

IS THE HYGIENE EFFECT BAD NEWS FOR THE CONTROL OF INFECTIOUS DISEASES?

Gerhard Walzl, MBChB, MMed, FLP(SA), PhD
Department of Medical Biochemistry, Faculty of Health Sciences, University of Stellenbosch, Tygerberg, South Africa

Tracy Hussell, BSc Hons, PhD
Centre for Molecular Microbiology and Infection, Department of Biochemistry, Imperial College of Science, Technology and Medicine, South Kensington, London, UK

Hendrik J Nel, BSc Hons, MSc
Department of Medical Biochemistry, Faculty of Health Sciences, University of Stellenbosch, Tygerberg, South Africa

SUMMARY

The relationship between bacterial exposure and the allergy epidemic has generated much interest. Additionally, a changing prevalence of auto-immune disease has been observed and also linked to the 'hygiene effect'. If exposure to infectious organisms has such a dramatic effect on the expression of allergic and auto-immune disease, then surely infection history should also have a profound impact on immune responses against subsequent infections. We here review the evidence from animal experiments and human studies and discuss possible mechanisms by which infection history can influence host responses. Immune suppression by regulatory cells, alteration in cytokine micro-environment, chemokine-induced redirection of effector cell trafficking, T-cell receptor cross-reactivity and bystander activation can come into play to change the phenotype or outcome of infection-induced immune responses. This may have important implications for vaccine design and testing. Additionally, the effect of helminth infection on the HIV and TB epidemics might have very important clinical significance and needs further research to allow greater understanding of these complicated interactions.

INTRODUCTION

The relationship between bacterial exposure and the allergy epidemic has generated much interest and was reviewed by Prescott¹ in a recent edition of this journal. The 'hygiene hypothesis' was first named by Strachan *et al.*² and refers to the relationship between hygiene (and associated levels of exposure to microbes and their products or effects on the immune system) and the increased incidence of allergy and atopy. Generally speaking, this hypothesis states that an increased awareness and application of more hygienic practices along with the use of antibiotics and vaccines in western societies has led to a decline in childhood exposure to and incurring of diseases that under 'normal' circumstances would have stimulated the immune system in such a way as to protect against allergic diseases. Epidemiological evidence to support the hypothesis has been published extensively, but it remains a highly controversial topic because of con-

flicting results brought about by intrinsic differences in study design and approach. In this review we examine the available evidence that similar mechanisms operate in the interaction between different microbes to influence the outcome of infectious diseases.

THE HYGIENE HYPOTHESIS AND ALLERGY

A protective role of early life infections in the development of atopy and asthma has been suggested by British cohort studies conducted since 1958. A consistent relationship was found between birth order and the risk of atopy inasmuch as the presence of older siblings appeared to protect younger children.^{3,4} Others have shown possible protection from allergy induced by early life-time lower respiratory tract infections,^{5,6} measles infection but not measles vaccination,⁷ positive tuberculin skin test responses⁸ and hepatitis A infection.⁹ However, others have found no protection by greater sibship size¹⁰ and an interesting observation relates to the high prevalence of asthma not only in developed communities but also in African cities¹¹ and among low-income minority groups in the USA.¹² It is unlikely that poor urban children in Africa are subjected to less crowding and therefore experience less infection than their rural counterparts.

What are the proposed immunological mechanisms for the protective effect of infections in the development of atopic disease? Atopy is related to the expression of allergen-specific responses with production of T helper 2 (Th2) cytokines such as interleukin 4 (IL-4) and IL-5, which promote IgE production and eosinophilia.¹³ In non-atopic individuals the T-cell system is biased to a Th1 phenotype with production of interferon-gamma (IFN- γ) and inhibition of Th2 cells. Further knowledge of the development of allergen-specific T-cell memory is important for the possible primary prevention of allergic disease. Fetal derived allergen-reactive T cells exhibiting a Th2 phenotype exist, which indicates intra-uterine T cell priming.¹⁴ Prescott *et al.*¹⁵ demonstrate the continuation of these fetal allergen-specific Th2 responses during infancy with a decreased capacity to produce IFN- γ in atopic neonates. They argue that environmental factors such as microbial agents exert their effects during early life and aid in the maturation of the adaptive immune response into a Th1-dependent system. Romagnani¹⁶ suggests that the rising prevalence of atopy is related to a reduction in childhood infections like tuberculosis with a resultant reduced production of cytokines antagonistic to Th2 cell differentiation. Animal studies support this concept of immune modulation by micro-organisms. Prior *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG) infection can suppress allergen-induced airway eosinophilia in mice in an IFN- γ dependent manner.¹⁷ In addition, Wang and Rook¹⁸ inhibited the established allergic response to ovalbumin in mice with killed *Mycobacterium vaccae*. The absence of microbial flora from the gut inhibits the maturation of the Th1 immune mechanisms and leads to life-long bias towards Th2 responses,¹⁹ which adds interesting support to the data on antibiotic use during the first two years of life as risk factor for the development of atopy.¹⁰

Correspondence: Dr Gerhard Walzl, Department of Medical Biochemistry, Faculty of Health Sciences, University of Stellenbosch, PO Box 19063, Tygerberg 7505, South Africa. Tel +27-21-938-9158, fax +27-21-938-9476, e-mail gwalzl@sun.ac.za

THE HYGIENE HYPOTHESIS AND AUTO-IMMUNE DISEASE

There has been a parallel increase in auto-immune disease and allergy over the past decades, especially in the developed world and in urban areas.²⁰ As in allergic disease, decreasing exposure to micro-organisms has been implicated as a possible cause for the increased expression of auto-immune disease.

Although it is generally accepted that autoreactive Th1 cells are important in auto-immune disease, the mechanisms leading to auto-immunity are not clear.²¹ Resting autoreactive T cells cannot induce disease whereas activated T cells can. Infections may play a role in initiation of these responses by non-specifically activating T cells.²² Molecular mimicry, superantigen activation of T cells expressing targeted β -chain alleles (V β), bystander activation and activation of lymphocytes by lymphotropic viruses are implicated. However, like the relationship between allergy and infections, a protective role for some infection, notably helminths and mycobacteria, has been postulated. This inverse relationship between infections and auto-immunity argues against a simple Th1 to Th2 shift as mechanism for the hygiene effect, as this should have been accompanied by a decrease in the prevalence of auto-immunity.

There have been reports of an inverse relationship between risk of type I diabetes mellitus in children and day care attendance.²³ Occurrence of infections in the first 6 months of life is also associated with a lower risk of diabetes, whereas infections in later life offer no advantage in this regard.²⁴ Mycobacterial infection and measles before the age of 7 protect from the development of multiple sclerosis as adults. These findings are from a retrospective case-matched study including 92 multiple sclerosis patients and 276 age- and sex-matched controls obtained from school health records in Copenhagen.²⁵ Animal studies also support the hygiene effect in auto-immune disease as BCG vaccination of C57BL/6 mice protects from experimental auto-immune encephalomyelitis (EAE).²¹ A 12 kDa purified protein derivative (PPD) belonging to the heat shock protein family was found to be important in this protective activity.^{26,27} The mechanism of protection might lie in shared T-cell epitopes with target antigen.

A regulatory T-cell subset (CD45RB^{low}CD38⁺) is required for mycobacterial protection from diabetes in mice. These cells trigger anergy or deletion of self-reactive peripheral lymphocytes.^{28,29}

In mouse experiments, *Schistosoma mansoni* ova were used to immunise prior to EAE induction and significant protection was observed, dependent on induction of a Th2 environment.²¹ Similarly, *Trypanosoma brucei brucei* infection protects from collagen-induced arthritis in rats,³⁰ malaria infection prevents lupus-like syndrome³¹ and *Trichuris trichiura* protects from inflammatory bowel disease in humans.³²

CD4+ T-HELPER MATURATION IN RESPONSE TO INFECTION

The differentiation path of T cells is determined by a variety of genetic and environmental factors. Naïve, uncommitted CD4⁺ T cells respond to their specific peptide-MHC class II complexes by secreting IL-2 and by proliferating before differentiating into T helper type 0 (Th0) cells. Th0 cells demonstrate both Th1 and Th2 functions but then differentiate into one of at least two effector phenotypes.³³ The cytokine micro-environment is one factor that plays an important role in determining the differentiation path. Antigen-presenting cells (APC), specifically macrophages and dendritic cells (DC), nat-

ural killer (NK) cells and CD8⁺ T cells are the source of these cytokines. Th1 and Th2 cells reciprocally inhibit each other.²¹ A schematic summary of Th1/Th2 maturation alternatives is shown in Figure 1. Other factors, such as antigen dose, DC maturational status and type of antigen also affect polarisation. Regulatory T (Treg) cells, however, inhibit the activity and differentiation of both Th1 and Th2 cells through inhibitory cytokines such as IL-10 and transforming growth factor- β (TGF- β) and through direct contact with these cells and APCs.^{34,35} This mechanism will be discussed in more detail later.

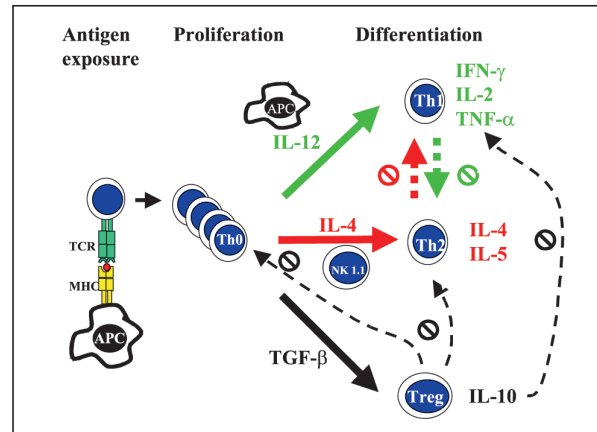


Fig. 1. Differentiation alternatives for Th0 cells. Naïve CD4⁺ T cells, after presentation of antigen through antigen-presenting cells (APC) and MHC class II complexes, proliferate in an IL-2 dependent manner and eventually differentiate into Th0 cells. The cytokine environment, among other factors, determines the downstream differentiation into Th1, Th2 or Treg effector CD4 cells characterised by their cell surface markers and specific cytokines production. Th1 and Th2 cells reciprocally inhibit each other while Treg cells control both these immune responses. Concurrent infections can influence differentiation of Th0 cells during the establishment of an inflammatory response by altering the cytokine micro-environment.

THE HYGIENE HYPOTHESIS AND INFECTIOUS DISEASES

The aim of this review is not to discuss the interaction between chronic infections, like HIV, and subsequent opportunistic infections, but rather to elucidate the less obvious effects of infection history on subsequent, unrelated immune responses against microbes.

More than a quarter of the world's population is infected with intestinal parasites.³⁶ Most helminth infections occur in the developing world where many individuals are co-infected with *M. tuberculosis*. Mycobacteria are controlled by Th1 responses with production of the cytokines IFN- γ , IL-2 and tumour-necrosis factor alpha TNF- α .³⁷ A protective response against gastrointestinal nematodes, however, is associated with production of type 2 cytokines, like IL-4, IL-5 and IL-13.³⁸ Down-regulation of Th1 responses by a Th2 type immune phenotype can occur and it has been postulated that helminth-induced Th2 bias of the immune system may lead to reactivation of latent tuberculosis.³⁹ Only a few prospective studies have, however, been published to support this hypothesis. Total serum IgE (associated with a Th2 response) and *Ascaris*-specific IgE are high in tuberculosis patients in some study communities;⁴⁰ this suggests that gastrointestinal worm infestation could be an important contributor to the TB problem. A

strong correlation between the incidence of TB and mean IgE levels is found in the Ravensmead/Uitsig community in the Western Cape⁴¹ and the most probable reason is intestinal parasite infection. It was also found that IgE levels decreased following successful treatment of TB,⁴¹ suggesting that the re-establishment of a Th1 response during successful control of TB infection might down-regulate Th2 responses. A Kenyan study reported reduced protective Th1 responses to BCG vaccination in children with prenatal sensitisation against filariae and schistosomes.⁴² Furthermore, cellular immune responses to PPD tuberculin are reduced in subjects with helminth infection but improve after deworming.⁴³ Clinical studies demonstrate altered responses to unrelated infections other than mycobacteria in helminth co-infected humans. In helminth-infected subjects peripheral blood mononuclear cells are more susceptible to HIV infection⁴⁴ and HIV/helminth co-infected patients have increased HIV disease severity (disease progression, higher plasma viral load and poor anti-retroviral response), which is reversible by helminth eradication.⁴⁵ Animal studies also show altered immune responses against unrelated antigens in helminth-infected hosts. In *Schistosoma mansoni*-infected mice, increased susceptibility to vaccinia virus with decreased Th1 cytokines and virus-specific CD8+ cytotoxic T-cell responses have been reported.⁴⁶

As discussed earlier, helminths may play a positive role in the prevention of manifestations of allergies⁴⁷ by blocking of mast cell Fc ϵ type I receptors by polyclonal IgE and resultant inhibition of degranulation. But helminth infection also leads to increased production of the immune-suppressive cytokines IL-10 and TGF- β and induction of regulatory lymphocyte subsets (Treg)³⁸ and this may be instrumental in lowering the prevalence of atopy in helminth-infected individuals. As Th1 responses are also suppressed by these cytokines, macrophages are deactivated and IFN- γ -induced nitric oxide synthetase-2 is inhibited,³⁷ helminth-induced immune suppression may theoretically play a role in altered immune responses against mycobacteria.

MECHANISMS OF INTERACTION BETWEEN INFECTIONS AND UNRELATED IMMUNE RESPONSES

Altered cytokine environment due to infections

Proliferation of T cells in a micro-environment rich in Th1 cytokines favours differentiation along the Th1 pathway and suppresses Th2 maturation. Two murine models demonstrate this phenomenon and will be discussed.

In the western world respiratory syncytial virus (RSV) infection is a common cause of infantile viral bronchiolitis and may possibly be involved in subsequent development of atopy and asthma.⁴⁸ A disastrous vaccination program took place in 1960 where infants who were vaccinated with formalin-inactivated RSV developed severe disease in later episodes of natural RSV infection, which were characterised by eosinophilic lung infiltrates and increased mortality.⁴⁹ A mouse model was developed to study this phenomenon.^{50,51} Infecting BALB/c mice with RSV leads only to mild illness and a dominant Th1 immune response, which resolves spontaneously in 7-10 days. However, sensitisation of these mice with the RSV attachment protein (G) before infection with RSV (G-primed RSV infection) leads to greatly enhanced illness. It is now believed that G protein priming took place in infants after inacti-

vated RSV vaccination. Data from the human vaccine studies show that older children were protected from the enhanced effects of post-vaccination RSV infection and this might reflect a more mature immune system, possibly due to 'immune educational' infections preceding vaccination. The effect of infection history was therefore examined with the use of a modified mouse model of G-primed RSV infection. Mice infected with influenza virus (which induces strong IFN- γ responses from CD4+ and CD8+ T cells) are protected from the Th2-driven eosinophilia and illness seen in the G-primed RSV infection.⁵² During influenza/G-RSV sequential infection, increased IFN- γ was observed by flow cytometry and, together with a reduction in IL-4 protein in lung lavage fluid, indicates a shift towards a Th1 phenotype. This suggests that an altered cytokine environment induced by prior infection can have important effects on subsequent unrelated immune responses against viruses.

The mouse model of *Cryptococcus neoformans*/BCG co-infection was used to study whether prior BCG exposure would alter subsequent Th2-mediated responses. *Cryptococcus* infection induces a strong Th2 response characterised by non-protective eosinophilic inflammation in certain mouse strains. Similarly to influenza/G-RSV infection, it was found that serum IFN- γ levels were increased during co-infection and ELISPOT assay showed a lower frequency of *C. neoformans*-specific IL-5-producing cells in BCG-infected mice. A dependence on Th1 cytokines for eosinophil reduction in the BCG/*C. neoformans* model was demonstrated in experiments with IFN- γ knockout mice. Intriguingly, live BCG was required for reduction of eosinophilia as heat-killed BCG increased eosinophil infiltration in the lung.

Conversely, T-cell proliferation in a micro-environment rich in Th2 cytokines favours differentiation along the Th2 pathway. *Schistosoma mansoni* infection decreases vaccinia virus-specific Th1 cytokine production and suppresses cytotoxic T lymphocyte (CTL) activity in mice.⁵³ Patients infected with *S. mansoni* mount a Th2-response to tetanus toxoid instead of a Th1 or Th0 response,⁵⁴ certain communities with a high prevalence of helminth infection respond to phytohaemagglutinin (PHA) stimulation with a Th2 instead of a Th1 response⁵⁵ and deworming of humans restores the ability of BCG vaccination to induce PPD-stimulated T-cell proliferation and IFN- γ production.⁴³

Regulatory T cells and infection

Immune deviation and changes in cytokine micro-environment alone cannot explain some of the epidemiological observations regarding infections and unrelated responses, as discussed earlier in the context of auto-immune disease. Recently, immune-suppressive mechanisms have generated much interest. T-cell-mediated immune regulation plays a main role in maintaining antigen-specific 'operational tolerance' *in vivo*, controlling T-cell homeostasis and regulating homeostatic expansion of T cells in immune deficient hosts.⁵⁶ Gershon and Kondo⁵⁷ first described suppression of antigen-specific immune responses in 1971 but specific molecular markers, the origin of these cells and the molecular basis of immune regulation remained uncertain. It is now clear, however, that these cells are enriched in the CD4+ populations, although CD8+, CD4-CD8- (double negative) and NK cells also possess regulatory properties.⁵⁶ Regulatory CD4+ cells express CD25, the α -subunit of the IL-2 receptor.⁵⁸ Although most of the literature concerning regulatory cells stems from allergic disease, auto-immune disease and transplantation tolerance work, some reports address the

role of these cells in infections. A yet unidentified non-B-, non-T cell suppresses virus-specific CTL in *Schistosoma*-infected mice via a soluble factor.⁵⁹ Tr1 cells are generated *in vivo* after repeated stimulation with IL-10 and IFN- α ⁶⁰ and produce IL-10, varying amounts of IFN- γ , IL-5 and TGF- β but no IL-2 or IL-4.⁶¹ They are associated with suppressed immune responsiveness during onchocerciasis infection (a filarial nematode causing visual impairment or blindness).⁶² CD25+CD4+ cells also contribute to Th2 polarisation during *S. mansoni* infection by suppressing Th1 response development.⁶³ During herpes simplex virus infection, regulatory cells decrease responses against this virus and against unrelated antigen.⁶⁴ In other animal models, CD4+CD45RB^{low} regulatory cells confer protection against inflammatory bowel disease⁶⁵ and this subset is also induced by a killed *M. vaccae* suspension and inhibits ovalbumin-induced airway eosinophilia via IL-10 and TGF- β .⁶⁶ However, the CD4+CD45RB^{low} phenotype, like the CD4+CD25+ phenotype in humans, is also a marker of activation and does not necessarily indicate regulatory activity. Functional properties like suppression of proliferation and the patterns of cytokine production are needed to categorise these cells. In summary, infection-induced regulatory cells are capable of suppressing responses against the causative organism and against unrelated pathogens. The importance of this observation has, however, not been conclusively demonstrated in humans and requires further studies.

Redirection of immune cell trafficking

Infectious foci can possibly play a role in protection from disease by sequestration or modification of inflammatory cells trafficking. Activated T cells travel to granulomas regardless of their antigenic specificity.²¹ In an auto-immune disease model, threshold levels of autoreactive T cells were not reached in the CNS because of redirection of inflammatory cells by strong chemokine gradients and/or shared addressins from distant inflammatory processes. The effect of previous infection on lung remodelling also warrants further investigation. No data exist to evaluate the effect of immune cell redirection during unrelated infections.

Cross-reactive immunity alters immune pathology

During a viral infection there is a rapid and dramatic increase in memory cells specific to unrelated pathogens.^{67,68} Selin and Walsh⁶⁹ have shown that within 3 days of an intraperitoneal vaccinia virus infection of a lymphocytic choriomeningitis virus (LCMV)-immune mouse there is a five times greater intraperitoneal CD8+ T-cell infiltrate than in corresponding LCMV-naïve animal. The proposed reasons for the enhanced recruitment of apparently unrelated memory cells include upregulation of chemokine receptors allowing non-specific recruitment to the site of an unrelated infection⁷⁰ or expansion of T cells cross-reactive between two viruses. Heterologous immunity describes the ability of memory T cells resulting from one viral infection to confer protection against a putatively unrelated heterologous virus.⁶⁷ Selin *et al.*⁶⁷ show that prior immunity to LCMV, Pichinde virus and MCMV is protective against a subsequent vaccinia virus infection. The protection depends on the sequence of viral infections and is not necessarily reciprocal. It is generally accepted that T cells are highly specific for peptide-MHC complexes but a single T-cell clone can recognise several different peptides from the same virus and even different proteins from different viruses.⁷¹⁻⁷³

Bystander activation and unrelated immune responses

Infections and their subsequent immune responses result in cytokine secretion that has the ability to stimulate non-specific cells.⁷⁴ IFN- γ , IL-2 and IL-15 in particular have been linked to memory CD8+ T-cell stimulation.^{74,75} Cytolytically active CD8+ T cells remain long after resolution of LCMV infection,⁷⁶ providing a means for rapid elimination of subsequent infection with homologous virus and this may also be true for activated CD8+ T cells specific for heterologous viruses. Limiting dilution assays for CTL precursors could only identify 10% of virus-induced CD8+ T cells as being virus-specific with other specificities included in the responses.⁷⁷ Additionally, cells were also elicited that lysed uninfected allogeneic targets.⁷⁸ The biological significance of bystander activation is, however, hard to establish. Many clones of virus-specific CTL cross-reacted with uninfected targets.⁷⁸ Zarozinski *et al.*⁷⁹ used transgenic mice with a limited, yet still diverse T-cell repertoire. T cells were capable of generating CTL responses against either H-2^d or H-2^k alloantigens but LCMV infection only induced H-2^k-reactive CTL, which argues against bystander activation. Selin⁶⁹ also found unanticipated cross-reactive CTL responses between putatively unrelated viruses. A decrease in bystander T cells, more pronounced among CD8+ T cells during unrelated responses, has been reported and these cells appear apoptotic.⁶⁹ An explanation offered for these conflicting findings regarding bystander activation is that cytokines during a second infection can stimulate memory CTL into cycle but that this leads to apoptosis in the absence of appropriate T-cell receptor (TCR) signalling.⁶⁹ IFN might also kill memory cells to free room in the lymphoid organs for later repopulation. The decrease in numbers of unrelated memory cells might therefore represent a homeostatic response to maintain the complex secondary lymphoid organ structures where stromal cells and APCs provide the interface for T-cell activation.

MHC class I tetramers are used to identify antigen-specific CD8+ T cells. These tetramers were used in an experiment where influenza-immune mice were challenged with G-primed RSV infection, as discussed ear-

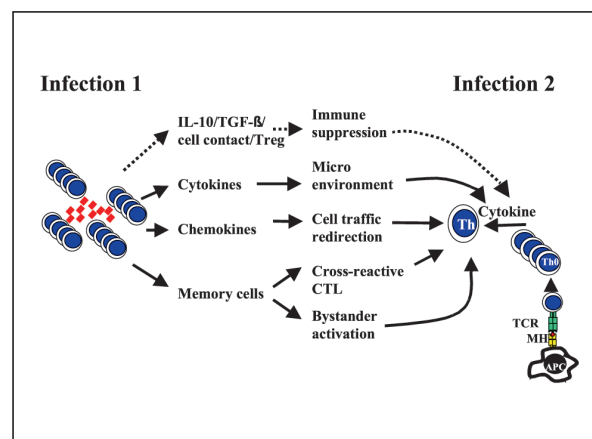


Fig. 2. Summary of the mechanisms by which infections can alter the outcome of unrelated immune responses. When Th0 cells differentiate into effector cells the stage has already been set by a prior infection and its accompanying immune response. Immune suppression by regulatory cells, alteration in cytokine micro-environment, chemokine-induced redirection of effector cell trafficking, T-cell receptor cross-reactivity and bystander activation can come into play to change the phenotype or outcome of the immune response.

lier.⁵² Influenza nucleoprotein (NP)-specific CD8+ T cells returned to the lung and expressed intracellular IFN- γ during this challenge. Memory cells are less dependent on co-stimulation compared with naive T cells,⁸¹ express higher levels of adhesion molecules and are activated by IL-2, TNF and IL-6.^{82,83} and the influenza-specific T cells may therefore represent bystander activation.⁵² G protein-induced eosinophilia is sensitive to IFN- γ ⁸⁴ and the return of influenza-specific IFN- γ -producing CD8+ T cells to the lung may therefore tip the balance away from an environment conducive to eosinophilia. This does not, however, imply expansion of influenza-specific cells during RSV infection, but could merely be the result of recruitment of these cells from other areas in the host through chemokines or cytokines during active RSV pneumonia.

The mechanisms by which infections can influence subsequent responses against other pathogens are summarised in Figure 2.

EFFECT OF INFECTION HISTORY ON SUBSEQUENT EXPOSURE TO MICRO-ORGANISMS: IMPLICATIONS FOR FUTURE RESEARCH

Accumulatively, the issues reviewed here have implications for vaccine design and vaccine testing as immunologically naïve infants will respond differently to vaccinations and subsequent exposure to pathogens than older individuals with more mature immune systems. The prevention of systemic infections by vaccines might also influence immune maturation as discussed in the context of the hygiene effect. Another aspect of interest is immune senescence, where memory to infectious agents wanes and where responses might revert back to a more naïve phenotype.

There is also an urgent need for properly designed studies to assess the influence of helminth infections on the HIV and TB epidemics. These diseases occur in similar geographical areas and socio-economic settings, and the impact of widespread worm infestation on HIV and TB disease expression has not been assessed comprehensively but could be substantial.

Another important question concerns the potential superiority of live vaccines over inactivated vaccines.^{85,86} In an era of increasing immune suppression due to the HIV pandemic (and to a much lesser extent due to a growing number of patients receiving immune suppressant therapy for auto-immune disease or organ transplantation) the use of a live organism in a vaccine poses a potential threat. Reports of systemic BCG infection have been published with a mortality exceeding 70%.⁸⁷

There has recently been a renewed interest in regulatory T-cell phenotypes⁸⁸ and as more information regarding these cells becomes available, their role in interactions between different pathogens needs to be addressed. Phenotypic and functional characteristics of these cells have only recently been defined in more detail and this knowledge has to be utilised in studies of pathogen-pathogen interaction. This is especially true for co-infection with pathogens known to induce regulatory/immunosuppressive immune responses, like gastrointestinal helminths and mycobacteria.

CONCLUSION

Infections can profoundly affect subsequent immune responses. As discussed in this review, infection history has a biologically significant impact on the immunopathology of subsequent infections and there are numerous mechanisms for these interactions that operate in concert. These interactions have to be con-

sidered when immune responses against other micro-organisms, allergens or self-antigens are investigated.

ACKNOWLEDGEMENTS

The financial support of the Centre of Excellence for Biomedical TB Research and the South African National Research Foundation is acknowledged.

REFERENCES

1. Prescott SL. Bacteria and the allergy epidemic: The culprit and the cure? *Current Allergy & Clinical Immunology* 2004; **17**(3): 108-114.
2. Strachan DP. Is allergic disease programmed in early life? *Clin Exp Allergy* 1994; **24**(7): 603-605.
3. Butland BK, Strachan DP, Lewis S, Bynner J, Butler N, Britton J. Investigation into the increase in hay fever and eczema at age 16 observed between the 1958 and 1970 British birth cohorts. *BMJ* 1997; **315**(7110): 717-721.
4. Strachan DP, Taylor EM, Carpenter RG. Family structure, neonatal infection, and hay fever in adolescence. *Arch Dis Child* 1996; **74**(5): 422-426.
5. Martinez FD, Stern DA, Wright AL, Taussig LM, Halonen M. Association of non-wheezing lower respiratory tract illnesses in early life with persistently diminished serum IgE levels. *Thorax* 1995; **50**: 1067-1072.
6. Von Mutius E, Martinez FD, Fritsch C. Prevalence of asthma and atopy in two areas of West and East Germany. *Am J Respir Crit Care Med* 1994; **149**: 358-364.
7. Shaheen SO, Aaby P, Hall AJ, et al. Measles and atopy in Guinea-Bissau. *Lancet* 1996; **347**(9018): 1792-1796.
8. Shirakawa T, Enomoto T, Shimazu S, Hopkin JM. The inverse association between tuberculin responses and atopic disorder. *Science* 1997; **275**(5296): 77-79.
9. Matricardi PM, Rosmini F, Ferrigno L, et al. Cross sectional retrospective study of prevalence of atopy among Italian military students with antibodies against hepatitis A virus. *BMJ* 1997; **314**(7086): 999-1003.
10. Farooqi IS, Hopkin JM. Early childhood infection and atopic disorder. *Thorax* 1998; **53**(11): 927-932.
11. Ng'ang'a LW, Odhiambo JA, Mungai MW, et al. Prevalence of exercise induced bronchospasm in Kenyan school children: an urban-rural comparison. *Thorax* 1998; **53**(11): 919-926.
12. Schenker MB, Gold EB, Lopez RL, Beaumont JJ. Asthma mortality in California, 1960-1989. Demographic patterns and occupational associations. *Am Rev Respir Dis* 1993; **147**(6 Pt 1): 1454-1460.
13. Romagnani S. Induction of TH1 and TH2 responses: a key role for the 'natural' immune response? *Immunol Today* 1992; **13**(10): 379-381.
14. Prescott SL, Macaubas C, Holt BJ, et al. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. *J Immunol* 1998; **160**(10): 4730-4737.
15. Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG. Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 1999; **353**(9148): 196-200.
16. Romagnani S. Regulation of the development of type 2 T-helper cells in allergy. *Curr Opin Immunol* 1994; **6**(6): 838-846.
17. Erb KJ, Holloway JW, Soback A, Moll H, Le Gros G. Infection of mice with *Mycobacterium bovis*-*Bacillus Calmette-Guerin* (BCG) suppresses allergen-induced airway eosinophilia. *J Exp Med* 1998; **187**(4): 561-569.
18. Wang CC, Rook GA. Inhibition of an established allergic response to ovalbumin in BALB/c mice by killed *Mycobacterium vaccae*. *Immunology* 1998; **93**(3): 307-313.
19. Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. *J Immunol* 1997; **159**(4): 1739-1745.
20. Black P. Why is the prevalence of allergy and autoimmunity increasing? *Trends Immunol* 2001; **22**(7): 354-355.
21. Sewell DL, Reinke EK, Hogan LH, Sandor M, Fabry Z. Immunoregulation of CNS autoimmunity by helminth and mycobacterial infections. *Immunol Lett* 2002; **82**(1-2): 101-110.
22. Wucherpfennig KW. Mechanisms for the induction of autoimmunity by infectious agents. *J Clin Invest* 2001; **108**(8): 1097-1104.
23. McKinney PA, Okasha M, Parslow RC, et al. Early social mixing and childhood Type 1 diabetes mellitus: a case-control study in Yorkshire, UK. *Diabet Med* 2000; **17**(3): 236-242.
24. Pundziute-Lycka A, Dahlquist G, Nystrom L, et al. The incidence of Type I diabetes has not increased but shifted to a younger age at diagnosis in the 0-34 years group in Sweden 1983-1998. *Diabetologia* 2002; **45**(6): 783-791.

25. Andersen E, Isager H, Hyllested K. Risk factors in multiple sclerosis: tuberculin reactivity, age at measles infection, tonsillectomy and appendectomy. *Acta Neurol Scand* 1981; **63**(2): 131-135.
26. Ben Nun A, Yossefi S, Lehmann D. Protection against autoimmune disease by bacterial agents. II. PPD and pertussis toxin as proteins active in protecting mice against experimental autoimmune encephalomyelitis. *Eur J Immunol* 1993; **23**(3): 689-696.
27. Ben Nun A, Mendel I, Sappier G, Kerlero dR. A 12 kDa protein of *Mycobacterium tuberculosis* protects mice against experimental autoimmune encephalomyelitis. Protection in the absence of shared T cell epitopes with encephalitogenic proteins. *J Immunol* 1995; **154**(6):2939-2948.
28. Martins TC, Aguas AP. Mechanisms of *Mycobacterium avium*-induced resistance against insulin-dependent diabetes mellitus (IDDM) in non-obese diabetic (NOD) mice: role of Fas and Th1 cells. *Clin Exp Immunol* 1999; **115**(2): 248-254.
29. Martins TC, Aguas AP. A role for CD45RB^{low} CD38+ T cells and costimulatory pathways of T-cell activation in protection of non-obese diabetic (NOD) mice from diabetes. *Immunology* 1999; **96**(4): 600-605.
30. Mattsson L, Larsson P, Erlandsson-Harris H, Klareskog L, Harris RA. Parasite-mediated down-regulation of collagen-induced arthritis (CIA) in DA rats. *Clin Exp Immunol* 2000; **122**(3): 477-483.
31. Hentati B, Sato MN, Payelle-Brogard B, Avrameas S, Ternynck T. Beneficial effect of polyclonal immunoglobulins from malaria-infected BALB/c mice on the lupus-like syndrome of (NZB x NZW)F1 mice. *Eur J Immunol* 1994; **24**(1): 8-15.
32. Elliott DE, Urban JF jun, Argo CK, Weinstock JV. Does the failure to acquire helminthic parasites predispose to Crohn's disease? *FASEB J* 2000; **14**(12): 1848-1855.
33. Romagnani S, Parronchi P, D'Elios MM, et al. An update on human Th1 and Th2 cells. *Int Arch Allergy Immunol* 1997; **113**(1-3): 153-156.
34. Athanassakis I, Vassiliadis S. T-regulatory cells: are we re-discovering T suppressors? *Immunol Lett* 2002; **84**(3): 179-183.
35. De Lafaille MAC, Lafaille JJ. CD4(+) regulatory T cells in autoimmunity and allergy. *Curr Opin Immunol* 2002; **14**(6): 771-778.
36. Chan L, Bundy DA, Kan SP. Genetic relatedness as a determinant of predisposition to *Ascaris lumbricoides* and *Trichuris trichiura* infection. *Parasitology* 1994; **108**(Pt 1): 77-80.
37. Flynn JL, Chan J. Immunology of tuberculosis. *Annu Rev Immunol* 2001; **19**: 93-129.
38. Yazdanbakhsh M, van den Biggelaar A, Maizels RM. Th2 responses without atopy: immunoregulation in chronic helminth infections and reduced allergic disease. *Trends Immunol* 2001; **22**(7): 372-377.
39. Bentwich Z, Kalinkovich A, Weisman Z, Borkow G, Beyers N, Beyers AD. Can eradication of helminthic infections change the face of AIDS and tuberculosis? *Immunology Today* 1999; **20**: 485-487.
40. Adams JF, Scholvinck EH, Gie RP, Potter PC, Beyers N, Beyers AD. Decline in total serum IgE after treatment for tuberculosis. *Lancet* 1999; **353**(9169): 2030-2033.
41. Beyers AD, van Rie A, Adams J, Fenhalls G, Gie R, Beyers N. Signals that regulate the host response to *Mycobacterium tuberculosis*. *Novartis Found Symp* 1998; **217**: 145-157.
42. Malhotra I, Mungai P, Wamachi A, et al. Helminth- and Bacillus Calmette-Guerin-induced immunity in children sensitized *in utero* to filariasis and schistosomiasis. *J Immunol* 1999; **162**(11): 6843-6848.
43. Elias D, Wolday D, Akuffo H, Petros B, Bronner U, Britton S. Effect of deworming on human T cell responses to mycobacterial antigens in helminth-exposed individuals before and after bacille Calmette-Guerin (BCG) vaccination. *Clin Exp Immunol* 2001; **123**(2): 219-225.
44. Shapira-Nahor O, Kalinkovich A, Weisman Z, et al. Increased susceptibility to HIV-1 infection of peripheral blood mononuclear cells from chronically immune-activated individuals. *AIDS* 1998; **12**(13): 1731-1733.
45. Galai N, Kalinkovich A, Burstein R, Vlahov D, Bentwich Z. African HIV-1 subtype C and rate of progression among Ethiopian immigrants in Israel. *Lancet* 1997; **349**(9046): 180-181.
46. Actor JK, Marshall MA, Eltoun IA, Buller RM, Berzofsky JA, Sher A. Increased susceptibility of mice infected with *Schistosoma mansoni* to recombinant vaccinia virus: association of viral persistence with egg granuloma formation. *Eur J Immunol* 1994; **24**: 3050-3056.
47. Lynch NR, Hagel I, Perez M, Di Prisco MC, Lopez R, Alvarez N. Effect of anthelmintic treatment on the allergic reactivity of children in a tropical slum. *J Allergy Clin Immunol* 1993; **92**(3): 404-411.
48. Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B, Bjorksten B. Asthma and immunoglobulin E antibodies after respiratory syncytial virus bronchiolitis: a prospective cohort study with matched controls. *Pediatrics* 1995; **95**(4): 500-505.
49. McIntosh K, Fishaut JM. Immunopathologic mechanisms in lower respiratory tract disease of infants due to respiratory syncytial virus. *Prog Med Virol* 1980; **26**: 94-118.
50. Alwan WH, Kozłowska WJ, Openshaw PJM. Distinct types of lung disease caused by functional subsets of antiviral T cells. *J Exp Med* 1994; **179**: 81-89.
51. Alwan, WH, Record FM, Openshaw PJM. Phenotypic and functional characterization of T cell lines specific for individual respiratory syncytial virus proteins. *J Immunol* 1993; **150**: 5211-5218.
52. Walz G, Tafuro S, Moss P, Openshaw PJ, Hussell T. Influenza virus lung infection protects from respiratory syncytial virus-induced immunopathology. *J Exp Med* 2000; **192**(9): 1317-1326.
53. Actor JK, Shirai M, Kullberg MC, Buller RM, Sher A, Berzofsky JA. Helminth infection results in decreased virus-specific CD8+ cytotoxic T-cell and Th1 cytokine responses as well as delayed virus clearance. *Proc Natl Acad Sci USA* 1993; **90**(3): 948-952.
54. Sabin EA, Araujo MI, Carvalho EM, Pearce EJ. Impairment of tetanus toxoid-specific Th1-like immune responses in humans infected with *Schistosoma mansoni*. *J Infect Dis* 1996; **173**(1): 269-272.
55. Bentwich Z, Weisman Z, Moroz C, Bar-Yehuda S, Kalinkovich A. Immune dysregulation in Ethiopian immigrants in Israel: relevance to helminth infections? *Clin Exp Immunol* 1996; **103**(2): 239-243.
56. Wood KJ, Sakaguchi S. Regulatory T cells in transplantation tolerance. *Nat Rev Immunol* 2003; **3**(3): 199-210.
57. Gershon RK, Kondo K. Cell interactions in the induction of tolerance: the role of thymic lymphocytes. *Immunology* 1970; **18**(5): 723-737.
58. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 1995; **155**(3): 1151-1164.
59. Marshall MA, Jankovic D, Maher VE, Sher A, Berzofsky JA. Mice infected with *Schistosoma mansoni* develop a novel non-T-lymphocyte suppressor population which inhibits virus-specific CTL induction via a soluble factor. *Microbes Infect* 2001; **3**(13): 1051-1061.
60. Levings MK, Sangregorio R, Galbiati F, Squadrone S, de Waal MR, Roncarolo MG. IFN-alpha and IL-10 induce the differentiation of human type 1 T regulatory cells. *J Immunol* 2001; **166**(9): 5530-5539.
61. Levings MK, Roncarolo MG. T-regulatory 1 cells: a novel subset of CD4 T cells with immunoregulatory properties. *J Allergy Clin Immunol* 2000; **106**(1 Pt 2): S109-S112.
62. Satoguina J, Mempel M, Larbi J, et al. Antigen-specific T regulatory-1 cells are associated with immunosuppression in a chronic helminth infection (onchocerciasis). *Microbes Infect* 2002; **4**(13): 1291-1300.
63. McKee AS, Pearce EJ. CD25+CD4+ cells contribute to Th2 polarization during helminth infection by suppressing Th1 response development. *J Immunol* 2004; **173**(20): 1224-1231.
64. Suvas S, Kumaraguru U, Pack CD, Lee S, Rouse BT. CD4+CD25+ T cells regulate virus-specific primary and memory CD8+ T cell responses. *J Exp Med* 2003; **198**(6): 889-901.
65. Powrie F, Leach MW, Mauze S, Menon S, Caddle LB, Coffman RL. Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi CD4+ T cells. *Immunity* 1994; **1**(7): 553-562.
66. Zuany-Amorim C, Sawicka E, Manlius C, et al. Suppression of airway eosinophilia by killed *Mycobacterium vaccae*-induced allergen-specific regulatory T-cells. *Nat Med* 2002; **8**(6): 625-629.
67. Selin LK, Varga SM, Wong IC, Welsh RM. Protective heterologous antiviral immunity and enhanced immunopathogenesis mediated by memory T cell populations. *J Exp Med* 1998; **188**(9): 1705-1715.
68. Flynn KJ, Riberdy JM, Christensen JP, Altman JD, Doherty PC. *In vivo* proliferation of naive and memory influenza-specific CD8(+) T cells. *Proc Natl Acad Sci USA* 1999; **96**(15): 8597-8602.
69. Welsh RM, McNally JM, Brehm MA, Selin LK. Consequences of cross-reactive and bystander CTL responses during viral infections. *Virology* 2000; **270**(1): 4-8.
70. Jung S, Littman DR. Chemokine receptors in lymphoid organ homeostasis. *Curr Opin Immunol* 1999; **11**(3): 319-325.
71. Anderson RW, Bennink JR, Yewdell JW, Maloy WL, Coligan JE. Influenza basic polymerase 2 peptides are recognized by influenza nucleoprotein-specific cytotoxic T lymphocytes. *Mol Immunol* 1992; **29**(9): 1089-1096.
72. Shimojo N, Maloy WL, Anderson RW, Biddison WE, Coligan JE. Specificity of peptide binding by the HLA-A2.1 molecule. *J Immunol* 1989; **143**(9): 2939-2947.
73. Kuwano K, Reyes VE, Humphreys RE, Ennis FA. Recognition of disparate HA and NS1 peptides by an H-2Kd-restricted, influenza specific CTL clone. *Mol Immunol* 1991; **28**(1-2): 1-7.

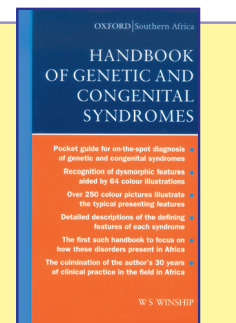
74. Biron CA. Cytokines in the generation of immune responses to, and resolution of, virus infection. *Curr Opin Immunol* 1994; **6**(4): 530-538.
75. Tough DF, Borrow P, Sprent J. Induction of bystander T cell proliferation by viruses and type I interferon *in vivo*. *Science* 1996; **272**: 1947-1950.
76. Selin LK, Welsh RM. Cytolytically active memory CTL present in lymphocytic choriomeningitis virus-immune mice after clearance of virus infection. *J Immunol* 1997; **158**(11): 5366-5373.
77. Moskophidis D, Assmann-Wischer U, Simon MM, Lehmann-Grube F. The immune response of the mouse to lymphocytic choriomeningitis virus. V. High numbers of cytolytic T lymphocytes are generated in the spleen during acute infection. *Eur J Immunol* 1987; **17**(7): 937-942.
78. Nahill SR, Welsh RM. High frequency of cross-reactive cytotoxic T lymphocytes elicited during the virus-induced polyclonal cytotoxic T lymphocyte response. *J Exp Med* 1993; **177**(2): 317-327.
79. Zarozinski CC, Welsh RM. Minimal bystander activation of CD8 T cells during the virus-induced polyclonal T cell response. *J Exp Med* 1997; **185**(9): 1629-1639.
80. Selin LK, Nahill SR, Welsh RM. Cross-reactivities in memory cytotoxic T lymphocyte recognition of heterologous viruses. *J Exp Med* 1994; **179**: 1933-1943.
81. Croft M, Bradley LM, Swain SL. Naive versus memory CD4 T cell response to antigen. Memory cells are less dependent on accessory cell costimulation and can respond to many antigen-presenting cell types including resting B cells. *J Immunol* 1994; **152**(6): 2675-2685.
82. Unutmaz D, Pileri P, Abrignani S. Antigen-independent activation of naive and memory resting T cells by a cytokine combination. *J Exp Med* 1994; **180**(3): 1159-1164.
83. Ehl S, Hombach J, Aichele P, Hengartner H, Zinkernagel RM. Bystander activation of cytotoxic T cells: studies on the mechanism and evaluation of *in vivo* significance in a transgenic mouse model. *J Exp Med* 1997; **185**(7): 1241-1251.
84. Hussell T, Baldwin CJ, O'Garra A, Openshaw PJM. CD8+ T-cells control Th2-driven pathology during pulmonary respiratory syncytial virus infection. *Eur J Immunol* 1997; **27**(12): 3341-3349.
85. Waris ME, Tsou C, Erdman DD, Day DB, Anderson LJ. Priming with live respiratory syncytial virus (RSV) prevents the enhanced pulmonary inflammatory response seen after RSV challenge in BALB/c mice immunized with formalin-inactivated RSV. *J Virol* 1997; **71**(9): 6935-6939.
86. Walz G, Humphreys IR, Marshall BG, Edwards L, Openshaw PJM, Shaw RJ, Hussell T. Prior exposure to live *Mycobacterium bovis* BCG decreases *Cryptococcus neoformans*-induced lung eosinophilia in a gamma interferon-dependent manner. *Infect Immun* 2003; **71**(6): 3384-3391.
87. Talbot EA, Perkins MD, Silva SF, Frothingham R. Disseminated bacille Calmette-Guerin disease after vaccination: case report and review. *Clin Infect Dis* 1997; **24**(6): 1139-1146.
88. Leving M, Sangregorio R, Sartirana C, et al. Human CD25+CD4+ T suppressor cell clones produce transforming growth factor beta, but not interleukin 10, and are distinct from type 1 T regulatory cells. *J Exp Med* 2002; **196**(10): 1335-1346.

Handbook of Genetic and Congenital Syndromes

Dr Bill Winship

May 2003, 264 pp, flexicover, 240 colour photo's, R310

The Handbook of Genetic and Congenital Syndromes provides an easily accessible reference to the more common genetic and congenital conditions in African patients. It is the first handbook to include colour illustrations and descriptions of genetic and congenital disorders in African children specifically, and is therefore an invaluable contribution to the effective diagnosis and health care of children in Africa. Includes an overview of the causes of genetic and congenital disorders and basic genetic principles. Fully illustrated chapters on dysmorphic features, selected chromosome disorders, single gene disorders, multifactorial disorders, trisomy disorders, and syndromes of unknown origin



Oxford Handbook of Clinical Medicine 6/e

Dr Murray Longmore, Dr Ian Wilkinson, and Dr Supraj Rajagopalan

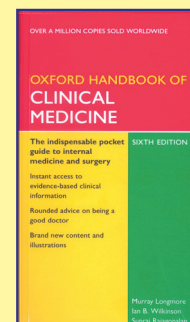
July 2004, 830 pp, flexicover, R210

The *Oxford Handbook of Clinical Medicine* covers all areas of internal medicine and provides rapid, on-the-spot access to evidence-based information on clinical management. Its characteristic style — combining incisive guidance with wit, a lucid style, and memorable epigrams — has been popular with generations the world over. There are a host of new features in this edition:

- new sections on common acute medical symptoms, and clinical skills
- a new 'cheat sheet' on commonly used drugs
- more colour plates
- more ECGs.

There are numerous other updates and improvements throughout, many suggested by extensive market research. The pages have been redesigned in full colour. A new junior co-author has ensured that the text is just right for its market and tells medicine 'as it is', and a panel of senior readers has approved every section. The OHCM is an utterly reliable 'friend in the pocket', any time of the day or night!

New edition



Forthcoming December 2004...

Handbook of Paediatrics 6/e

Dec 2004, flexicover, provisionally priced at R250

Orders: Contact Jackie at Medical Book Seller,
PO Box 3784, Tygervalley 7536.

Tel 083 303 8500. Fax (021) 975-1970.

E-mail: jackie@medbookseller.co.za

