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REVIEW

Vaccination and heterologous immunity: educating the immune system

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This review discusses three inter-related topics: (1) the immaturity of the neonatal and infant immune response; (2) heterologous immunity, where prior infection history with unrelated pathogens alters disease outcome resulting in either enhanced protective immunity or increased immunopathology to new infections, and (3) epidemiological human vaccine studies that demonstrate vaccines can have beneficial or detrimental effects on subsequent unrelated infections. The results from the epidemiological and heterologous immunity studies suggest that the immune system has tremendous plasticity and that each new infection or vaccine that an individual is exposed to during a lifetime will potentially alter the dynamics of their immune system. It also suggests that each new infection or vaccine that an infant receives is not only perturbing the immune system but is educating the immune system and laying down the foundation for all subsequent responses. This leads to the question, is there an optimum way to educate the immune system? Should this be taken into consideration in our vaccination protocols?

Keywords: Crossreactive, Heterologous immunity, Neonatal, T cells, Vaccines

Introduction

This review begins by briefly summarizing the limited literature that is available on neonatal and infant immunity in mice and humans to give an appreciation of the 'immaturity' of neonatal immune responses and how they differ from adults. The second part reviews the extensive adult mouse and human research on heterologous immunity—that is, the effects of prior immunity on immune responses to new infections, which can be either innate or adaptive effects, beneficial or detrimental. This review focuses predominantly on the latter aspect of heterologous immunity; that mediated by memory T cell crossreactivity. Innate heterologous immunity has been previously reviewed elsewhere.[1] The concept of memory T cells being crossreactive with new pathogens suggests that with each new infection we would educate the immune system increasing our library of memory responses with an ability to respond not only to the original infecting antigens, but potentially to many others we have not yet experienced. These crossreactive memory responses can potentially enhance and alter innate responses. The third part reviews the epidemiological evidence for heterologous immunity playing a role during vaccination of children suggesting that each vaccine is educating your immune response to react to not only the vaccine antigens, but also to unrelated pathogens.

Maturation of the neonatal immune system

Neonates are highly susceptible to death from infection because they lack protective immunological memory and are still developing and undergoing a process of colonization of their skin and gut with commensal microbial flora. Disparities in the innate, cellular and humoral arms of the immune system all play a role in the reduced immune responses of neonates to infection. To combat the susceptibility of neonates, three vaccines have been administered worldwide to newborns (first 24 hours of life): the hepatitis B virus (HBV) vaccine, the BCG vaccine and the oral poliovirus vaccine, in an effort to further protect this highly susceptible group.[2] The immune system of a neonate and infant is significantly different than that of an adult, making understanding of how the neonatal/infant immune system matures, and whether and how vaccination or infections play a vital role in educating the immune system in order to optimize vaccine strategies vital.

Neonatal innate immunity

As their exposure to antigens in utero is limited, neonates lack immunological memory and are reliant on passive maternal antibody and their innate immune responses as their first line of defense.[3] However, the innate immune system of the neonate is functionally distinct from that of an adult. For example, blood-derived monocytes from human infants have reduced production of cytokines such as IFNα, IFNγ and IL-12, upon toll-like receptor (TLR) stimulation, but have an increase in IL-10 and the T helper (Th)17-inducing cytokines, IL-6 and IL-23.[4] Antiviral cytokines, such as type I IFNs are protective in young children. For example, susceptibility to severe herpes simplex virus (HSV) infection correlates with low TLR-mediated type I IFN production in children

aged 0–2 months.[5] In addition to the direct antiviral mechanisms, inflammatory cytokines triggered through innate receptors also control the differentiation of the adaptive immune response and memory development. Direct effects of type I IFN are required for CD8 T cell expansion during lymphocytic choriomeningitis virus (LCMV) infection in mice, as IFNα receptor-deficient CD8 T cells have decreased survival and limited effector functions.[6]

Neonatal adaptive immunity

Compared to adults, neonates also have altered adaptive responses. Antibody responses are reduced, of shorter persistence and with decreased affinity maturation and altered IgG isotype skewing.[7] The T cell responses in neonates differ from that of adults by decreased number of T cells in the spleen,[8] frequencies of T cell subsets such as CD4 and CD8,[9] responsiveness to stimulation and T cell receptor (TCR) diversity,[10] resulting in altered T cell responses after infection. Neonatal T cells were thought to be unresponsive until 1996, when three seminal papers showed that neonatal T cells were more prone to Th2-skewing.[8,11,12] However, it is still not completely understood why this happens during some infections but not others. For example, high doses of murine leukemia virus induced a Th2 response in neonatal mice, while low doses allowed for the induction of adult-like cytotoxic CD8 T cell and Th1-skewed CD4 T cell responses.[11] These differences may in part be due to the altered inflammatory responses that are found in neonates, but there are also T cell intrinsic differences between adults and neonates.

The majority of the peripheral T cells in neonates are recent thymic emigrants (RTE), which are defective in developing Th1 responses[13–15] and may explain Th2 skewing in some situations. RTE from neonates undergo increased IL-7-dependent proliferation in the periphery compared to RTE in adult mice.[15] In a co-transfer model of Listeria monocytogenes, neonate and adult transgenic CD8 T cells were allowed to respond to infection within the same inflammatory environment.[16] Neonatal cells were found to proliferate and differentiate faster than adult cells after infection.[16] During Listeria infection, neonatal CD8 T cells had a higher frequency of cytotoxic granzyme B+ cells with a higher expression of effector phenotype markers, including killer-like lectin receptor G1 (KLRG1), early during infection. However, neonatal CD8 T cells showed minimal proliferation in response to a secondary infection.[16] These studies show that in addition to the influences of the skewed inflammatory environment, neonatal CD8 T cells are intrinsically programed to respond to antigens differently than adult cells.

Neonatal TCR repertoire

Neonatal T cells also differ in the diversity of their TCR. During T cell development, diversity is accrued in the TCR repertoire by VDJ recombination, $\alpha\beta$ pairing of the TCR, and non-coding nucleotide insertions and deletions in the complementary determining region (CDR) 3 of the TCR. These CDR3 insertions and deletions are mediated by the enzyme terminal deoxynucleotidyl transferase (TdT), which is responsible for up to 90% of the TCR diversity.[17] In humans, this enzyme is expressed in the thymus during the third trimester, but in mice it is turned on between days 4 and 5 of age, with increased CDR3 lengths not detected in single positive thymocytes until day 8 of age.[18] As mice age the CDR3 length of their TCR increases.[10] Shorter CDR3 regions have been associated with increased crossreactivity.[19] T cell clones specific to influenza A virus (IAV)-NP366 were isolated from wildtype and TdT knock out (ko) adult C57BL/6 mice and assessed for their ability to lyse targets coated with a library of peptides. TdT ko clones were able to recognize a greater number of peptides, indicating that they were more crossreactive.[19] In contrast, adult studies with IAV-M1-epitope-specific CD8 T cell responses in human leucocyte antigen (HLA)-A2+ individuals showed increased alanine/glycine representation within longer CDR3 loops and associated with increased flexibility and increased crossreactivity.[20] Instead, TdT ko T cells, with shorter CDR3 regions, can respond to more antigens and this is attributed to a greater interaction of the TCR with the α chain of major histocompatibility complex class I instead of the peptide.[19] The increased crossreactivity of the neonatal T cell repertoire may allow for smaller populations of T cells to react against a wide range of pathogens,[19] but perhaps with lower affinities.

Exactly what the mechanisms are that lead to maturation of the neonatal immune system have not been well studied. It would be logical to assume that exposure to infectious pathogens would contribute to this maturation, both by developing memory T and B cell repertoires and by changes in the innate responses, perhaps some developmental while others are environmentally induced. For instance, Swain and colleagues observed that transferring memory IAV-specific CD4 cells into naive mice could prime an IAV response without the need for TLR activation.[21] Data from research into heterologous immunity of adult mice and humans, as well as epidemiological data from vaccination studies of children, suggest that the sequence of infection or vaccination can lead to either detrimental or beneficial effects on subsequent unrelated infections. These data would be consistent with the idea that they play a role in the education of the immune system and that there may be a more optimum vaccination strategy, which would be beneficial to the maturation of the immune system.

Heterologous immunity and T cell crossreactivity

Heterologous immunity refers to a phenomenon whereby a previous exposure of an immune system to one pathogen will alter the host response to a second heterologous pathogen. The mechanisms of heterologous immunity can vary, but the focus of this review is on experimental systems where both beneficial and detrimental heterologous immunity are mediated by crossreactive T cells.[22,23] More than 20 years of research have delineated basic principles of heterologous immunity, such as (1) T cell crossreactivity is quite common between unrelated pathogens and alters T cell immunodominance;[24] (2) networks of crossreactive T cells alter the efficacy of the T cell response and influence protective immunity and immunopathology;[25–30] (3) T cell crossreactivity can lead to narrowing of the T cell repertoire and generation of viral escape mutants;[31] (4) the private specificity of crossreactive T cells can determine an individual's disease outcome in regards to protective immunity and immunopathology;[31–33] (5) heterologous immunity can reduce the effectiveness of vaccines due to immunodominant skewing of undesired T cell responses;[29,34] (6) peptide-dependent interventions or cytokine

blocking therapy can be used to prevent severe pathology caused by heterologous immunity;[25,30,34] and (7) the immune response to each new pathogen impacts the frequencies, distributions and activities of memory T cells to previous infections.[22,35,36]

The contention is that heterologous immunity is the norm, not the exception, but given the diversity in human genetics, MHC and infection histories, is it too complicated an issue to address? There have been a number of studies that have already correlated several human disease syndromes to crossreactive epitopes presented by a single MHC molecule.[27,37–46] For detrimental heterologous immunity, a pathogenic epitope response might be dominant and not be obscured by other protective epitopes and MHC molecules. Recent papers show high frequencies of virus-specific memory T cells in non-immune individuals and a crossreactive epitope spanning many types of herpes viruses.[40,47] Clear networks of crossreactive epitopes and patterns of beneficial or detrimental heterologous immunity have been defined in specific virus sequences in mouse models (Supplementary Figure 1).[24–34,48–53] For example, in the C57BL/6 mouse ($H2^b$), CD8 T cell crossreactive epitopes have been defined between LCMV and Pichinde virus (PICV), LCMV and vaccinia virus (VACV), LCMV and murine cytomegalovirus (MCMV), LCMV and IAV, IAV and MCMV, and PICV and VACV, and complex networks of mouse or human T cell crossreactivity can exist between two viruses.[23,24,27,28,34,54–56] Furthermore, structural studies on crossreactivity between LCMV and VACV epitopes can pinpoint the target of crossreactivity and render the OVA SIINFEKL epitope crossreactive with LCMV by an amino acid substitution.[57,58] Crossreactivity is an essential feature of TCR, and the positive selection of T cells in the thymus is mediated through self-peptides that crossreact with a substantial repertoire of foreign epitopes.

The hierarchy of T cell responses to immunodominant epitopes in immunologically naive genetically identical mice is very consistent,[53–56] but the amino acid sequences of the TCRs responding to these epitopes differ from mouse to mouse; these are sometimes called 'private' specificities.[57–60] In a T cell repertoire, that which is common between individuals, be it TCR VA usage or a common amino acid sequence or 'motif', is a public specificity; that which is different between individuals, such as a CDR3 sequence, is a 'private' specificity. Thus, genetically identical hosts have, as a consequence of random DNA recombination events, genetically different immune systems, and this diversity of TCR usage poses a challenge when one considers whether an epitope-specific T cell response may be crossreactive with another epitope. This is because the expanded clones of virus-specific T cells in different individuals may have different private specificities, such that one individual may have a repertoire crossreactive between two epitopes, whereas the other individual may not. We have shown that private specificity is an integral part of heterologous immunity by demonstrating that even genetically identical mice use different crossreactive T cell responses, resulting in tremendous variability in disease.[50,61,62] The private specificity of TCR repertoires in the memory pool has an impact on heterologous immunity, where subsets of a crossreactive T cell repertoire are mobilized to control the heterologous viral infection.[31–33,56]

Studies revealing 'private specificities' of T cell responses showed that if memory cells from one LCMV-immune mouse were adoptively transferred into three naive congenic recipients later challenged with heterologous viruses, those three mice showed a similar epitope hierarchy as the original donor and differed from that of a set that received cells from an alternate donor.[32] In vivo findings in mice showed that a high affinity crossreactive response between LCMV and PICV would lead to narrowing of the TCR repertoire during the heterologous infection.[31] However, during a human study with less similar crossreactive epitopes, Epstein–Barr virus (EBV)-$BMLF1_{280}$ and IAV-$M1_{58}$, presumably lower affinity, led to broadening of the crossreactive TCR repertoire during infectious mononucleosis.[59]

In general, in vivo studies in mice have shown that an infection with an unrelated virus will induce the formation of new memory cells specific to the second virus and will delete memory cells specific to the previously encountered virus.[35,60] This is a permanent change that remains for the lifetime of the mouse, unless viral antigens remain present or are re-introduced, though this has never been sufficiently studied in humans. Two models could explain this loss in memory T cell frequency: the passive attrition model, whereby old memory cells are lost simply by their competition with newly formed memory cells for survival niches in the immune system after immune response silencing, or the active attrition model, whereby there is a directed apoptosis of the pre-existing memory cells.[60–62] Most of the in vivo data support the active attrition model.[61,63,64] Many viral infections in mouse and human induce a substantial loss in lymphocyte and leukocyte numbers in the early stages of infections.[65] A substantial loss in CD8 and CD4 T cell number during the first 5 days of LCMV infection parallels the levels of type 1 IFN production, does not occur in type 1 IFN receptor ko mice,[66] is blocked by antibody to type 1 IFN[67] and is mimicked by IFN-inducing toll receptor agonists, such as poly I:C.[66] The apoptotic loss of memory occurs prior to cell division.[63,66,68] An in silico virtual immune system model, IMMSIM, which unlike a biological system can selectively turn off either active or passive attrition, predicted that without the active attrition and apoptosis of memory T cells early in infection, crossreactive T cells might overwhelmingly dominate the response to an infection.[61,63] By selectively reducing the frequency of memory cells at the beginning of an immune response, there actually may develop a more diverse T cell response to a new pathogen, and that diversity may be beneficial to the host for control of the pathogen. This prediction was further validated in vivo in elderly mice as they undergo less IFN-driven T cell attrition than do young mice.[69] Elderly mice have more dominant expansion of their crossreactive T cells in both IAV-immune mice challenged with LCMV (Wlodarcyzk and Selin, unpublished data) and LCMV-immune mice challenged with PICV.[69]

The VACV infection of the LCMV-immune mice model combines aspects of beneficial protective heterologous immunity with severe immunopathology associated with detrimental heterologous immunity**,** suggesting that there is a fine balance between these responses. When LCMV-immune male mice are infected with VACV intraperitoneally there is a substantial reduction in VACV titer; however, some of these mice develop severe panniculitis, in the form of inflammation and necrosis of visceral fat tissue.[22,30,33] This type of abdominal fat pathology is seen in human syndromes of unknown etiology, such as Weber-Christian disease or lupus erythematosis, and erythema nodosum, a more benign and more common form of panniculitis, involves inflammation of subcutaneous fat tissue sometimes seen after vaccinations with VACV, human papilloma virus (HPV) and HBV.[30,70–73] When LCMV-immune mice are infected intranasally with VACV, there is again a reduction of viral titers in the

lung, but some present with the pathological picture of bronchiolitis obliterans,[25] comparable to a lethal human disease of unknown etiology. Both the protective heterologous immunity and the severe immune pathology are dependent on IFNγ in this system. LCMV and VACV share a complex matrix of CD8 T cell crossreactivity, which has been defined and studied molecularly.[28,52,57] Herein, adoptive transfer studies showed that T cell private specificity of crossreactive memory responses plays a major role in the protection and immunopathology.[32,33]

The IAV+LCMV respiratory infection mouse model is noteworthy in that there are both higher titers of LCMV and much greater lung pathology in mice previously infected with IAV, both detrimental effects of heterologous immunity. Crossreactive epitopes have been defined between these viruses and have shown that severity of pathology directly correlated with the frequency of crossreactive responses in the IAV-immune mice (Supplementary Figure 1). Notably, severe pathology can be prevented by immunizing with viral epitope mutants, by peptide tolerization against the epitope or by blockade of IFNγ.[26,34] In addition, T regulatory cells induced by IAV seem to play a role in dampening the overall T cell response to LCMV.[51] Interestingly, in both the IAV+LCMV and the LCMV+VACV systems, there is evidence that the cytokine responses early in infection as part of the innate responses are dramatically altered in immune versus naive mice during the acute viral infection.[26] This would suggest that prior infection could alter innate responses to a subsequent new pathogen and potentially play a role in the maturation of innate responses in the neonate.

LCMV/PICV simultaneous infection

Co-infections with two or more pathogens can occur after mosquito bites, contaminated needle sticks, combination vaccines or simultaneous administration of multiple vaccines, but little is known about the dynamics of acute and memory T cell responses after these acute co-infections. Co-infections led to highly variable CD8 T cell responses, with different immunodominance hierarchies between mice simultaneously infected with LCMV and PICV or LCMV and VACV instead of the more reproducible hierarchies seen in singly infected mice (Kenney et al., unpublished data). Further, there was decreased immune protection and enhanced immunopathology upon re-challenge of LCMV/PICV co-infected immune mice with either virus, where neutralizing antibody is not a confounding variable. Crossreactive T cell responses between the different viruses were associated with skewed T cell hierarchies and were shown to contribute to immune pathology on challenge. These experiments suggest that the magnitude and character of memory CD8 T cells generated in response to simultaneous co-infections differ substantially from those induced by single immunization. These results underscore that a better understanding of human T cell responses during co-infection or co-vaccination by two or more viruses is needed. Knowledge in this regard could contribute to optimizing strategies for currently used vaccines, for predicting who will be at increased risk for immunopathology and for designing therapeutic interventions.

Translation into human infections

A network of crossreactive CD8 T cell responses has been defined between EBV and IAV in humans (Supplementary Figure 1).[27,28] These responses were activated and proliferated during severe infections like acute infectious mononucleosis (IM) or influenza A infection. Unique EBV/IAV crossreactive populations have been identified in different HLA-A2+ patient populations, suggesting that crossreactive patterns may be predicted during human infections (Supplementary Figure 2). The percentage atypical lymphocytes and severity of IM correlated with increased frequencies of crossreactive IAV-M1/EBV-BMLF1, memory IAV-M1[27] and acute BMLF1 CD8 T cell responses and EBV load (Aslan and Selin, unpublished data). This suggests that the M1/BMLF1 crossreactive responses may drive severity of IM. During a recent influenza pandemic, studies in severely symptomatic HLA-A2+ patients acutely infected with IAV also demonstrated crossreactive responses with EBV peptides (Mishra and Selin, unpublished data). CD8 T cells were also examined in the unusual 5% of EBV sero-negative (EBV-SN) and PCR-negative middle-aged adults aged 30–60 years old who have resisted infection with EBV. Five such HLA-A2+individuals (Watkin et al., unpublished data) had evidence of crossreactivity between IAV-specific CD8 T cells and EBV-BMLF1 peptide. These observations suggest that both detrimental and beneficial heterologous immunity may be a factor in human EBV infections. The EBV-SN middle-aged adults had highly unique M1 TCR repertoires with dominant clones and unusual TCR sequences, perhaps enabling them to provide protective rather than detrimental immunity to EBV. Mouse studies have not yet addressed whether crossreactive memory responses can lead to sterilizing immunity at very low dose exposures with some viruses in mice.

All of these studies on heterologous immunity in adult humans and mice would suggest that the immune system is constantly evolving and that an individual's history of infections can strongly impact the outcome of subsequent new infections. This would suggest that the infections and vaccinations that we have early in life will form the bedrock that will influence all of our subsequent immune responses for the rest of our life. Epidemiological studies examining disease outcome following particular childhood vaccinations would tend to support this contention.

Epidemiological evidence of heterologous immunity during childhood vaccination

Remarkable epidemiological data have demonstrated that in areas in low-income countries, live vaccines like BCG or attenuated measles virus can have protective effects, decreasing morbidity and mortality to unrelated pathogens that may be due to reduced susceptibility to infections.[1] In contrast, the killed vaccine against diphtheria, tetanus and pertussis (DTP) has been associated with detrimental effects such as increased mortality to infections unrelated to the vaccine. These results are very reminiscent of the findings in the studies of immune responses during heterologous infections described earlier in the paper.

Measles vaccine

The studies on standard measles vaccine suggest that this vaccine has beneficial effects beyond protection against measles. Its implementation significantly improved child survival in Africa and may have enhanced resistance to unrelated infections. Comparative studies showed that two doses of measles vaccine

at 4.5 and 9 months of age reduced the mortality to infections other than measles by 30% in children as compared to one dose of measles vaccine at 9 months.[74–76] The vaccine was especially successful in children who were additionally protected by maternal antibodies to measles present at the time of immunization. Other studies suggest that measles vaccine has a positive non-specific heterologous effect reducing mortality to unrelated infections such as pneumonias and diarrheal infections, but this non-specific beneficial effect was eradicated if the measles vaccine was followed by an inactivated DTP vaccine.[76,77]

BCG vaccine

Similar to measles vaccine, BCG immunizations provided their recipients with positive heterologous immunity effects on mortality. Based on the trials led in Europe and the USA in the 1940s and 1950s, BCG vaccine decreased mortality by 25% from other infectious causes unrelated to the vaccine.[78] Whereas in low birth weight neonates it reduced the mortality by almost 50% as demonstrated by fewer cases of sepsis and respiratory infections.[79,80] This is similar to results in adult mouse models of heterologous immunity where it was demonstrated that BCG-immune mice were more resistant to vaccinia virus infection and that this resistance was mediated by IFNγ-producing CD4 T cells.[81] Interestingly, Steenhuis and others showed that in children, by 18 months of age, immunization with BCG resulted in lowered numbers of children with eczema, a Th2-immune mediated disease.[82]

Vaccinia vaccinations

Due to the fact that vaccinia immunizations generate very strong immune responses, it is probable that it may also have heterologous effects on the human immune system. After the eradication of smallpox, WHO in 1980 decided to stop the recommended smallpox vaccinations. However, the observational studies in rural Guinea-Bissau indicated that the presence of vaccinia scars were associated with increased survival rates among adults.[79,83] It was also shown that it may decrease the risk of asthma and malignant melanoma.[84] In Danish studies, smallpox immunizations lowered the number of infectious disease hospitalizations.[85]

DTP vaccine

In contrast to the vaccines described above, application of some inactivated vaccines may have negative effects. One of them is the DTP vaccine. Observational studies showed that after DTP vaccinations in Guinea-Bissau in the 1980s, all-cause mortality increased twofold compared to the non-vaccinated group.[86,87] Interestingly, the age and gender of the vaccinated group played an important role since DTP immunization of girls at 2 months of age resulted in sixfold higher mortality than in the unvaccinated female cohort between 2 and 6 months old. Also, the pediatric hospital mortality was increased when DTP was administered after measles vaccine compared to measles vaccine alone.[1] This suggests that the order of immunization is important, since application of BCG or measles vaccine reduced the negative effect of DTP vaccine[88].

Live measles virus and BCG promote strong Th1 responses, which may help mature the neonatal immune response that is Th2 prone, whereas the killed vaccine, DTP, promotes strong Th2 responses. Each of these vaccines may condition the entire immune system to become polarized in the same direction due to the reactivation of crossreactive memory CD8 or CD4 T cells. Stimulation of pre-existing memory crossreactive T cells skewed to produce a certain pattern of cytokines will result in rapid production of those cytokines, which then can drive newly responding naive T cells down similar pathways or else have some influence on innate cytokine production that could polarize both innate and adaptive immune responses. Vaccinations early in life may condition the entire immune system to become polarized in the same direction[1] as that particular vaccine. Thus, it may be of concern that the majority of the 20 vaccines given in the first 2 years of life are killed vaccines, prone to giving Th2-type responses given to a Th2-prone neonate or infant. Could this contribute to the increased rates of asthma or eczema?

The mechanisms mediating these heterologous effects of vaccines in children have yet to be determined. Although BCG has been shown to have a beneficial effect against VACV infection in mouse models, killed vaccines such as DTP have yet to be studied in mouse models of heterologous immunity. However, based on the large body of accumulating evidence of heterologous cross-reactive T cell immunity and innate heterologous immunity,[1] it is likely these mechanisms are playing a role in the human vaccine non-specific effects.

Conclusions

Exactly what the mechanisms are that lead to maturation of the neonatal immune system have not been studied. It would be logical to assume that exposure to infectious pathogens would contribute to educating the immune system both by developing memory T and B cell repertoires and by changes in the innate responses with some perhaps developmental while others are environmentally induced. Certainly the environment has a major impact as adult germ free mice are known to have extremely poor immune responses to pathogens. Education by environmental influences such as development of the microbiome and infections will alter the neonatal/infant immune response and this interrelationship has evolved over millions of years and only recently have we begun to intervene in this maturation process with vaccinations. These vaccinations have been crucial in preventing tremendous morbidity and mortality by common childhood infections, but if immune responses to each new pathogen do not occur in isolation like in a naive mouse, but are part of an ever-evolving continuum, this would suggest that some vaccination strategies might be more optimum than others in educating the immune system. Research into heterologous immunity of adult mice and humans, as well as epidemiological data from vaccination studies of children, suggest that the sequence of infection or vaccination, as well as the sex of the infant, can lead to either detrimental or beneficial effects on subsequent unrelated infections. These data would be consistent with the idea that both infections and vaccines play a role in the education of the immune system and that there may be an optimum vaccination strategy which will be beneficial to maturation of the immune system. This is in need of further study. WHO at a recent Strategic Advisory Group of Experts meeting acknowledged that vaccines may have heterologous or non-specific effects and that this needs further

investigation. In fact, clinical trials[89–91] have begun in both Denmark and Australia to study the heterologous effects of BCG at birth.

Supplementary data

Supplementary data are available at Transactions Online (http://trstmh.oxfordjournals.org).

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