OPINION

Harnessing the beneficial heterologous effects of vaccination

Helen S. Goodridge[1], S. Sohail Ahmed[2], Nigel Curtis[3], Tobias R. Kollmann[4], Ofer Levy[5], Mihai G. Netea[6], Andrew J. Pollard[7], Reinout van Crevel[8], Christopher B. Wilson[9]

[1]Cedars-Sinai Medical Center, 8700 Beverly Boulevard, Los Angeles, CA 90048, USA. e-mail: helen.goodridge@csmc.edu

[2]VisMederi Srl, via Fiorentina 1, Siena, Italy 53100. e-mail: ahmed@vismederi.com

[3]Department of Paediatrics, The University of Melbourne and Murdoch Children's Research Institute, Royal Children's Hospital Melbourne, Parkville, Australia. e-mail: nigel.curtis@rch.org.au

[4]Division of Infectious Disease, Department of Paediatrics, University of British Columbia, CFRI A5-175, 950 W 28th Ave, Vancouver, BC V5Z4H4, Canada. e-mail: tkollm@mac.com

[5]*Precision Vaccines Program*, Division of Infectious Diseases, Boston Children's Hospital and Harvard Medical School, Boston, MA, USA. e-mail: ofer.levy@childrens.harvard.edu

[6]Department of Internal Medicine and Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, The Netherlands. e-mail: mihai.netea@radboudumc.nl

[7]Oxford Vaccine Group, Department of Paediatrics, University of Oxford and the NIHR Oxford Biomedical Research Centre, Oxford, UK. e-mail: andrew.pollard@paediatrics.ox.ac.uk

[8]Department of Internal Medicine and Radboud Center for Infectious Diseases, Radboud University Medical Center, PO Box 9101, 6500 HB Nijmegen, The Netherlands. e-mail: reinout.vancrevel@radboudumc.nl

[9]Global Health Program, Bill and Melinda Gates Foundation, 500 5th Ave N, Seattle, WA 98105, USA. e-mail: chris.wilson@gatesfoundation.org

Clinical evidence strongly suggests that certain live vaccines, in particular BCG and measles vaccines, can reduce all-cause mortality, likely via protection against non-targeted pathogens in addition to the targeted pathogen. The underlying mechanisms are currently unknown. We discuss how heterologous lymphocyte activation and innate immune memory could promote protection beyond the intended target and consider how vaccinologists could leverage heterologous immunity to improve outcomes in vulnerable populations, in particular the very young and the elderly.

The goal of vaccination is to induce an immune response against one or more antigens that results in persistent antibody production (to maintain circulating and mucosal antibody levels) and/or more efficient (faster and/or increased) mobilization of adaptive immune cells (T and B lymphocytes) when an individual subsequently encounters a pathogen containing those antigens months or years later. Antigens can be delivered as purified molecules (e.g. subunit vaccines) or in a more complex format (e.g. as components of live attenuated or inactivated microbes). Purified antigens are generally poorly immunogenic, and therefore adjuvants are used to boost their immunogenicity. Whole microbes often have intrinsic adjuvant activity due in part to their immunostimulatory molecules, such as cell wall components and nucleotides that engage pattern recognition receptors (PRRs). However, even vaccines that deliver intact microbes may be enhanced by an additional adjuvant, especially in the case of inactivated vaccines. Aluminum salts (alum) are the most commonly used adjuvants in human vaccines, but microbe-derived components and their synthetic congeners, and/or oil-in-water emulsions that engage PRR pathways are increasingly attractive alternatives in the development of new vaccines [1, 2].

Vaccines have classically been thought to generate specificity and memory via activation of antigen-induced adaptive immune responses mediated by T cells and B cells, and adjuvants may promote these responses by stimulating antigen acquisition and immunogenic presentation by antigen presenting cells (APCs) of the innate immune system (principally dendritic cells, DCs). However, as discussed below, accumulating evidence from clinical and laboratory studies indicates that heterologous activation of lymphocytes and innate immune memory mechanisms also shape the host response to vaccination.

Moreover, several lines of evidence suggest that certain vaccines influence immune responses against either other vaccines, or pathogens not targeted by the vaccine. These effects have variously been called ‘heterologous’, ‘non-specific’ or ‘off-target’ effects. As defined in Box 1,

we herein use the term ‘heterologous’ to describe a vaccine that is designed to target a specific pathogen, but also impacts the host’s response to unrelated pathogens (or potentially to the host itself), with unanticipated effects on morbidity and mortality that are not attributable to prevention of the disease(s) targeted by the vaccine. We also use the term ‘heterologous’ to describe the activation of lymphocyte responses (antigen-specific or non-specific) that are directed against non-target antigens.

In this article we discuss how complex effects of antigens and adjuvants underlie immune responses to vaccines, with parallels to the development of immunity following natural infection. Moreover, we consider how these effects could account for heterologous clinical effects of vaccination, and reflect on the implications of vaccine-induced heterologous immunity for the optimization of immunization programs.

Heterologous effects of vaccination

A major goal of modern vaccinology is to influence the magnitude, quality and durability of the T and B cell response using adjuvants, viral vectors, virus-like particles and other formulations and delivery vehicles to enhance immune responses [2]. The aim is to generate protective immunity in immunologically naïve or less immunocompetent populations (especially the very young, who are the target of most vaccines, and the elderly) by boosting responses against ‘weak’ antigens and inducing responses that are more broadly protective against a range of microbial strains (e.g. influenza).

In addition to providing immune protection against their intended targets, vaccines may have other beneficial effects, such as broadening the diversity of cross-clade protection within an individual species. For example, the MF59-adjuvanted influenza vaccine provided expanded

coverage to include related but antigenically distinct influenza strains in addition to the target strain [3]. Vaccines may also provide broader benefit by preventing opportunistic secondary infections. Indeed, a recent population-level analysis describing the post-disease immunosuppressive effects of measles infections argued that measles vaccination provides protection against not only measles virus, but also the suppressive shadow that the disease casts on the immune system, thereby also reducing non-measles mortality [4].

Moreover, there is increasing evidence that some vaccines can broadly enhance immune responses to a range of distinct pathogens or other vaccines (see Boxes 2 and 3), which indicates that immune protection may be influenced by prior exposure to unrelated microbes or microbial components. The strongest evidence of such effects in humans relates to the apparent ability of the Bacille Calmette-Guerin (BCG) vaccine (live attenuated *Mycobacterium bovis*) to reduce all-cause infant mortality in high mortality settings [5-7] (see Table 1). Similar benefits have been reported for other live attenuated vaccines, specifically measles vaccine and oral polio vaccine [8, 9]. The observed reductions in all-cause mortality appear to extend beyond the direct protection induced by these vaccines against their target pathogens, and likely reflect the induction of resistance to unrelated pathogens via enhancement of heterologous adaptive immune responses and/or antigen non-specific innate immune mechanisms. These positive heterologous effects of certain live attenuated vaccines may not be limited to resource-poor settings. Indeed, certain live attenuated vaccines, such as measles, smallpox and polio vaccines, may also reduce infection-related hospitalization in developed countries by providing protection against unrelated pathogens [10-13].

Heterologous effects do not just influence immune responses to pathogens, but have also been harnessed in the treatment of non-invasive bladder cancer, where local instillation of BCG is used to induce anti-tumor responses [14]. Negative heterologous effects have also been reported,

including an association between the AS03-adjuvanted pandemic influenza vaccine that was widely used in Europe during the 2009 H1N1 influenza pandemic [15-17] and the development of narcolepsy in some children and infants [18, 19]. It has also been suggested that inactivated vaccines (such as DTP) may either have negative heterologous effects or oppose the positive heterologous effects of live vaccines [5], although evidence for this is currently limited [7].

The above examples of heterologous effects indicate that the immunological consequences of infectious diseases, and the vaccines that prevent them, may influence the host's immediate and future interaction with antigens from unrelated organisms or the host. These observations provide both opportunities and challenges in the design of vaccines and vaccination programs, and call for a deeper, evidence-based understanding of the mechanisms underlying immune responses to both infection and vaccination. While it is apparent that the nature of the antigen and adjuvant can have profound effects on the immune response to that antigen, it is less clear how they can also influence subsequent immune responses to other antigens, resulting in heterologous effects.

Heterologous lymphocyte responses

Antigen cross-reactivity resulting from molecular mimicry may be responsible for some heterologous effects (see Figure 1). For example, adult humans (but not neonates) have been shown to possess memory T cells reactive to viral antigens to which they had not been exposed (including HIV, HSV and CMV) and cross-reactive to antigenic peptides from non-targeted microbes [20]. The development of narcolepsy in some patients who received the AS03-adjuvanted influenza (H1N1) pandemic vaccine may also be due to cross-reactivity, in this case to host antigens [21]. Specifically, molecular mimicry between a fragment of one of the influenza antigens (nucleoprotein) and a portion of the human brain receptor that promotes wakefulness (hypocretin

receptor 2) provides a plausible explanation for this heterologous effect, although other mechanisms have been suggested [22]. Interestingly, during the same H1N1 influenza pandemic, a spike in childhood narcolepsy cases was also observed in China, where vaccination rates were very low [23]. It is possible that the same antigen cross-reactivity mechanism (i.e. between the nucleoprotein of the infectious virus and the host hypocretin receptor 2) was responsible for the increased narcolepsy in this population, but this has not yet been demonstrated. Antigen cross-reactivity cannot, however, explain all the diverse heterologous effects observed with other vaccines.

Heterologous lymphocyte activation may account for some of the heterologous effects.

'Bystander' activation of unrelated B cells and/or T cells following infectious or vaccine-induced immune stimulation may also help to sustain pre-existing antigen-specific immunity (see Figure 1). Microbial components or cytokines may stimulate polyclonal activation of T cells or antibody production by memory B cells. Indeed, a recent report demonstrated that memory CD8 T cells can be re-activated by inflammatory monocyte-derived IL-15 and IL-18 [24]. Similarly, it has been reported that immunizing adults with tetanus toxoid not only increases tetanus antibody concentrations, but also stimulates increased levels of measles and *Toxoplasma gondii* antibodies, either through enriched T cell help or other polyclonal stimuli [25, 26]. However, others failed to see a substantial impact of heterologous immunization on pre-existing antibody titers in humans [27] or on memory B cell responses in mouse models [28]. Thus, while polyclonal stimulation of B cell responses could contribute to sustained antibody responses throughout life, the evidence for this is inconsistent. When given at or around the same time as other vaccines, BCG may enhance the antibody response to those vaccines, acting as or like an adjuvant, although the specific effects/outcomes are a matter of debate [29-31]. In any case, this is not likely to be the main mechanism for the beneficial heterologous effects of BCG or measles vaccine in infants because they often lack pre-existing immunity to most pathogens and durability of

antibody responses is more limited than at older ages, perhaps attributable in part to suboptimal support in the bone marrow [32, 33].

Cytokine production has also been suggested to underlie the heterologous effects of BCG (see Figure 1b). Experiments conducted half a century ago demonstrated infection-induced "cross-protection" between unrelated bacterial pathogens, and these pioneering observations played a crucial role in defining the concept of cell-mediated immunity [34]. This classical form of cross-protection is mediated by lymphocytes that produce interferon-γ (IFN-γ) after stimulation by the first pathogen encountered (e.g. BCG), thereby activating macrophages. This generates a state of heightened innate immunity against a secondary infection, which wanes rapidly once the initial pathogen is eliminated [35].

Some, but not all, studies in humans have reported that peripheral blood mononuclear cells harvested at various time points after BCG immunization exhibit increased production of IFN-γ and other pro-inflammatory cytokines upon *in vitro* stimulation with mitogens or unrelated antigens [36]. Enhanced *in vitro* IFN-γ production following measles vaccination has also been reported in some studies [36]. In contrast, several studies have consistently shown reduced mitogen-induced proliferative responses following vaccination [36]. These apparently divergent results may reflect variation in randomization, controls, antigenic stimuli and the *in vitro* assays used. It is therefore difficult to draw robust conclusions regarding the impact of live attenuated vaccines on cytokine induction from the available data.

An enhanced pro-inflammatory cytokine milieu after measles and BCG vaccination might contribute to the increased protective immunity and reduced all-cause mortality observed with these vaccines. However, a clear pattern has not been determined for the antigenic requirements, timing, nature or persistence of these effects, nor their influence on subsequent antibody or T cell

responses. Moreover, there is currently no direct evidence in humans that such immunological phenomena are the basis for the epidemiological observations of reduced mortality following live vaccines in high mortality settings. Indeed the immunological mechanisms underlying both the mycobacteria-targeting and the heterologous effects of BCG remain incompletely defined despite over 50 years of laboratory and animal studies highlighting its immunomodulatory properties [37-39].

Innate immune memory

In addition to the impact of lymphocyte-derived cytokines such as IFN-γ on innate immune cells, recent evidence suggests that innate immune cells, especially monocytes/macrophages and natural killer (NK) cells, possess intrinsic memory characteristics and may make a greater contribution to the heterologous protective effects of vaccines than T and B cell-based adaptive immune mechanisms.

Of note, immunization with certain live microbes or microbial components that activate innate immune cells can protect mice against lethal infection with distinct pathogens. For example, heterologous immunity is induced by fungal β-glucans against infection with *Staphylococcus aureus* bacteria [40, 41], by the peptidoglycan component muramyl dipeptide against *Toxoplasma* parasites [42], and by CpG oligodeoxynucleotide against sepsis, *Escherichia coli* meningitis, *S. pneumoniae* and rotavirus [43-45]. The broad characteristics of this protection implicate innate immune mechanisms rather than classical antigen-specific adaptive immune memory.

Bacteria, fungi, parasites and viruses can exert long-term immunomodulatory effects that have been demonstrated, using T and B cell-deficient or -depleted animals, to be independent of adaptive immunity. BCG vaccination of animals induces T and B cell-independent protection

against secondary infections with *Candida albicans* or *Schistosoma mansoni* [46, 47], demonstrating that innate immune mechanisms mediate at least some of the heterologous protective effects of vaccination in these models. Moreover, protection against disseminated candidiasis (CA-6 strain of *C. albicans*) conferred by an attenuated (PCA-2) strain of *C. albicans* is dependent on macrophages [48] and pro-inflammatory cytokines [49], and is induced in athymic mice and *Rag1*-deficient animals, demonstrating a T and B cell-independent mechanism [50, 51]. Latent infection of mice with gamma-herpesvirus also increases T cell-independent resistance to unrelated bacterial pathogens such as *Listeria monocytogenes* and *Yersinia pestis* [52], due to sustained IFN-γ production and systemic macrophage activation. Similarly, the helminth parasite *Nippostrongylus brasiliensis* induces a long-term macrophage phenotype that damages the parasite and induces protection from re-infection independently of T and B lymphocytes [53].

'Trained immunity', whereby the responses of innate immune cells such as monocytes and macrophages are primed by prior exposure to the same or an unrelated stimulus [54] (see Figure 2), may underlie some of the heterologous effects of vaccination. For example, exposure of monocytes or macrophages to *C. albicans* or β-glucan enhances their subsequent response to stimulation with unrelated pathogens or pathogen-associated molecular patterns [51]. Innate immune memory can induce various functional programs, some characterized by a combination of suppressive and stimulatory effects. This is best exemplified by lipopolysaccharide (LPS)-induced 'tolerance'. LPS confers long-lasting innate immune effects on monocytes and macrophages, resulting in reduced inflammatory cytokine production upon either re-stimulation with LPS or subsequent exposure to unrelated microbial components. However, in contrast to its effects on inflammatory cytokine production, anti-microbial responses are primed by prior LPS exposure [55]. While the functional program may be different from that induced by β–glucan or other vaccine adjuvants, conceptually the long-term functional reprogramming of innate immume cells by LPS can therefore be considered similar to trained immunity. Thus innate

memory is shaped by a combination of positive and negative effects at the molecular level (often simultaneously within the same cell – Figure 2b) that ultimately influence subsequent responses to related or unrelated pathogens.

Epigenetic reprogramming likely underlies these effects. LPS stimulation of macrophages induces gene-specific chromatin modifications that simultaneously silence genes coding for inflammatory molecules (tolerance) and promote the expression of genes coding for anti-microbial molecules (priming) [55]. Monocyte training by *C. albicans* and β-glucan is also accompanied by significant reprogramming of chromatin marks, and blockade of histone methylation has been shown to impair the induction of trained immunity [51, 56]. Metabolic shifts in the trained monocytes from oxidative phosphorylation to glycolysis ('Warburg effect') are also important for the induction of trained immunity [57]. Several metabolites may function as co-factors for epigenetic enzymes [58], and investigation of the impact of changes in cellular metabolism on the epigenetic program of innate immune cells is an important focus of ongoing research.

Autophagy has also been implicated in the development of trained immunity. A recent study showed that epigenetic reprogramming of monocytes by BCG *in vitro* is dependent on autophagy [59]. Blocking autophagy abrogated the *in vitro* training effect of BCG, and monocyte training *in vitro* and *in vivo* was also influenced by genetic variation in the autophagy gene *ATG2B*. Moreover, the same polymorphism correlates with responsiveness to intravesical BCG instillation in non-invasive bladder cancer patients [59].

NK cells can also respond more vigorously after previous encounters with pathogens or other activation events. Indeed, the heterologous protective effects of BCG vaccination may be mediated in part by activation of NK cells. NK cells from BCG-vaccinated individuals exhibit

enhanced pro-inflammatory cytokine production in response to mycobacteria and other unrelated pathogens, and in mice, NK cells help mediate the heterologous protective effects of BCG against *C. albicans* [60]. Moreover, several recent studies have reported that NK cells develop adaptive immune characteristics after infection with murine cytomegalovirus (mCMV) [53, 61-63]. NK cells bearing the Ly49H receptor persist for months in lymphoid and non-lymphoid organs after mCMV infection, and upon re-infection, these 'memory' NK cells undergo a secondary clonal expansion, rapidly degranulating and releasing cytokines, thereby inducing a protective immune response [53]. NK cell memory may also involve the IL-12/IFN-γ axis [63] and the activation of the co-stimulatory molecule DNAM-1 (DNAX accessory molecule-1, CD226) [64].

Evidence in support of NK cell memory is almost entirely derived from rodent studies [65], but a recent report showed that simian immunodeficiency virus infection and viral vectored-vaccines induce NK cell memory in macaques that can persist for up to 5 years [66]. However, this immunity, like NK cell memory induced by mCMV or hapten sensitization in mice, was specific to the priming agent. Thus unlike the heterologous monocyte/macrophage effects, NK cell memory may provide pathogen specificity. mCMV-induced NK memory, for example, appears to be specific for mCMV but not EBV [67], and to confer impaired heterologous immunity against influenza and *L. monocytogenes* [68, 69]. Depending on the conditions, NK cells are therefore able to employ both trained immunity, which may be mediated by epigenetic changes [62, 70], and antigen-specific memory.

An important aspect to be considered regarding trained immunity is the lifespan of innate immune cells, particularly monocytes and the macrophages derived from them. In humans, trained monocytes can be observed in the circulation for at least three months after BCG vaccination [60]. This observation suggests that reprogramming also takes place at the level of progenitor cells to account for the persistence of modified populations of these relatively short-

lived cells (Figure 2c). Indeed, innate immune memory effects of microbial exposure can be transferred from hematopoietic stem and progenitor cells to their progeny. For instance, hematopoietic stem and progenitor cells exposed to Toll-like receptor 2 agonists generate macrophages that produce lower amounts of inflammatory cytokines and reactive oxygen species [71]. Moreover, NK cells prime monocytes for regulatory function in the bone marrow in response to *T. gondii* infection, an effect that is mediated at least in part by IFN-γ programming of monocyte progenitors [72]. Epigenetic imprinting of hematopoietic progenitors may enable chronic persistence of training/tolerance effects. Indeed, UV radiation of the skin induces PGE_2-mediated systemic immunosuppression due to modulation of DC function, and this effect can be transferred to naïve mice upon serial bone marrow transplantation due to long-lasting epigenetic reprogramming of DC progenitors (presumably hematopoietic stem cells) [73-75].

Impact of age on heterologous immunity

The heterologous effects of infection and vaccination are likely impacted by age. Indeed, the beneficial heterologous effects of BCG were most evident when given early in life [7]. Moreover, narcolepsy following the monovalent AS03-adjuvanted H1N1 pandemic influenza vaccine was primarily observed in children and adolescents, and decreased as the age of the subjects approached adulthood [76-78]. In contrast, in elderly subjects a related vaccine enhanced protection against some influenza subtypes compared to the non-adjuvanted trivalent seasonal influenza vaccine and decreased all-cause morbidity and mortality, despite not meeting the main criteria for efficacy in this clinical study [79, 80]. A recent study reported that the early immune response of healthy adults to the monovalent AS03-adjuvanted H1N1 vaccine changes significantly after the age of ~35 years [81].

The immune system undergoes development throughout childhood. Th1-associated cytokines (TNF-α, IL-12, IFN-γ), NK cell cytotoxicity, antigen-specific Th1 responses and high affinity antibody production are all low during the neonatal period and gradually increase over time (see Box 4). Immune function is shaped in early life by colonization with commensal microbes and exposure to pathogens and vaccines. Given this immune ontogeny and that to date most beneficial heterologous effects have been noted with pediatric vaccines given in early life, innate immune memory mechanisms may play a particularly prominent role in early life, likely exceeding that of heterologous lymphocyte activation [82]. Indeed, blood cells isolated 4 weeks after neonatal BCG immunization exhibit increased TLR-induced cytokine production *in vitro*, suggesting that BCG may accelerate the development of innate cytokine responses [83].

Among the elderly, decreased innate and adaptive immune function underlies increased susceptibility to infection and reduced vaccine efficacy [84, 85]. Thymic involution, restricted lymphocyte clonality and declining anti-microbial innate immune function contribute to sub-optimal responses to many vaccine antigens and adjuvants. Little is known about the heterologous effects of vaccination in elderly populations or the impact of immunosenescence on heterologous lymphocyte responses and trained immunity. Dysregulated cytokine responses associated with immunosenescence following a lifetime of microbial exposure may lead to diminished trained immunity, but this remains to be investigated.

Perspectives for vaccinology

The studies and mechanisms described above present vaccinologists with exciting opportunities, as well as a number of challenges, in relation to the design of new vaccines and the development of vaccination programs. Positive heterologous effects mediated by innate and/or adaptive immune mechanisms could be leveraged to confer broader protection against a range of

pathogens. If a ‘super-protective’ state could be induced by vaccination, even if only for a brief window of exquisite infectious susceptibility such as the neonatal period, important improvements in health could be realized. On the other hand, the potential for negative heterologous effects must also be considered in order to improve vaccine efficacy and safety.

High quality randomized controlled trials are needed to establish heterologous effects (both pathogen-specific and non-specific) of vaccines in diverse populations e.g. to define the impact of age, genetics, geographical location, and sociological factors. These studies must be accompanied by thorough characterization of immunologic correlates of clinically observed heterologous effects, including phenotypic and molecular changes in monocytes, NK cells, lymphocytes and other leukocytes. Model organisms should also be employed to establish the role of proposed immune mechanisms in the observed effects. Bioinformatics tools will enable the refinement of vaccine antigens to exclude those that are potentially cross-reactive, thus limiting the negative effects. In addition, positive and negative heterologous effects could be predicted by taking advantage of new technologies such as human *in vitro* platforms that model age-specific immunity [1] and powerful systems biology approaches [81, 86, 87] that can now be employed using small sample volumes [88].

Defining mechanisms of heterologous lymphocyte activation and innate immune memory in early life and the elderly is of particular importance because infection-induced mortality is highest in the first year of life and aging-associated susceptibility to infection is placing increasing pressure on healthcare resources in the developed world. Given the burden of infection in those at the extremes of age, an effective plan is needed that engages funding agencies and other stakeholders to mobilize the resources required to leverage these newly discovered immunologic pathways to reduce infection-induced morbidity and mortality.

Box 1 | **Definitions: heterologous effects and mechanisms.**

A vaccine that confers protection against unrelated pathogens, in addition to the target pathogen, is described as having heterologous effects (see Box 2 and Table 1 for examples). Heterologous effects of vaccination may persist for long periods (see Box 3). Deleterious (negative) heterologous effects are also possible if vaccination impairs the ability of the host to combat infection with non-targeted pathogens. Vaccines may also have heterologous effects (positive or negative) that are directed against host tissues, such as induction of an anti-tumor response or autoimmune disease.

In some cases, heterologous effects can be attributed to antigen cross-reactivity, whereby lymphocytes specific for the vaccine antigen also recognize other antigens due to molecular mimicry. However, most heterologous effects of vaccination cannot be explained by molecular mimicry.

Heterologous effects of vaccination may alternatively be mediated by heterologous immune responses that are not specifically directed against the vaccine antigen (see Figures 1 and 2). Heterologous lymphocyte responses include the broad effects of cytokines produced by activated T cells (e.g. macrophage activation by IFN-γ) and activation of 'bystander' lymphocytes that are specific for non-targeted antigens. Heterologous immune responses can also involve lymphocyte-independent activation of innate immune cells. These effects may persist as a result of 'innate memory' mechanisms involving macrophages or NK cells. For example, epigenetic reprogramming due to sustained changes in gene expression and cell physiology, without permanent genetic changes (mutations or recombination), underlies 'trained immunity'.

Box 2 | **Heterologous effects of vaccination**

The strongest evidence for heterologous effects of vaccination relates to the apparent ability of certain live attenuated vaccines to enhance immunity in an antigen non-specific manner, resulting in reduced all-cause mortality that likely reflects the induction of resistance to unrelated pathogens [5, 89]. Specifically, the World Health Organization (WHO) Strategic Advisory Group of Experts (SAGE) on Immunization recently concluded that available evidence suggests a possible beneficial effect of immunization with Bacille Calmette-Guerin (BCG; a live attenuated *Mycobacterium bovis* strain) and live attenuated measles vaccine on all-cause mortality in high risk populations during the first weeks of life [5, 7] (see Table 1). Oral polio vaccine has also been reported to reduce all-cause mortality in high mortality settings [9].

Such benefits of live attenuated vaccines may not be limited to resource poor settings. A retrospective analysis of epidemiological data from Spain suggested that BCG given at birth may decrease hospitalization due to sepsis and respiratory infection [10]. Also, cohort studies from Denmark have suggested that measles, smallpox and oral polio vaccines reduce the rate of infection-related hospitalization [11-13].

Research is ongoing to determine whether other types of vaccines, including inactivated vaccines, also exhibit heterologous effects and to evaluate the potential for negative as well as positive effects [5].

Vaccines can also have heterologous effects (positive or negative) on host tissues. For example, the anti-tumor effects of BCG have been leveraged to treat non-invasive bladder cancer [14]. Other vaccines have been implicated in the development of autoimmunity, such as the increased incidence of narcolepsy among children who received the AS03-adjuvanted H1N1 influenza

vaccine [18, 21].

Box 3 | **Durability of heterologous effects of vaccination.**

Data describing the durability of heterologous protection following vaccination are limited. In the randomized controlled trials that provide some of the strongest evidence of a beneficial effect, BCG given at birth in a high-risk population in Guinea-Bissau was associated with a 58% reduction in all-cause mortality over the first few days, whereas the reduction over the first month and first year of life was 48% and 21% respectively [90]. This might suggest that protection waned rapidly. However, because those who did not receive BCG at birth subsequently received it (from 6 weeks of age onwards), it is also possible that the apparent reduction in benefit over time was related to a gain in protection in the control group rather than waning protection in those who received the vaccine at birth.

Similarly, in a study of children who received their first dose of measles vaccine at 4.5 or 9 months of age, there was a greater reduction in all-cause mortality between 4.5 and 9 months of age (33%) than between 9 and 36 months of age (17%), but the confidence intervals overlapped and those who did not receive measles vaccine at 4.5 months did receive the vaccine at 9 months of age [8]. Thus, although the data suggest that protection is greatest in the first weeks to months after receipt of BCG or measles vaccine, the apparent diminution in protection after the first months may be due in part to confounding factors.

At least one study suggests that heterologous effects can persist for more than a decade. An epidemiological study of 10-14 year old children in Spain reported 69.6% fewer incidents of hospitalization due to respiratory infections not attributable to tuberculosis among those who had received BCG at birth, compared with those who had not received the BCG vaccine [10].

Mechanistic studies are consistent with the possibility that at least some heterologous effects may be durable. Heterologous responses of lymphocytes may persist for decades in memory lymphocyte populations, and innate immune memory may persist for extended periods due to epigenetic reprogramming of long-lived differentiated cells (e.g. tissue-resident macrophages) or hematopoietic progenitors. Indeed, some heterologous responses to non-mycobacterial stimuli have been reported to remain strongly elevated 1 year after BCG vaccination of healthy adults [91].

Box 4 | **The immune system in early life.**

Humans (unlike rodents) have high numbers of T and B lymphocytes by mid-gestation, but development of the immune system continues for many years after birth [92]. A 'layered' immune system, comprising a mix of fetal liver-derived and adult-like bone marrow-derived leukocytes, exists in early life [1].

Regulatory T cells dominate *in utero* [93], and at birth almost all T and B lymphocytes are phenotypically and functionally naïve. Regulatory and Th2 responses are favored during the neonatal period, and a progressive shift to Th17 and then type I interferon responses follows over the first year of life. Th1 responses gradually develop later. Newborns exhibit impaired PRR-stimulated APC production of Th1-supporting cytokines [1], consistent with the high susceptibility of neonates to infection with intracellular pathogens, which declines throughout infancy [84].

Infants also have a lower capacity to mount robust and durable antibody responses. T cell-dependent antibody responses reach near adult competence by 1 year of age, and T cell-independent responses by 2-4 years of age [94]. NK cells in infants are approximately half as effective as adult NK cells at killing most target cells, although their cytotoxic capacity can be augmented by cytokines that promote Th1 responses and approaches that of adult cells by one year of age [92]. Limitations in neutrophil mobilization in response to systemic infection and mononuclear phagocyte function (including their responsiveness to IFN-γ) may also contribute to susceptibility to infection in the neonatal period.

Postnatal immune development is profoundly impacted by interactions with microbes, in particular colonization of the host with the microbiota, which is initially acquired from the mother following vaginal delivery and matures thereafter in an age- and environment-dependent

manner [95-98], as well as by exposure to pathogens and live vaccines. Of note, human newborn and adult monocytes exhibit distinct secretomic (proteomic) responses to adjuvants and licensed adjuvanted vaccines *in vitro* that demonstrate age- and adjuvant-specific correlations to adjuvanted vaccine-induced transcriptomic responses *in vivo* [99]. Remarkably, administration of TLR agonists to newborn mice enhances subsequent cytokine and phagocytic responses to polymicrobial sepsis associated with enhanced survival [100]. Moreover in humans, early inflammatory/innate immune-activating events such as histologic chorioamnionitis, early-onset sepsis, and BCG immunization are associated with reduced risk of late onset sepsis [82, 83]. These observations raise the possibility that broadly similar, but age-distinct, mechanisms may contribute to beneficial trained immunity in human newborns and infants.

Figure 1 | **Heterologous lymphocyte responses. a** | Some heterologous effects may be attributed to antigen-specific mechanisms that generate lymphocytes whose antigen receptors are cross-reactive for distinct microbial or host antigens due to molecular mimicry. Alternatively, a vaccine may induce bystander activation of memory lymphocytes to sustain levels of antibodies directed against other targets. **b** | Heterologous lymphocyte responses can induce protection against unrelated pathogens via antigen-specific bystander lymphocytes or induction of antigen non-specific innate mechanisms such as cytokine production to activate macrophages.

Figure 2 | **Mechanisms of innate memory in monocytes/macrophages.** Initial exposure of monocytes/macrophages or their progenitors to certain microbial stimuli induces epigenetic marks and metabolic changes that persist for extended periods and influence the subsequent responses of these cells to the same or distinct stimuli. **a** | Priming/training increases the subsequent response, whereas tolerance reduces responsiveness. **b** | Priming/training and tolerance can occur simultaneously within the same cell, with expression of some genes being promoted and others suppressed. **c** | Innate memory effects may be long-lived due to the persistence of self-renewing macrophages in tissues, or exposed progenitors that continue to yield monocytes and macrophages with altered function for extended periods. Innate memory may be maintained indefinitely or wane over time.

Table 1 | **Impact of BCG and measles vaccines on all-cause mortality.**[*]

Vaccine	Country (year)	Sample size	Observed % reduction in all-cause mortality (95% CI)	References
BCG	Canada (1933-1945)	609	6% (-32, 33)	[101]
BCG	Guinea Bissau (2002-2008)	2343	45% (11, 66)	[90, 102]
BCG	Guinea Bissau (2002-2008)	105	72% (-37, 94)	[90]
BCG	USA (1935)	3008	9% (-99, 59)	[103]
BCG	USA (1941)	451	58% (-35, 87)	[104]
Measles	Guinea Bissau (2002-2008)	6417	33% (-19, 62)	[8]
Measles	Guinea Bissau (1989-1999)	300	0% (-392, 80)	[105]
Measles	Guinea Bissau (1989-1999)	8511	6% (-67, 47)	[106]
Measles	Nigeria (1961)	1962	59% (15, 86)	[107]

CI – confidence interval

*Randomised trials of the impact of BCG and Measles vaccine on all-cause mortality identified in a systematic review conducted for the World Health Organisation [7]. Data were derived from the 2014 Strategic Advisory Group of Experts (SAGE) on immunization report: www.who.int/immunization/sage/meetings/2014/april/3_NSE_Epidemiology_review_Report_to_SAGE_14_Mar_FINAL.pdf

Further information

Systematic review of the non-specific effects of BCG, DTP and measles containing vaccines:

www.who.int/immunization/sage/meetings/2014/april/3_NSE_Epidemiology_review_Report_to_SAGE_14_Mar_FINAL.pdf

Systematic review of the non-specific immunological effects of selected routine childhood immunizations:

http://www.who.int/immunization/sage/meetings/2014/april/2_NSE_Immunology_review_version_for_SAGE_JLC.pdf

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Author biographies

Helen S. Goodridge received her Ph.D. from the University of Glasgow, U.K. After postdoctoral training there and at Cedars-Sinai Medical Center, Los Angeles, USA, she joined the Cedars-Sinai faculty and is currently an Associate Professor. Her research focuses on the production and function of myeloid cells in infectious and inflammatory diseases, cancer and aging.

S. Sohail Ahmed received his M.D. degree from the University of Texas Medical School in Houston where he completed his general medicine residency training and clinical immunology/rheumatology subspecialty training. In 2006, he joined Novartis Pharmaceuticals (Translational Medicine) and in 2011, he was appointed Global Head of Clinical Sciences at Novartis Vaccines & Diagnostics Srl. He is currently affiliated with VisMederi Srl in Siena, Italy.

Nigel Curtis completed medical training in Cambridge and London, received his PhD from the University of London, and did further clinical and laboratory training in London and Vancouver before moving to Melbourne, Australia. He is Head of Infectious Diseases at the Royal Children's Hospital Melbourne, Professor at the University of Melbourne, and Leader of the Infectious Diseases & Microbiology Research Group at Murdoch Children's Research Institute. His current research focuses on the heterologous effects of BCG vaccination.

Tobias R. Kollmann completed his MD and PhD at the Albert of Einstein College of Medicine and his residency and fellowship at the University of Washington, Seattle. He is now consulting physician in pediatric infectious disease at BC Children's Hospital, Canada. His research focuses on development of the immune system, with special emphasis on vaccines for early life. His

team has developed high-throughput platforms to dissect molecular events in small samples from newborns and small infants.

Ofer Levy received his MD and PhD (Microbiology) from New York University. He completed his residency in Pediatrics and fellowship in Pediatric Infectious Diseases at Boston Children's Hospital, along with postdoctoral research training at the Brigham & Women's Hospital. He is currently staff physician and Director of the *Precision Vaccines Program* at Boston Children's Hospital and Associate Professor of Pediatrics at Harvard Medical School. His laboratory focuses on employing human *in vitro* systems and adjuvant discovery to develop vaccine formulations tailored to those at the extremes of life - the very young and the elderly.

Mihai G. Netea studied medicine in Cluj-Napoca, Romania and completed his PhD at the Radboud University Nijmegen, The Netherlands. After postdoctoral training at the University of Colorado, he returned to Nijmegen to finish his clinical training as an infectious diseases specialist. He currently heads the division of Experimental Medicine, Department of Internal Medicine, Radboud University Medical Center. His main research interests are sepsis and immunoparalysis, pattern recognition of fungal pathogens, primary immunodeficiencies in the innate immune system, and the study of the memory traits of innate immunity.

Andrew J. Pollard, FRCPCH PhD, is Professor of Paediatric Infection and Immunity at the University of Oxford, UK. He trained in paediatric infectious diseases in the UK and Canada. His research includes the design, development and clinical evaluation of vaccines in the UK and Nepal. He has supervised 23 PhD students and has published over 300 manuscripts.

Reinout van Crevel is an internist-infectious diseases specialist. His primary research focus is the interaction of mycobacteria with innate host defense, combining patient cohorts, mainly in

Indonesia, with in vitro studies. He leads a consortium on the interaction between diabetes and tuberculosis, with field work and laboratory studies in eight different countries.

Chris B. Wilson, M.D., is Director of the Global Health Discovery & Translational Sciences program at the Bill & Melinda Gates Foundation. He is a member of the Advisory Committee to the Director, NIH and the Advisory Council of the National Institute of Allergy & Infections Diseases, and an elected fellow of the American Association for the Advancement of Science.

Table of contents summary

Clinical evidence, including results from randomized controlled clinical trials, strongly suggests that certain live vaccines can reduce all-cause mortality, likely due to protection against non-targeted pathogens. This review examines the potential immunological mechanisms underlying these effects.

Key Points

- A vaccine that confers protection against unrelated pathogens in addition to the target pathogen is described as having heterologous effects. For example, some live vaccines (in particular BCG and measles vaccine) have been shown to reduce all-cause mortality in high risk populations.
- Heterologous effects may persist for long periods (months or even years).
- Most heterologous effects cannot be attributed to antigen cross-reactivity.
- Heterologous effects may be mediated by heterologous lymphocyte activation or by innate immune memory mechanisms such as ‘trained immunity’.
- The age of the vaccinated individual impacts the immune response and therefore the heterologous effects. It is particularly important to study heterologous effects and mechanisms in infants and the elderly.
- Leveraging heterologous effects could reduce infection-induced morbidity and mortality in vulnerable populations.

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Figure 1

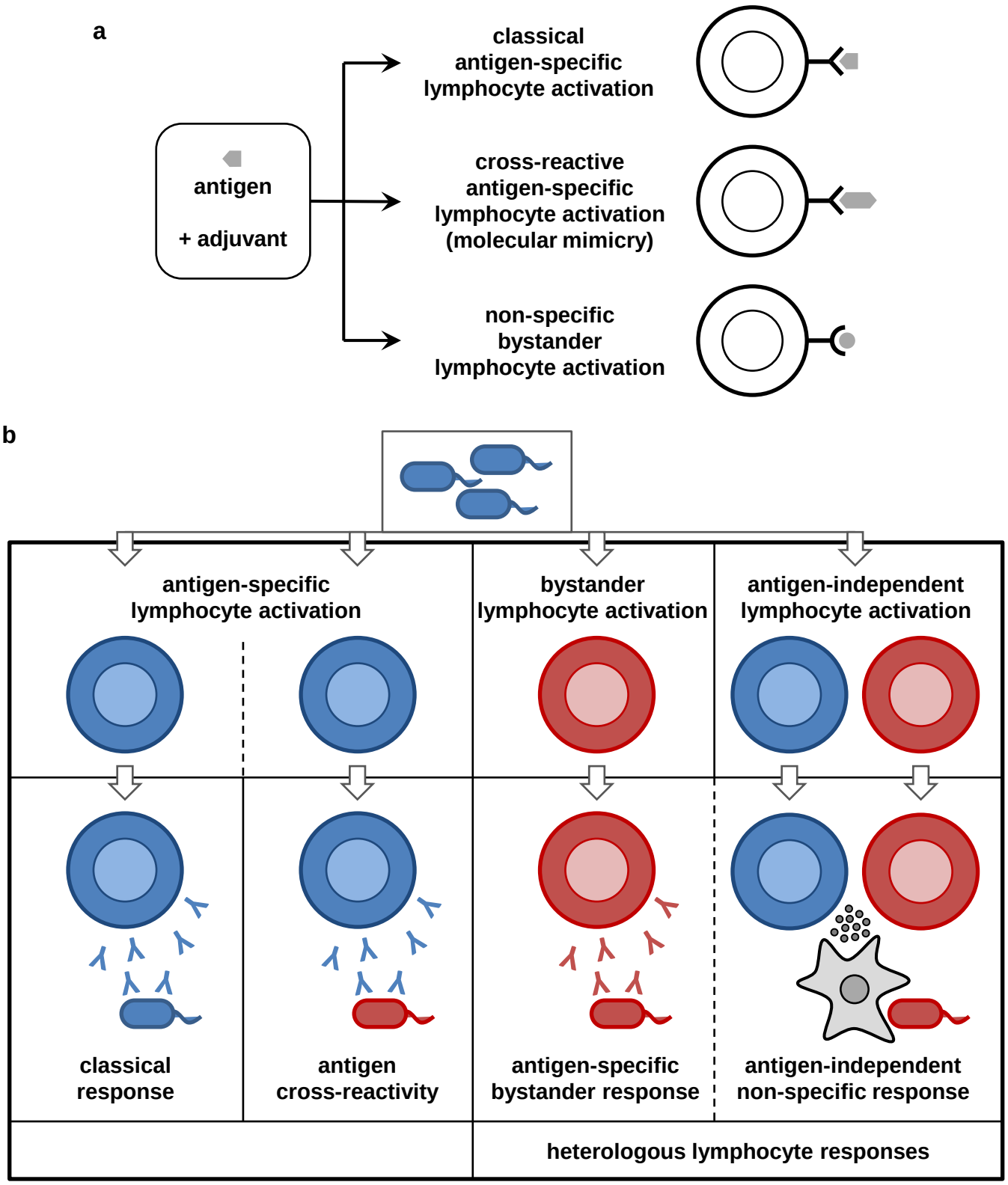
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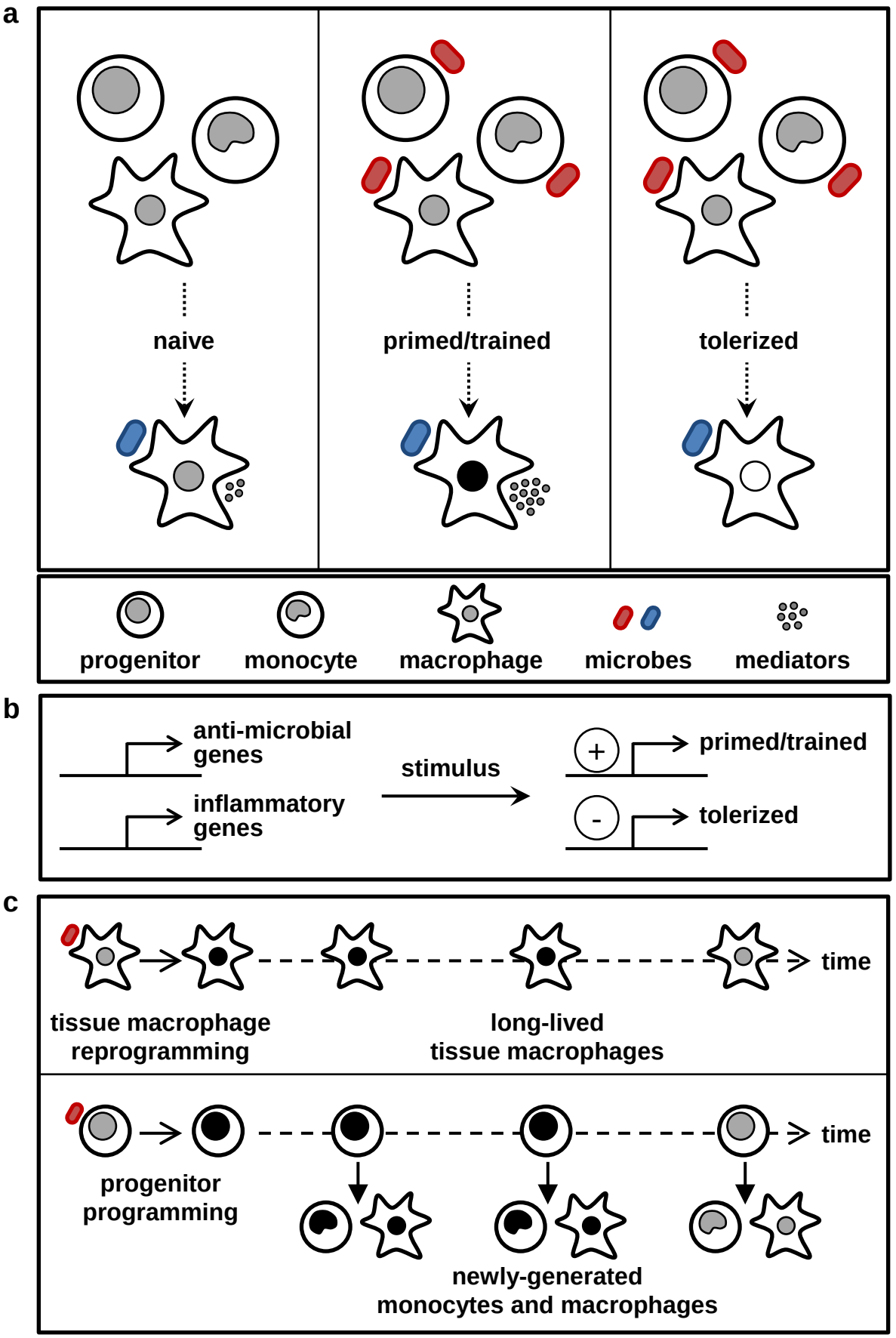
Figure 2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