterologous immunity in human EBV and murine vaccinia virus infections. *J. Immunol.* 184, 2825–2838
45 Nie, S. *et al.* (2010) Pathological features of heterologous immunity are regulated by the private specificities of the immune repertoire. *Am. J. Pathol.* 176, 2107–2112
46 Kim, S.K. *et al.* (2005) Private specificities of CD8 T cell responses control patterns of heterologous immunity. *J. Exp. Med.* 201, 523–533
47 Wlodarczyk, M.F. *et al.* (2013) Anti-IFN-γ and peptide-tolerization therapies inhibit acute lung injury induced by cross-reactive influenza a-specific memory T cells. *J. Immunol.* 190, 2736–2746
48 Bahl, K. *et al.* (2010) Analysis of apoptosis of memory T cells and dendritic cells during the early stages of viral infection or exposure to toll-like receptor agonists. *J. Virol.* 84, 4866–4877
49 Selin, L.K. *et al.* (1999) Attrition of T cell memory: selective loss of LCMV epitope-specific memory CD8 T cells following infections with heterologous viruses. *Immunity* 11, 733–742
50 Brehm, M.A. *et al.* (2002) T cell immunodominance and maintenance of memory regulated by unexpectedly cross-reactive pathogens. *Nat. Immunol.* 3, 627–634
51 Nie, S. *et al.* (2009) Resistance to vaccinia virus is less dependent on TNF under conditions of heterologous immunity. *J. Immunol.* 183, 6554–6560
52 Su, L.F. *et al.* (2013) Virus-specific $CD4^+$ memory-phenotype T cells are abundant in unexposed adults. *Immunity* 38, 373–383
53 Yang, H.Y. *et al.* (1985) Necrosis of adipose tissue induced by sequential infections with unrelated viruses. *Am. J. Pathol.* 120, 173–177
54 Di Giusto, C.A. and Bernhard, J.D. (1986) Erythema nodosum provoked by hepatitis B vaccine. *Lancet* 2, 1042
55 Ojaimi, S. *et al.* (2009) Quadrivalent Human Papillomavirus recombinant vaccine associated lipoatrophy. *Vaccine* 27, 4876–4878
56 Requena, L. and Requena, C. (2002) Erythema nodosum. *Dermatol. Online J.* 8, 4
57 Smoller, B.R. *et al.* (1990) An unusual cutaneous manifestation of Crohn's disease. *Arch. Pathol. Lab. Med.* 114, 609–610
58 Cornberg, M. *et al.* (2007) Protection against vaccinia virus challenge by CD8 memory T cells resolved by molecular mimicry. *J. Virol.* 81, 934–944
59 Beaumier, C.M. *et al.* (2008) Cross-reactive memory $CD8^+$ T cells alter the immune response to heterologous secondary dengue virus infections in mice in a sequence-specific manner. *J. Infect. Dis.* 197, 608–617
60 Mongkolsapaya, J. *et al.* (2003) Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever. *Nat. Med.* 9, 921–927
61 Wedemeyer, H. *et al.* (2001) Cross-reactivity between hepatitis C virus and Influenza A virus determinant-specific cytotoxic T cells. *J. Virol.* 75, 11392–11400
62 Urbani, S. *et al.* (2005) Heterologous T cell immunity in severe hepatitis C virus infection. *J. Exp. Med.* 201, 675–680
63 Sheridan, S.L. *et al.* (2012) Number and order of whole cell pertussis vaccines in infancy and disease protection. *JAMA* 308, 454–456

64 Akira, S. *et al.* (2006) Pathogen recognition and innate immunity. *Cell* 124, 783–801
65 Bowdish, D.M. *et al.* (2007) Macrophage receptors implicated in the "adaptive" form of innate immunity. *Microbes Infect.* 9, 1680–1687
66 Pham, L.N. *et al.* (2007) A specific primed immune response in *Drosophila* is dependent on phagocytes. *PLoS Pathog.* 3, e26
67 Rodrigues, J. *et al.* (2010) Hemocyte differentiation mediates innate immune memory in *Anopheles gambiae* mosquitoes. *Science* 329, 1353–1355
68 Little, T.J. *et al.* (2003) Maternal transfer of strain-specific immunity in an invertebrate. *Curr. Biol.* 13, 489–492
69 Witteveldt, J. *et al.* (2004) Protection of *Penaeus monodon* against white spot syndrome virus by oral vaccination. *J. Virol.* 78, 2057–2061
70 van den Burg, H.A. and Takken, F.L. (2009) Does chromatin remodeling mark systemic acquired resistance? *Trends Plant Sci.* 14, 286–294
71 Conrath, U. (2011) Molecular aspects of defence priming. *Trends Plant Sci.* 16, 524–531
72 Slaughter, A. *et al.* (2012) Descendants of primed *Arabidopsis* plants exhibit resistance to biotic stress. *Plant Phys.* 158, 835–843
73 Bistoni, F. *et al.* (1986) Evidence for macrophage-mediated protection against lethal *Candida albicans* infection. *Infect. Immun.* 51, 668–674
74 Sun, J.C. *et al.* (2009) Adaptive immune features of natural killer cells. *Nature* 457, 557–561
75 Lopez-Verges, S. *et al.* (2011) Expansion of a unique CD57(+)NKG2Chi natural killer cell subset during acute human cytomegalovirus infection. *Proc. Natl. Acad. Sci. U.S.A.* 108, 14725–14732
76 Paust, S. *et al.* (2010) Critical role for the chemokine receptor CXCR6 in NK cell-mediated antigen-specific memory of haptens and viruses. *Nat. Immunol.* 11, 1127–1135
77 Beeson, P.B. and Elizabeth, R. (1947) Tolerance to bacterial pyrogens: I. Factors influencing its development. *J. Exp. Med.* 86, 29–38
78 Draisma, A. *et al.* (2009) Development of endotoxin tolerance in humans *in vivo*. *Crit. Care Med.* 37, 1261–1267
79 Foster, S.L. *et al.* (2007) Gene-specific control of inflammation by TLR-induced chromatin modifications. *Nature* 447, 972–978
80 Tribouley, J. *et al.* (1978) Effect of Bacillus Callmette Guerin (BCG) on the receptivity of nude mice to *Schistosoma mansoni*. *C. R. Seances Soc. Biol. Fil.* 172, 902–904 (in French)
81 van't Wout, J.W. *et al.* (1992) The role of BCG/PPD-activated macrophages in resistance against systemic candidiasis in mice. *Scand. J. Immunol.* 36, 713–719
82 Clarke, T.B. *et al.* (2010) Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. *Nat. Med.* 16, 228–231
83 Bistoni, F. *et al.* (1988) Immunomodulation by a low-virulence, agerminative variant of *Candida albicans*. Further evidence for macrophage activation as one of the effector mechanisms of nonspecific anti-infectious protection. *J. Med. Vet. Mycol.* 26, 285–299
84 Vecchiarelli, A. *et al.* (1989) Protective immunity induced by low-virulence *Candida albicans*: cytokine production in the development of the anti-infectious state. *Cell. Immunol.* 124, 334–344
85 Quintin, J. *et al.* (2012) *Candida albicans* infection affords protection against reinfection via functional reprogramming of monocytes. *Cell Host Microbe* 12, 223–232
86 Barton, E.S. *et al.* (2007) Herpesvirus latency confers symbiotic protection from bacterial infection. *Nature* 447, 326–329
87 Tenenbaum, J.B. *et al.* (2011) How to grow a mind: statistics, structure, and abstraction. *Science* 331, 1279–1285
88 Aaby, P. *et al.* (2012) Vaccine programmes must consider their effect on general resistance. *BMJ* 344, e3769
89 Chen, A.T. *et al.* (2012) Loss of anti-viral immunity by infection with a virus encoding a cross-reactive pathogenic epitope. *PLoS Pathog.* 8, e1002633
90 Durrant, W.E. and Dong, X. (2004) Systemic acquired resistance. *Annu. Rev. Phytopathol.* 42, 185–209
91 Jaskiewicz, M. *et al.* (2011) Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response. *EMBO Rep.* 12, 50–55
92 Moret, Y. and Siva-Jothy, M.T. (2003) Adaptive innate immunity? Responsive-mode prophylaxis in the mealworm beetle, *Tenebrio molitor*. *Proc. Biol. Sci.* 270, 2475–2480
93 Benn, C.S. (2012) Combining vitamin A and vaccines: convenience or conflict? *Dan. Med. J.* 59, B4378
94 Aaby, P. and Benn, C.S. (2009) Assessment of childhood immunisation coverage. *Lancet* 373