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JOURNAL OF THE ALLERGY SOCIETY
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Respiratory infections and their effect on the paediatric lung microbiome

The influence of placental microbiomes on immunology and allergy

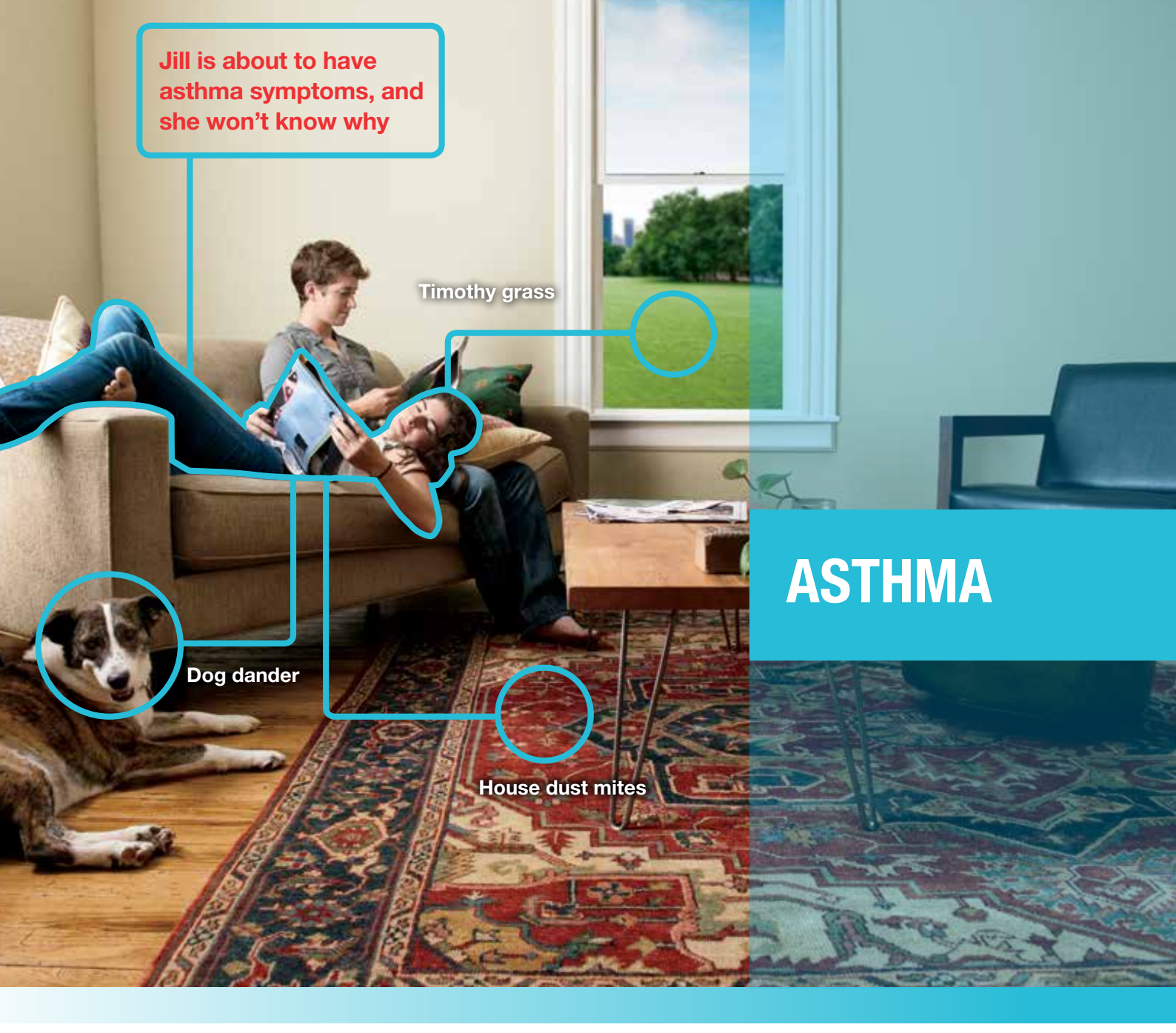
Running short of breath: a case study of hard-metal lung disease

The tangled web of the immune system

Allergy immunotherapy update

Allergy and the microbiome

Ethics and the microbiome



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GUEST EDITORIAL

THE FINE BALANCE OF THE MICROBIOME, IMMUNOLOGY AND ALLERGY – MAY THE MICROBIOME ALWAYS BE WITH YOU

The human microbiome – the collection of microorganisms (viral, bacterial, fungal, parasitic) living in symbiosis on and in human beings, and disturbances of which may result in an imbalance of immunological forces, leading to a shift in the disease–health balance – has recently become the focus of much scientific endeavour.

Nowadays, almost every meeting or conference about allergy and autoimmunity is likely have a session on the influence of the microbiome on immunology and the development of allergy or autoimmunity. A large body of data on the subject is being generated: some based on experimental evidence in animal models, some using complex molecular techniques such as 16s RNA, and some epidemiological and clinical data that have been formulated into what seems like sometimes competing and sometimes synergistic forces that dictate the propensity of the immune system to tolerance or not. Teasing away the sensationalism and understanding the shifts in the immune response and the mechanisms that the human microbiome has on allergy are critical to proper disease classification and management.

This edition of the journal brings together a collection of excellent articles from otolaryngologists, allergists and pathologists (chemical pathologists, histopathologists, microbiologists and virologists) who are practising at the interface of the microbiological environment. Each article has attempted to examine and review the influences of the human microbiome on immunology and allergy. Drs Justyna Wojno (microbiologist), Fierdoz Omar (chemical pathologist) and Elloise du Toit (medical microbiologist, University of Cape Town) introduce the topic with an excellent review of allergy and the microbiome. Their article has a special emphasis on the gut microbiome and allergic disease and reviews some of the literature highlighting the microbiome in the African context.

Following this is a short review that I have written on the influence of the microbiome on immunology. It is intended to remind us of the fine balance that the human immune response treads between health and disease and what the bottom line actually is for the influence of microbes and external allergens on the development of a robust tolerance. Drs Allison Glass (virologist) and Sharona Seetharam (microbiologist) focus on the respiratory tract; they present data on respiratory infections in paediatric patients referred from private practice. They reflect on the epidemiology of these respiratory infections and how these influence the microbiome and ultimately affect paediatric health.

A growing area of interest is given an airing in the review article penned by Saloshini Ramsamy together with Dr Vanessa Shahibdeen and Professor Colleen Wright (histopathologist in the Department of Anatomical Pathology, University of the Witwatersrand). They review how inflammation and infection during pregnancy may affect immunity in new-born infants.

Finally, Dr Tamara Jaye, an allergist in private practice, presents insights into inducing tolerance with her up-to-date and insightful review of recent changes in allergy immunotherapy. There are many remaining unknowns with the induction of tolerance to aeroallergens, in particular the impact of antigen-specific tolerance on how well unrelated allergens are tolerated. Does treating house-dust mite allergy result in the control of grass-pollen allergy? And, if so, what is the final pathway that controls the induction and maintenance of tolerance? This is a major insight waiting in the wings, much like the recent revolution in the therapeutic strategy to control food allergy: switching the paradigm from avoidance to understanding and inducing oral tolerance in food allergies. In the words of Yoda: 'Always with you to induce tolerance may the microbiome be.'

Prof Eftyhia Vardas

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Review Article

ALLERGY AND THE MICROBIOME

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ABSTRACT
Over the past few decades, the rise in the prevalence of allergic conditions has been notable. The literature suggests an association between the human microbiota and the development of allergic conditions such as asthma and atopic dermatitis. Patients with atopy exhibit alterations in the relative levels of 'beneficial' and potentially harmful bacteria compared to healthy controls. One proposed mechanism is that changes in gut microbiome composition and function, which are altered by various environmental exposures, influence the healthy development of the immune system and thereby affect immune tolerance. Specific species of commensal bacteria and their functions as well as biologically active metabolites may play either a protective or a pathogenic role in the development of allergy or asthma. This article aims to summarise briefly the human gut, skin and respiratory microbiome and the link to allergic conditions as well as to consider the African data.

INTRODUCTION: THE HISTORY OF MICRIOBIOTA AND THE HUMAN HOST

Trillions of commensal microbes reside on the human skin, mucous membranes and the gastrointestinal and respiratory tracts. Although human beings have co-evolved with these microbes, this complex relationship is not yet fully understood. Human beings and their resident commensal microbes, also known as the 'normal flora', have a symbiotic relationship. Microbes obtain nutrients and residence from the host while human beings also benefit significantly. For instance, the normal gastrointestinal flora assist the host with the digestion of food, the production of vitamins, the training and development of the immune system and with colonisation resistance.

Many environmental factors can influence the composition and function of human microbiota. Improved technology such as high-throughput DNA sequencing and bioinformatics analysis tools has allowed researchers to gain greater insight into the composition and function of previously non-culturable microorganisms.[1] This technology has made it possible to assess the effect that environmental factors can have on the microbiota as well as the ability to assess the changes in the human microbiota in certain disease states.

Most microbiome research has been carried out on the gut microbiota. The gut microbiota can be affected by many internal and external factors, including diet, genetics, birth mode, hormones, medication, pollutants and age.[2] These factors can cause a functional and taxonomic imbalance of gut flora, known as 'dysbiosis'. There is increasing evidence that the human microbiota in the human gastrointestinal tract as well as in the skin and the respiratory tracts, for example, contribute to health and disease. Many disease states, including atopy/asthma and allergy, have been associated with dysbiosis. The potential contribution of the microbiota to the increase in the rates of asthma, AD and food allergy has come under the spotlight. The human gut microbiome plays an important role in the development of the immune system and immune tolerance. Immune tolerance is a healthy response to an allergen whereas an allergic state is an excessive response. Asthma and other atopic conditions are classically associated with the hyper-activation of the T helper 2 (Th2) arm of adaptive immunity.

The role played by early-life exposure to microorganisms in the susceptibility to allergic conditions has been known and documented for years. Strachan observed an inverse relationship between hay fever and family size due to unhygienic contact with older siblings and proposed that the frequency of exposure to beneficial microbes may be protective.[3] The 'hygiene hypothesis' that was proposed soon after states that the rise in allergic conditions may be a consequence of the reduction in microbial exposure in early life. Other exposures that are associated with enhanced microbial exposure in early life, such as living on a farm,[4] a lack of early antibiotic exposure, vaginal delivery, and exposure to pets during pregnancy seem to be associated with lower rates of allergy.[5,6,7] There may be a critical period in early life when disruptions in microbial colonisation may affect immunological development and maturation and therefore affect allergic sensitisation, with

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TABLE I: DEFINITIONS
Atopy: A predisposition to developing certain allergic reactions such as allergic rhinitis (AR), asthma and atopic dermatitis (AD).
Coloniser/commensal: A microorganism that forms part of the normal microbial flora of its host and exists in a relationship in which one organism derives benefits from another organism without necessarily either hurting or helping it.
Colonisation resistance: When commensal organisms prevent the host or microbial community from being colonised by exogenous and potentially pathogenic microorganisms.
Dysbiosis: An imbalance of microbial composition and function that may be associated with disease.
Diversity: Microbial diversity: the number and abundance distribution of distinct microbial types. The two main components include richness (the number of microbial species) and evenness (the abundance of microbes relative to others). A healthy microbiome is generally associated with greater diversity. *Alpha diversity:* a measure of the diversity in a sample. *Beta-diversity:* a term for the comparison of the diversity of two samples to each other (dissimilarity between samples).
Microbiome: Collective genetic material of the microbiota in a certain community. This term is often used interchangeably with microbiota.
Microbiota: The human microbiota refers to the collection of microorganisms (bacterial, archaeal, fungal and parasitic) and viruses that inhabit the human body.
Micro-organism: A microscopic organism not visible to the naked eye.
Pathogen: A microorganism that causes disease.
Relative abundance: The number of organisms of a particular kind relative to the total number of organisms in a given community.
Symbiosis: The interaction between two different organisms living in close association, usually to the advantage of both organisms.
Taxonomy (taxa/taxon): The science of classification where organisms are grouped and systematically categorised. Taxa are a set of groups into which organisms can be classified. Taxa can be ranked, forming a hierarchy. Example: *Proteobacteria* is a major *phylum* of gram-negative bacteria. They include a wide variety of pathogens, such as *Escherichia, Salmonella*, and many other notable *genera*. Within each Genus there may be various species associated with illness i.e., *Escherichia coli*.

subsequent development of atopy in later life. The mechanisms involved in this process may be related to immune training and/or excessive histamine production by the resident populations of organisms in these patients. The resulting allergic effects may be local in the gut or shape patterns of immunological responses to allergen exposure at distant anatomical sites such as the mucous membranes.

GASTROINTESTINAL MICROBIOME

Recent advances in the field of allergology/immunology and the human microbiome have improved our understanding of the importance of the gut microbiota in the development of a healthy response to allergens (immune tolerance).

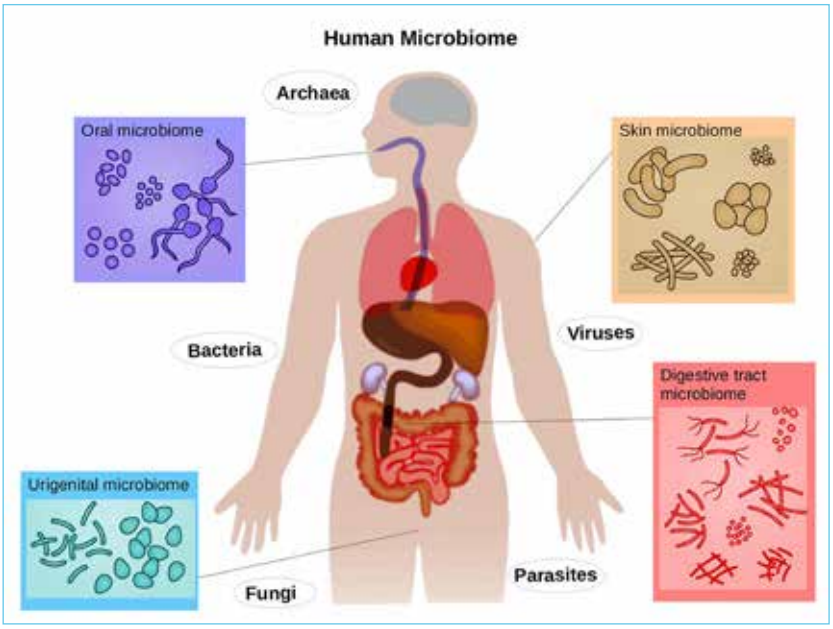

Figure 1: The human microbiome differs at different anatomical sites (© Lara Marks, WhatIsBiotechnology.org)

Childhood allergy has been linked to changes in the relative abundance of specific gut microbial species in early life. The specific species of intestinal commensal bacteria and their relative abundances may play a protective or pathogenic role in the development of allergy/asthma. As an example, children who developed allergic conditions were significantly less often colonised by organisms such as *Lactobacillus rhamnosus, Lactobacillus casei* and *Bifidobacterium adolescentis* during their first two months of life. These organisms, together with other well-known probiotic strains such as *Bifidobacterium longum*, appear to be protective.[8,9] Other bacteria in the human gut, such as *Escherichia coli, Morganella morganii* and *Lactobacillus vaginalis*, can produce histamine in large quantities. Histamine-producing bacteria appear to be more abundant in patients with asthma and severe asthma, compared to controls, and this may influence host immunological processes.[10]

THE RESPIRATORY AND SKIN MICROBIOME

While it was long believed that the lungs are sterile, the presence of large numbers of bacterial genomes have been described in recent literature.[11] Evidence suggests that the microbiome in the lungs of healthy subjects (mostly *Bacteroidetes*) differs from that in patients with obstructive lung disease (mostly *Proteobacteria* – a major phylum containing many species associated with respiratory illnesses)

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in their lower respiratory tracts (including *Haemophilus spp, Moraxella, Neisseria* and *Streptococcus*).[12,13,14,15] The bacterial load is also higher in these patients when compared with normal subjects.[16] A direct effect of microbiota on the development of allergic responses and allergic-airway inflammation in asthma has been shown in mice exposed to farm dust or specific bacteria associated with farm dust (*Acinetobacter lwoffii* F78 and *Lactococcus lactis* G121). Allergic-airway responses are reduced in these mice.[17,18] Toll-like receptor 4 signalling and A20 activation in airway epithelial cells are thought to be the mechanism by which this occurs.[17,19]

Next-generation sequencing has demonstrated remarkable microbiome diversity in healthy skin at the genus level, with *Staphylococcus spp*, and the *Corynebacterium* and *Propionibacterium* genera representing 60 per cent of the bacterial load.[15,20] Host factors such as age, body region and skin pigmentation affect this bacterial composition.[15]

In patients with atopic dermatitis (AD), the diversity appears to be lost, with an increased *S. aureus* abundance. However, the microbiome diversity improves with emollient and inflammatory therapy, suggesting that skin epithelial function maintains the normal microbiome.[21,22]

Importantly, commensal skin microbes (such as *Staphylococcus epidermidis* and *Staphylococcus hominis*) are associated with protection against skin pathogens such as *S. aureus*. In fact, the replacement of the dysbiotic microbiome with commensals from normal skin has been shown to correct the skin barrier and host defence against skin pathogens such as *S. aureus* (by inhibiting growth and biofilm formation).[23,24] The mechanism by which the skin barrier is enhanced appears to be via Toll-like receptor 2, inducing keratinocyte-derived antimicrobial peptides and increased tight junctions.[25,26] This suggests that microbiome transplants similar to faecal transplants performed in patients with recurrent *Clostridium difficile* infections may benefit patients with AD.

Similarly, the application of appropriate probiotics and prebiotics, topically, may be a future consideration in skin and lung conditions involving microbiome dysbioses, as has been the case in the GIT.

MICROBIOME AND ALLERGY IN THE AFRICAN CONTEXT

Very little literature is available regarding allergy and the microbiome in Africa. Given the increasing rate of caesarean sections, the changing childhood diet (westernisation) and the more frequent use of antibiotics in Africa (greater accessibility) as well as genetics – all known to affect the gut microbiome – further exploration in the African setting is needed.[27]

Microbiome and allergy literature in Africa has focused mainly on AD. Globally, the incidence of AD is on the rise, but the prevalence of such atopic conditions is still lower in Africa.[28] This could be explained by the fact that may populations are still rural and therefore people are living more closely to their microbe-enriched environment, with daily and significant contact with animals and plants. In contrast, people of African descent living in Western countries show a high risk for severe AD compared to African people remaining in Africa.[28] These differences can be reflected in and possibly explained by the differences in the microbiota of rural African versus westernised populations.

Grzeskowiak et al (2012) compared the gut microbiota of six-month-old infants from rural Malawi and urban Finland. They found that the Malawian infants' gut microbiome differed significantly from that of the Finnish infants. The study showed that the Malawian infants had a significantly greater proportion of total *Bifidobacterium spp*, higher *Bacteroides-Prevotella* group and C*lostridium histolyticum* group than the Finnish infants, but no *Bifidobacterium adolescentis*, *Clostridium perfringens*, *Staphylococcus aureus*, or Akkermanisa-like bacteria, which were detected in the Finnish infants.[29] More specifically, *Bifidobacterium longum* and *Bifidobacterium bifidum*, which possess immunomodulatory properties, were found to be significantly higher in the gut of Malawian infants than in those of Finnish infants. The preponderance of these bacterial species could also reflect risk of disease, because allergic infants are more often colonised by *Clostridium difficil*e and *Staphylococcus aureus* and less by *Bifidobacteria.*

In 2017, Mahdavinia et al compared the stool microbiome of 38 South African children between the ages of 12 and 36 months, 29 of whom had AD (17 of these were sensitised to at least one food). No difference in alpha diversity or in the relative abundance of any taxa was found between the two groups. However, the Phylum Actinobacteria (and genera *Blautia* and *Bifidobacterium*) was found to be higher (not significantly so) in cases with food sensitisation than cases without food sensitisation, and in the controls.[30] A later study compared the gut microbiome of 83 rural South African children, 36 with AD and 47 controls.[31] In this case, the authors showed that the beta diversity differed significantly between the two groups.

A study of children from Burkino Faso showed that their gut microbiome contains a significantly higher microbial richness and biodiversity compared to European children.[32] The genera *Xylanibacter*, *Prevotella*, *Butyrivibrio* and *Treponema* were found exclusively in children from Burkino Faso. These organisms are able to produce high levels of short-chain fatty acids (SCFAs), which are thought to be beneficial to the host. Indeed, SCFAs have been shown to play a protective role against allergic diseases,[33] and the Burkino Faso cohort showed high faecal SCFA levels.

Zimmerman et al (2010) invesigated the effect of iron fortification on the gut microbiome of anaemic African children. These children have an unfavourable ratio of certain *Enterobacteriaceae* to *Bifidobacteria* and *Lactobacilli*, which may be enhanced by iron fortification. This dysbiotic microbiota has been associated with increased gut inflammation and the initiation or perpetuation of allergic inflammation.[34]

CONCLUSION

It is clear that a complex interaction between the host and the mucosal *microbiome* at various anatomical sites is associated

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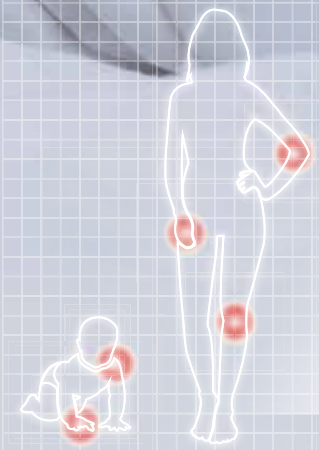

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with various disease entities, including allergy. The role of microbiota manipulation through the use of probiotic/pre-biotic preparations to prevent disease or enhance health needs to be explored further. Manipulation by means of targeted environmental or behavioural modifications is another strategy by which the microbiota can be manipulated. Examples include the avoidance of elective caesarean sections, unnecessary antibiotic use and increased exposure to pets at an early age as well as dietary interventions.

A patient-targeted approach in which microbial populations are assessed and then corrected by either re-population or manipulation may be the future of personalised preventive or therapeutic medicine. This re-population would need to be specific for the anatomical site in question – that is the skin, the respiratory tract or the gut – and occur at the crucial time points to ensure the greatest benefits. This exact personalised protocol has yet to be elucidated.

DECLARATION OF CONFLICT OF INTEREST
The authors have no potential conflict of interest related to this article.

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Review Article

THE TANGLED WEB OF THE IMMUNE SYSTEM: ALLERGY AND THE MICROBIOME

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INTRODUCTION

Allergic diseases are a major health problem especially in rapidly developing and high-income societies. They require chronic treatment and have a significant impact on quality of life (QoL) of the individuals, families and communities affected by them; in addition, they are a burden on limited health resources. What really causes allergic disease and why do some human beings develop them, whereas others do not? There are multiple layers to this immunological enigma that have been partially unravelled over the past few years. The development of atopy – or a genetic predisposition to allergic diseases and asthma – has been examined and, undoubtedly, allergic inflammation in an individual is a delicate interplay between their genetic milieu (which includes conventional chromosomal DNA and epigenetic DNA) and the environment.[1]

EPIDEMIOLOGY

Robust epidemiological evidence supports the increasing incidence of allergy[2] and asthma worldwide[3] over the past few decades. The hypothesis for these changes in global allergy epidemiology is simple; in its most basic form, it is postulated that a decrease in the frequency of infections leads to an increase in allergic conditions.[4] This idea developed further and became formalised as the 'hygiene hypothesis' in 2000. The hypothesis stated that the decrease in microbial exposure – specifically the loss of the symbiotic microbiological environment that evolves with the individual from early foetal life, birth and the crucial neonatal/early infancy period – resulted in the loss of protection from the development of allergic diseases.[5]

Animal models and some human-cohort data have demonstrated clear associations between decreased exposure to infectious organisms and 'clean' environments, on the one hand, and increased antibiotic use and caesarean section rates, on the other. Decreased exposure to environmental allergens and viral, bacterial, helminth loads can lead to significant differences in the rate of allergic-disease development.[6] But there is some confusion because this relationship is not clear cut, and certainly not linear, for not all infections are good. Indeed, some infections are detrimental and lead to disease. These include viral respiratory-tract infections in infants with respiratory syncytial virus or rhinovirus type C, both of which affect bronchial smooth musculature and result adversely in persistent wheeze and asthma in later life.[7]

ENVIRONMENT

The biome does not work in isolation: lifestyle changes and modern living conditions, with their associated reduction in allergen exposure, are fundamental to the loss of normal immunological tolerance to non-threatening environmental allergens.[9] Epidemiological evidence accumulated from many countries has shown that the rate and pattern of this increase in allergic diseases is skewed, with significantly higher prevalence and more severe disease occurring in higher-income countries.[3] The 'hygiene hypothesis' and the 'biome depletion' theories[5] are two sides of the same coin. Both refer to the impact that modern lifestyles have had on the normal microbiological flora (the human biome); indeed, many of the risk factors for allergic disease affect the microbiome of the placenta, skin, nasopharynx, lung and gut in the immunologically important early postnatal period (see Figure 1).

Recently, a well-designed study clearly demonstrated this concept in human beings and it has shown the protective effect of a traditional farming environment on the development of allergic sensitisation and asthma in two communities living in the United States: the Amish and the Hutterites. For religious reasons, these two communities have chosen to remain isolated from modern lifestyles and from marrying outside of their specific groups.[9] Because of their isolation, these communities afforded the opportunity for a natural human experiment which allowed the comparison of the effects of one critical factor – different farming practices – on children of similar genetic background and lifestyles and their development of allergy and asthma.

Both the Amish and the Hutterites are farming communities; however, the Amish use traditional methods whereas the Hutterites use industrialised methods. On the one hand, the Amish traditional farming environment was found to offer protection against the development of allergies; on the other hand, Hutterite children showed almost six times higher allergic sensitisation and four times higher asthma rates.[9] This study also analysed the microbial composition and endotoxin levels in household dust from these two communities. This showed that dust from Amish households had higher median endotoxin levels and significant differences in microbial composition when compared with the Hutterite examples.[9] Crucially, analysis indicated that the numbers and gene-expressing profiles of innate cells of periphery involved in the innate immune response

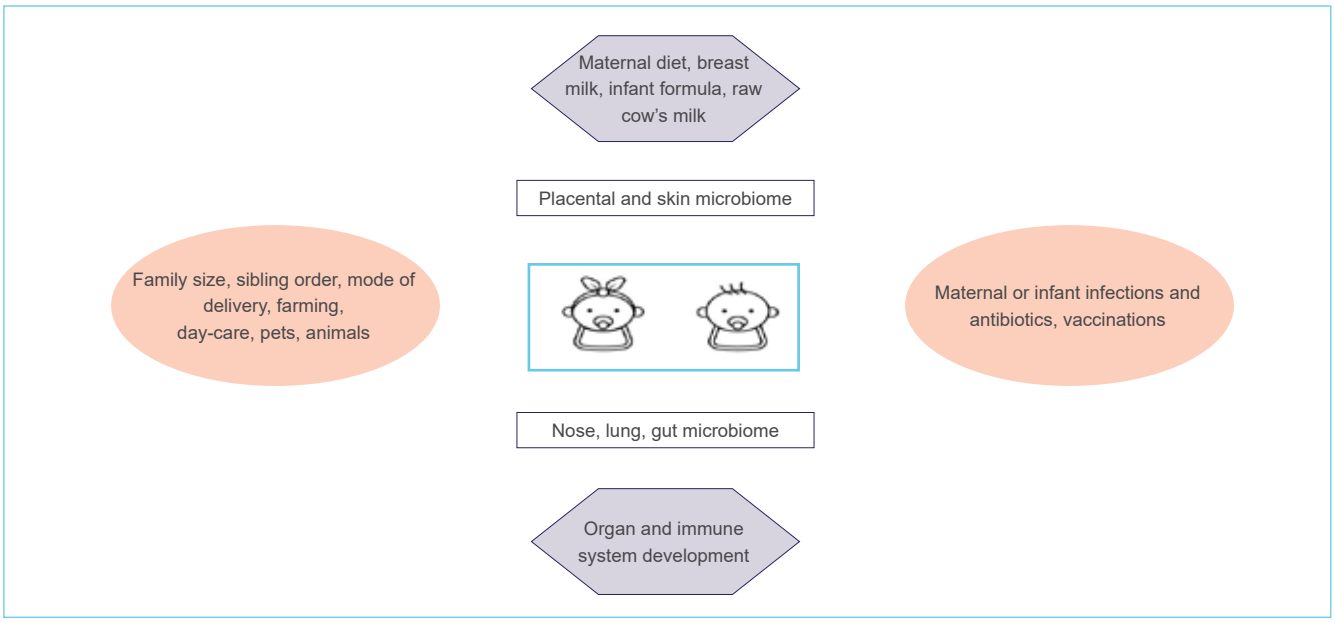

Figure 1: Factors that have an impact on the microbiome and increase the risk for the development of allergy

(monocytes, neutrophils and eosinophils) were different in the two groups of children.

The Amish children also displayed higher numbers of functional genes that limit the multiple inflammatory pathways essential in the innate immune responses against viruses. Sustained microbiological exposure leads to reduced expression of HLA-DR on monocytes and drives immature neutrophils from the bone marrow, both of which were confirmed in the Amish children.[9] These children also showed normal levels of T*regs* (regulatory T cells) and IL-10 that normally function to balance immune effects.

A study from South Africa of Xhosa adolescents has similarly demonstrated significant associations between polymorphisms in IL-10 and IL-4 genes, on the one hand, and susceptibility to allergy and asthma, on the other.[10] This study is a major step forward in describing allergy phenotypes in people of black ethnicity, although the definitions cannot be definitive because social and economic environments may confound ethnicity.

Stein et al,[9] in their study, then elegantly confirmed the findings observed in human beings by providing an immunological mechanism for the discrepancy in allergic disease in the children from these two communities. This was done by using a mouse model lacking *MyD88* and *Trif* adaptor proteins, which disables multiple pathways of the innate immune response and, more specifically, disables those pathways that mediate signalling by microbial products through toll-like receptors (TLRs). Dust samples from the Amish households suppressed the induction of airway inflammation in the mouse model of allergic asthma compared with the dust samples from Hutterite households.[9]

IMMUNOLOGY

The immunology of allergic disease is complex and the mechanistic details of the processes that lead to allergic disease are still being elucidated;[11] but the loss of innate, cellular and humoral immune-regulatory pathways is involved. The immune system functions by being able to receive and interpret signals internally from cells that make up the body, from environmental agents and from microorganisms, either pathogenic or commensal. Allergic reactions to usually innocuous environmental agents (aeroallergens and foods) interact with parts of the innate immune system that plays a pivotal role in shaping immune responses.

The structure of the allergenic substance is the key initiating factor, with many allergenic substances being lipid-binding proteins (e.g. lipocalins of pets and lipid-transfer proteins of plants) and some being glycoproteins (e.g. peanut Ara h2). These proteins interact with pathogen-recognition receptors such as the TLRs on antigen-presenting cells, which push the immune system towards Th2 inflammation tissue injury, remodelling and chronicity.[12]

The first step in the complex interplay which leads to allergy is *sensitisation*. During sensitisation, allergen-specific IgE antibodies are produced and bind to the high-affinity FcεRI receptors on the surface of mast cells and basophils. During this phase, effector Th2 cells produce IL-4, IL-5 and IL-13. IL-4 and IL-13 induce class-switching to ε immunoglobulin heavy chains in B cells, IgE memory B-cell expansion and the production of allergen-specific IgE antibodies.[13] On re-encountering the allergen at a later time during the *effector phase* the stage has been set, the allergen causes cross-linking of the IgE-FcεRI receptor complexes on basophils and mast cells which release mediators (e.g. histamine, prostaglandins, IL-3, IL-4, IL-5, IL-13) that are responsible for the immediate hypersensitivity reactions. Inflammation in allergy is controlled by adaptive allergen-specific CD4+ helper T cells (T_H2 cells) and by group 2 innate lymphoid cells (ILCss) that drive IgE synthesis, eosinophilis, mucous

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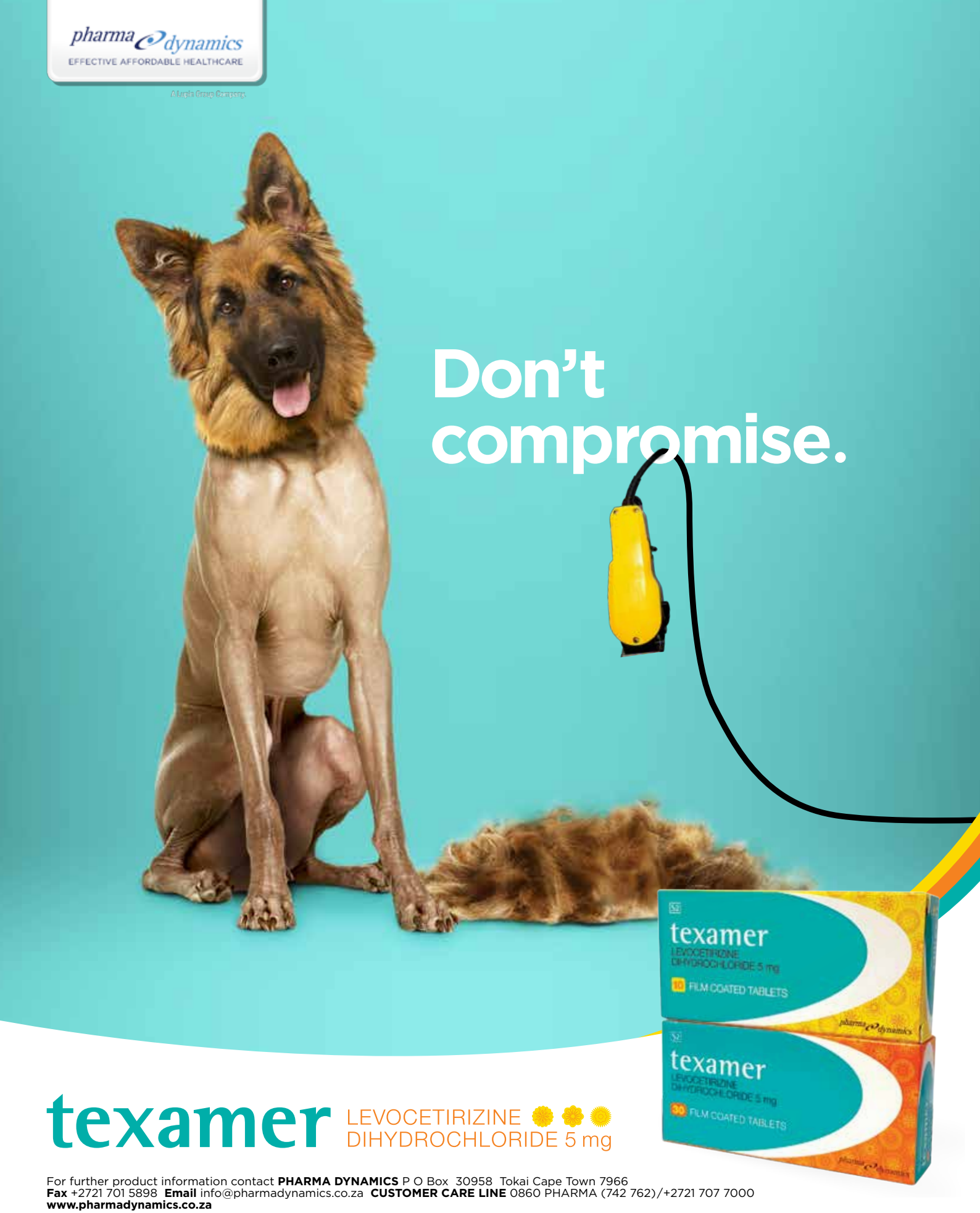

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production and smooth-muscle contraction via interleukins (IL-4, IL-5, IL-13, IL25, IL33).[13]

The leading concept in trying to understand the immunological mechanisms of allergy has always depended on the concept of a T_H1–T_H2 balance. T_H1 cells are involved in infections and autoimmunity; T_H2 cells are involved in allergic disease; each set has reciprocal roles in regulating one another. It has been suggested that the loss of infectious pressure resulted in the loss of T_H1 cells and therefore in a rebound increase in T_H2 cells.[11] Unfortunately, this was way too simplistic and, what is more, the discovery of ILCs changed the perception of T cells as the effectors of immunity; accordingly, ILC2 cells took over the limelight and were shown in animal models to contribute significantly to TH2 inflammation.[13]

TOLERANCE AND REGULATORY T CELLS (T*regs*)

Allergic diseases occur because of a failure to develop tolerance towards a specific allergen, which results in allergen-specific T_H2 cells, IgE and cytokine production. T*reg* cells are the enforcers and regulators of tolerance to both self-antigens and innocuous environmental allergens. They are a distinct CD4+ T-cell subset identified by a marker required for their differentiation known as Forkhead Box 3 or FOXP3.[14] Some T*reg* cells are induced in the thymus and are involved chiefly in autoimmune diseases; a second subset, induced peripherally, are directed against microbial antigens or environmental allergens. These T*reg* subsets are characterised by non-overlapping T-cell receptors so there is a clear division of labour between cells that regulate responses to self-antigens and non-self-antigens.

T*regs* produce immune-regulatory cytokines IL-10 and TGF-β (transforming growth factor β). IL-10 is an anti-inflammatory cytokine that inhibits effector T-cell activation by suppressing stimulatory pathways; it also does so by suppressing the antigen-presenting capacity of dendritic cells (DCs) and eosinophil activation, by so doing interrupting both early and late allergic responses. TGF-β has a wide range of functions that include suppressing B- and T-cell proliferation and differentiation, control of airway inflammation and remodelling.[14]

In mice, alternations in the microbiome diversity with decreased fibre intake led to defective T*reg* cells.[11] In human beings, deep sequencing techniques show that allergen-specific FOXP3 T*reg* cells dominate in heathy individuals; however, in allergic individuals, T*reg* cells and memory/effector T_H2 cells exist together. The influence of the microbiome is demonstrated by the induction of T*regs* butyrate, a short-chain fatty acid produced by commensal bacteria (*Bacteriodes, Bifidobacterium, Fecalibacterium* and *Enterobacteria*). Further research is needed to determine how cleaner environments and modern lifestyles promote the loss of T*regs* and how microbes can promote allergen-specific T*regs* and dampen TH2 inflammation.

The importance of tolerance and these T*reg* mechanisms are illustrated further in establishing tolerance in allergy immunotherapy. This therapy requires, as a first step, basophil and mast-cell desensitisation to degranulation, the formation of allergen-specific T*reg* cells expressing multiple suppressor factors, and decreased eosinophil, mast-cell and basophil migration in the affected tissues.[8]

MECHANISMS OF INFLUENCE OF THE MICROBIOME

Multiple studies in both animal models and human beings have shown that disruption of the normal complex microbiological commensals on the skin, oral/nasal mucosa, gastrointestinal tract and lung leads to disease susceptibility. Associations with disease have been postulated as a function of the lack of or the change in diversity of the microbiome.[15]

Acquiring a functional microbiome during delivery, infancy and childhood is essential in maintaining homeostasis and preventing susceptibility to allergic disease. Experiments in mouse models of atopy show that dysbiosis, especially at the time of weaning, may have detrimental effects, leading to the development of atopy.[16] Increasing evidence also suggests that any effects on the microbiome may occur even earlier during gestation. Here, the use of maternal antibiotics and the significant impact of the mode of delivery show the significant differences in microbiological flora for vaginally delivered infants compared to those delivered by caesarian section.[7] During the postnatal period, a Th2-predominant immunological environment exists as a consequence of maternal–foetal tolerance. This is normal and assists in organ development; however, it allows easy sensitisation of the infant, given the immunological environment.

Commensal microbiological flora in the gut work through different immune cells of either the innate or the adaptive immune systems to influence allergic responses. Microbiological flora promote IgA production by T*reg*-cell-dependent MyD88-dependent mechanisms that allows T*regs* in the gut to differentiate into follicular T-helper cells (T_{FH}). Th2 cells predominate in situations where antibiotics deplete commensal bacteria. These, however, are usually held in check by the MyD88 microbial-dependent suppression of IgE.[16]

Bacterial metabolites produced by the bacterial fermentation of dietary fibre such as acetate propionate and butyrate (short-chain fatty acid) increase the production of T*reg* cells via a specific receptor FFAR2 (G protein-coupled receptor, GPR43) in the case of acetate proprionate. In the case of butyrate, which is a histone deactylase (HDAC) inhibitor, this increases FOXp3 protein acetylation, which in turn increases the stability and suppressive function of intestinal T*regs*.[5] IL-22, which decreases gut permeability and therefore oral allergen uptake, is promoted by the presence of gut *Clostridia* species that allow the development of oral tolerance by the production of IL-10 and control systemic IgE production by decreasing IL-4 production from CD4+ T cells.[7] Evidence suggests that microbiota promote the expansion of the ROR-γt T*reg* cell and the regulation of DC activations; a deficiency of these cells therefore leads to a Th2 environment.

CONCLUSION

More than sufficient and convincing evidence confirms the significant impact on human health and disease of the

environment and, in particular, the commensal microbiological environment that constitutes the microbiome. The challenge now is that the pathways and complex interactive immunological mechanisms that have been teased out need to be distilled into a common understanding of the fine balance that exists between them. This information can then be used with confidence in providing directed and novel interventions for the primary prevention of allergic diseases through the use of probiotics and microbiome supplementation. It could also be used to direct new therapies towards these diseases of immune disruption.

DECLARATION OF CONFLICT OF INTEREST
The author declares no conflict of interest

This article has been peer reviewed.

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Review Article

RESPIRATORY INFECTIONS AND THEIR EFFECT ON THE PAEDIATRIC LUNG MICROBIOME

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ABSTRACT
Respiratory tract infections, especially viral infections in children are a major cause of morbidity and mortality in many developing countries. The effect of infection and its potential sequelae are a consequence of the interaction of the lung microbiome, the pathogen and the immune system. The epidemiology of viral infections and *Bordetella pertussis* observed over a two-year period in a private-sector laboratory is presented. We then describe the paediatric respiratory microbiome on a basic level and briefly describe the effect of some of these pathogens on the developing paediatric microbiota.

INTRODUCTION
The human microbiome refers to the microbiota (all microorganisms including viruses, bacteria and fungi) existing in the human body and the habitat they colonise.[1] Microbiomes, be they in the lung or gut, are very complex with respect to the types and quantities of microbe present. Sequencing techniques have revealed that the lung microbiome is a diverse system that varies from the anterior nares to the distal airways with different combinations of diverse species. These differences may be due to genetics, environmental factors, anatomical factors, mucosal characteristics, immunity and microbe–microbe interaction. Emerging evidence suggests that the microbiome shapes the immune response therefore influencing the balance between health and disease.[2] Acute infections, especially viral, that disrupt the established microbiome may contribute to the development of bacterial pneumonia or asthma.[2, 3]

Viral infections account for 45–75% of childhood community-acquired pneumonias. These infections are responsible for significant morbidity and mortality in children under the age of five years and are a leading cause of paediatric hospital admissions, particularly in developing countries.[4] The number of recognised viruses causing clinically significant respiratory tract infections has increased due to improvements in molecular testing. Viruses associated with respiratory tract infections include influenza viruses A and B, parainfluenza viruses (PIV) 1 to 4, respiratory syncytial virus (RSV), human metapneumovirus (hMPV), adenovirus, human coronaviruses (hCoV), rhinovirus, enteroviruses and human bocavirus (hBoV).[5] Testing a respiratory specimen with a multiplex **polymerase chain-reaction** (**PCR**) panel improves the likelihood of identifying the cause of a lower respiratory tract infection. Such testing:

- guides treatment decisions;
- prevents unnecessary investigations or procedures;
- minimises the unnecessary use of antibiotics;
- facilitates infection control, and
- provides knowledge of seasonality that influences healthcare planning.[6]

However, molecular testing has also brought about various dilemmas in the interpretation of results. These include ascertaining the dominant pathogen in mixed viral or viral–bacterial infections, the effect on the lung microbiome for future health and interactions with the host response.[3]

In the South African private healthcare sector, molecular diagnostics have been increasingly used for the investigation of community-acquired pneumonia. Respiratory specimens, usually nasopharyngeal swabs/aspirates, are submitted for testing on various panels that consist of common viruses and bacteria such as *Bordetella pertussis.*

STATISTICS
Data from 54 834 respiratory samples sent to a South African laboratory operating in the private healthcare sector were collated over a two-year period to ascertain the epidemiology of respiratory viruses and *Bordetella pertussis*. Specimen numbers indicate an increase in testing during March which correlated with the peak RSV season. Further peaks in specimen numbers occurred during the winter months correlating with the influenza season. Fifty-five per cent of specimens were from children under the age of five years. Multiple viruses were detected in 17% of specimens.

RESPIRATORY SYNCYTIAL VIRUS
RSV is associated with bronchiolitis, pneumonia and an increased risk of developing asthma.[7] RSV was detected

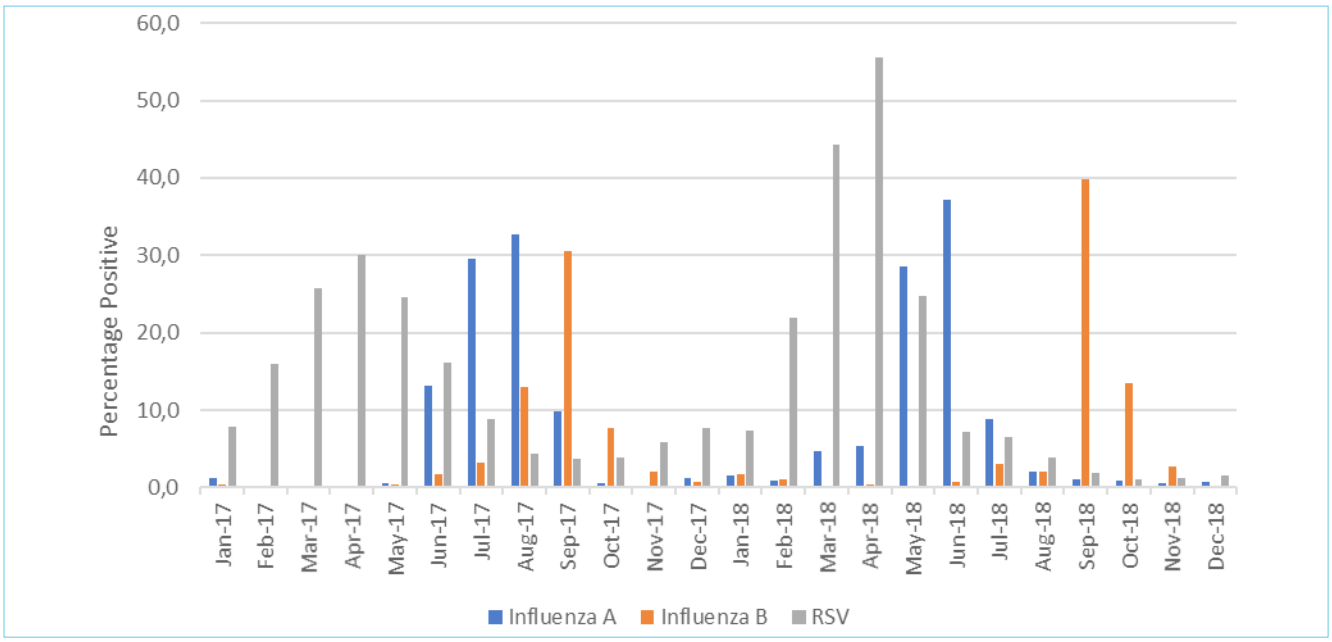

Figure 1: Influenza A, influenza B and RSV cases for 2017–2018

throughout the reported period, but the peak season was from February to May, preceding the influenza season (see Figure 1). During these peak months, RSV was detected in 16–55% of samples submitted for testing.

INFLUENZA A AND B

Influenza A and B cause seasonal epidemics that occur during the winter months (see Figure 1). Influenza A typically precedes influenza B. The influenza B season often extends into springtime. During the influenza season, influenza was detected in 14–46% of samples. Sporadic cases occur throughout the rest of the year and may be associated with travel to the northern hemisphere.

RHINOVIRUS

Rhinovirus causes upper respiratory tract infections, otitis media, asthma exacerbations and pneumonia.[5,8] Rhinovirus was the most prevalent virus, detected throughout the year in 10–33% of specimens.

ADENOVIRUS

Adenovirus infections are associated with pharyngitis, otitis media, bronchiolitis and pneumonia.[8,9] Adenovirus was detected throughout the year in 10–16% of specimens, making it the third most commonly detected virus after rhinovirus and RSV.

ENTEROVIRUS

Enterovirus infections may result in upper respiratory tract infections, bronchiolitis and pneumonia.[10] Enterovirus infections were detected throughout the year in 2–14% of specimens. Detection was above 10% in February–March 2017, September 2017 and November–December 2018.

PARAINFLUENZA VIRUSES

PIV types 1–4 were detected throughout the year with intermittent peaks (see Figure 2). PIV-3 was responsible for 54.8% of PIV infections over the two-year period. PIV-3 is more commonly associated with pneumonia than the other types.[5] A majority of patients represented in the described data were hospitalised and more likely to have lower respiratory tract infections, which may account for the predominance of PIV-3.

HUMAN METAPNEUMOVIRUS

hMPV is responsible for upper respiratory tract infections, bronchiolitis, pneumonia and exacerbation of asthma.[5] hMPV was detected in 1–7% of specimens on a monthly basis. There was a marked peak in April 2018 when it was detected in 18% of specimens.

HUMAN CORONAVIRUSES

HCoV-229E, hCoV-HKU1, hCoV-NL63 and hCoV-OC43 are associated with upper respiratory tract infections, although they may cause pneumonia in children.[5] HCoV were detected in 1–13% of specimens monthly. Seasonal peaks occur during the winter months.

BORDETELLA PERTUSSIS

Pertussis (whooping cough) is a respiratory infection caused by the bacterium *Bordetella pertussis*. It is an endemic infection that occurs in periodic cycles of 3–5 years. Immunity follows either vaccination or infection, but is not lifelong. Patients at risk include the unimmunised or partially immunised comprising mostly infants, those with impaired immune systems and those with chronic lung diseases. The most severe infections occur in infants, which lead to multiple pathogenic changes in the lower respiratory tract.[11,12,13]

In 2017, 62% of positive results were from children <5 years old with 53% <1 year old. Adults accounted for 9% of cases. In 2018, 67% of positive results were from children <5 years old with 69% <1 year old. Adults accounted for 23% of cases.

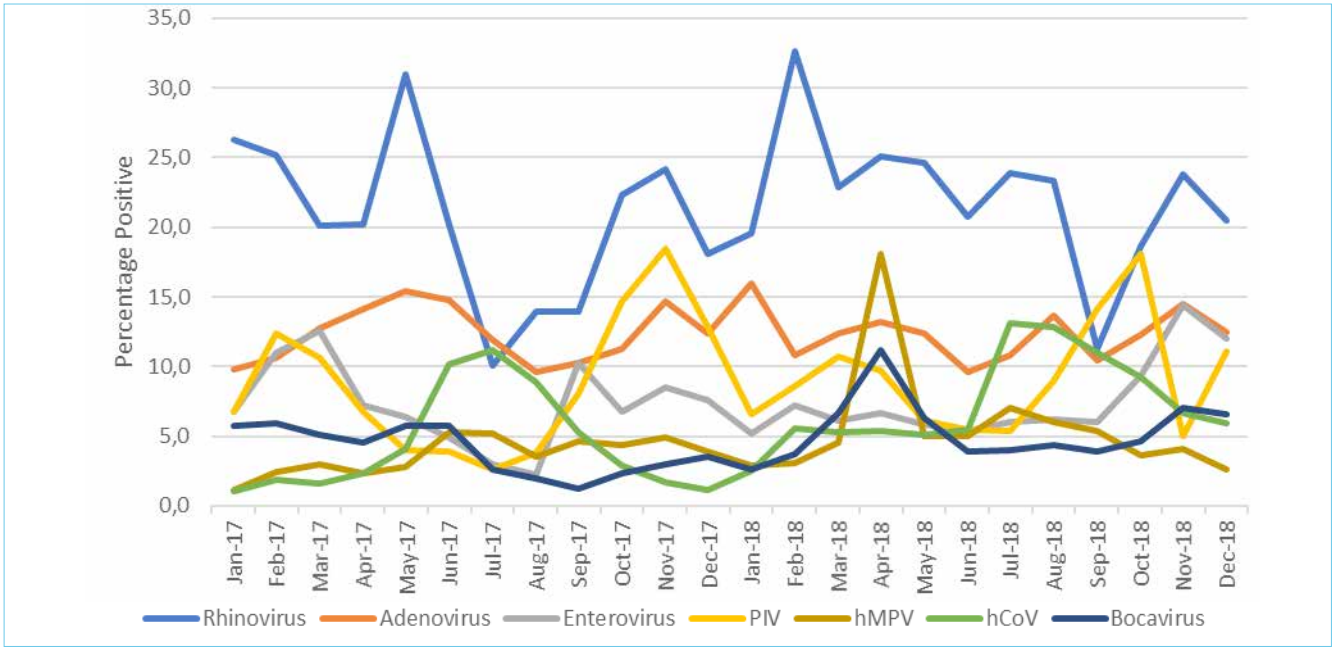

Figure 2: hCoV, PIV, rhinovirus, adenovirus, enterovirus, bocavirus and hMPV cases for 2017–2018

The number of requests for *Bordetella pertussis* testing between 2017 to 2018 increased markedly (1 631 specimens submitted in 2017 vs 8 853 specimens submitted in 2018). The percentage of specimens testing positive in 2017 and 2018 was 2.8% and 2.4%, respectively. This suggests that the increase in reported cases is due to an increase in testing rather than an increase in the number of infections as the detection rates were similar. However, in August of 2018, the National Institute of Communicable Diseases (NICD) reported a generalised increase in the number of diagnosed cases in South Africa from January 2018.[14] Our data suggest that the increase in diagnosed cases is due to an increased awareness with a subsequent increase in testing.

INFLUENCE OF INFECTIONS ON THE PAEDIATRIC LUNG MICROBIOME

The symbiotic relationship between the immune system and microbiome allows for appropriate and timely responses to infection. For an infection to occur in a site with an established microbiome, either displacement and/or elimination of the healthy state and changes in immunity are needed. Infection can occur either with a commensal (member of the microbiota) or an external pathogen. Any disturbance or stress on the system can cause an imbalance (dysbiosis) that impacts on overall health. Acute mucosal infections are characterised by dysbiosis associated with significant shifts in the microbiota leading to enhanced invasive properties and increased inflammation with tissue damage.

The microbiome seen later in life is shaped primarily by its make-up and dynamics during infancy. Newborns usually acquire a microbiota resembling their mother's and this is dependent on the mode of delivery. Infants delivered by caesarean section are usually colonised with skin flora such as *Staphylococcus*, *Streptococcus* and *Corynebacterium* species, whereas those delivered vaginally acquire *Lactobacillus* or *Prevotella* species. Over the first six months of life this changes with a gradual decline of *Streptococcus, Corynebacterium* and *Staphylococcus* to the dominance of *Pneumococcus, Haemophilus* and *Moraxella* species.[15,16,17,20] The stages of development, for example, birth to infancy and factors such as choice of feeds, that is, breastmilk or formula, all influence the spectrum of microbiota that develop to maintain respiratory health. A longitudinal study of more than 200 infants showed that breastfed babies had an abundance of gram-positive bacteria such as *Corynebacterium* and lower numbers of *Staphylococcus* species in comparison to those who were formula-fed at six weeks. These changes in the microbial patterns were associated with lowered susceptibility to infections and development of wheeze in breastfed infants.[20]

Nasopharyngeal colonisation and its subsequent microbiome has been touted as one of the determinants of infection severity, spread to lower airways and chronic sequelae. Multiple studies have shown that microbial profiles in early airway colonisation with high abundance of species such as *Pneumococcus* and *Haemophilus* species predispose infants to acute severe respiratory tract infections such as bronchiolitis and pneumonia compared to those with more 'stable' profiles or other species.[16,17] The microbial dynamics associated with stable profiles have been described as the early presence in large numbers of *Corynebacterium*/*Dolosigranulum* followed by *Moraxella* species.

Viral infections may impair innate immune responses and mechanical mechanisms that clear bacteria in the lung through dysregulation of both alveolar macrophages and neutrophils. The degree of mucous production, reduction in mucociliary clearance and impairment of cellular repair impacts on the subsequent dysbiosis in the various microbial loads. This is a possible explanation as to why infection severity, co-infection with viruses and bacteria and development of chronic respiratory sequelae differ from individual to individual.[2,11,15]

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PATHOGENS AND THEIR EFFECT ON THE LUNG MICROBIOME

In the respiratory tract, viruses can either cause primary infections, predispose to superinfection by bacteria or co-infect with bacteria.

RSV

Certain viruses such as RSV have been studied quite extensively to determine the factors determining acute infection, disease progression and long-term sequelae such as wheeze and asthma. Changes in the microbiome composition were studied in a cohort with no, mild, moderate and severe RSV infection, respectively. Hospitalisation and an exaggerated immune response were noted in the presence of higher predominance of *H influenzae* and *Streptococcus* species at the time of infection. The severity of infection is increased with bacterial co-infections such as *Moraxella* species. In addition, numerous studies have described the association between *Moraxella* respiratory tract colonisation and an increased severity of RSV infection, with RSV being reciprocally capable of altering the respiratory microbiome of the host.[7,15,16,18,20] Consequences include changes in the abundance of various species increasing predisposition to future infection and co-infections with other viruses such as hMPV and rhinovirus.[21]

BORDETELLA PERTUSSIS

B pertussis is a human-specific pathogen that utilises multiple virulence factors, for example, pertussis toxin, fimbriae and secretion systems, working in synergy to produce infection after adherence to the mucosal epithelium.

In vitro mouse studies have shown that nasal-cavity colonisation by this organism can be inhibited by certain murine microbiota, for example, *B bronchiseptica, Klebsiella* and *Staphylococcus* species. Conversely, the descent of *Bordetella pertussis* from the upper respiratory tract, where it colonises the nasopharynx, to the lower respiratory tract is potentially facilitated by microbiota such as *S mitis, S oralis, N subflava* or *Corynebacterium* species. The underlying mechanisms of these organism interactions, which include various virulence factors such as secretion systems, have not yet been well established. This study suggests that the organism has mechanisms to outcompete other species within its ecological niche, but not at the point of colonisation. Given that human upper respiratory tract microbiota is similar to that found in mice, manipulation of the nasal flora might prevent successful *B pertussis* colonisation and establishment of lower respiratory tract infection.[11,17,19]

Future research directed at interventions aimed at either mechanisms of maintenance or improvements to the 'healthy' respiratory microbiome are being studied. The ecological diversity of the microbiome based on age, nutrition and geographic location makes it difficult to establish a 'one size fits all' concept to prevent or treat infection. A necessary first step is the identification of the core processes necessary for host–microbe symbiosis. Delineating what the 'healthy' microbiota looks like and the impact of modifiable factors such as antibiotic use could provide a baseline. Current sequencing and non-culture-based microbiological techniques could further define which interactions or microbial relationships to target. These could possibly lead to new strategies, adjuvant therapies and vaccines to decrease morbidity and mortality with early intervention.

GLOSSARY

Microbiota: microorganisms inhabiting a specific niche or site
Microbiome: symbiosis between the human host, its resident microbes and its interactions

DECLARATION OF CONFLICT OF INTEREST

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Review Article

THE INFLUENCE OF PLACENTAL MICROBIOMES ON IMMUNOLOGY AND ALLERGY AND THE EFFECT OF PLACENTAL INFLAMMATION ON IMMUNITY IN NEWBORNS

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ABSTRACT

Stillbirth remains a global adverse pregnancy outcome affecting approximately three million pregnancies each year. Most stillbirths that occur in low- and middle-income countries are reported to be as a result of infections, which account for approximately 50% of deaths.[1] In South Africa, despite a substantial decrease in infant mortality rates over the past decade, in 2018 the infant mortality rate was still 36.4 per 1 000 live births.[2] The protection of the newborn against infection is dependent upon the innate immune system as their capacity for adaptive immune response is limited. Infants are susceptible to infection as a result of insufficient memory-effector B-cells and effector-memory T-cells. During pregnancy, placental infection can lead to the transfer of an antigen to the foetus, which results either in infection of the foetus (sepsis) or in a foetal immune response,[3] with increased risk of preterm birth, neurological sequelae or stillbirth.[1] During pregnancy there is cross-over of the maternal gut microbiome to the placenta and into the amniotic fluid. This exposure to bacteria plays a role in the development of the immune system and, in turn, the subsequent quality of life. It has been postulated that this initial bacterial exposure has a direct impact on the immune tolerance and the response of the child to allergies and autoimmune diseases.[4] The placental microbiome may certainly play a role in the aetiology of diseases experienced by children and adults.

Key words: placental microbiome, immunity, stillbirth, chorioamnionitis, villitis

ANATOMY AND FUNCTION OF THE PLACENTA

The placenta is a temporary organ of both maternal and foetal origin formed during pregnancy. It is an oval-shaped organ consisting of the foetal membranes: chorion and amnion (forming the amniotic sac enclosing the foetus), the parenchyma (contains connective tissue, chorionic villi and trophoblast) and decidua capsularis (close to the maternal side).[5]

The trophoblast comprises placental cells that permit the transfer of oxygen and carbon dioxide and the removal of waste between the foetus and the maternal blood. The placenta is responsible for metabolising many products which are exchanged between the maternal and the foetal interface. The sole purpose of the placenta is to support the growth and developmental requirements of the foetus during gestation.[6,7]

PLACENTAL INFLAMMATION

The consequences of placental inflammation are severe and cause significant foetal and neonatal morbidity and mortality.[8] Preterm birth is a major complication of inflammation associated with the foetal inflammatory response syndrome (FIRS). FIRS can lead to cardiorespiratory, neurological and renal complications that adversely influence the quality of life (QoL), should the neonate survive.[9]

Inflammation may reach the placenta via several routes. The commonest of these routes ascends from the lower female

genital/urinary tract, but is contiguous with the uterus or fallopian tubes; here, haematogenous and iatrogenic diagnostic/therapeutic procedures may occur. Inflammation of the chorioamnion and decidua basalis is referred to as chorioamnionitis and deciduitis. Inflammation of the villous tree is known as villitis.[10]

The origin of placental inflammation lies in either an organism (viral, bacterial or fungal), a host immune response or an unknown agent.[10] During a chronic inflammatory response, infiltration of various cells into the different placental compartments is observed. An increased presence of lymphocytes and plasma cells is seen in the decidua basalis. This is accompanied by a mixture of lymphocytes and eosinophils in the chorioamnion together with lymphocytes and histocytes in the villous tree and occasionally in the intervillous space (intervillositis).[11]

CHORIOAMNIONITIS

Chorioamnionitis (CA) is usually an ascending infection caused most commonly by bacteria and is estimated to be present in approximately 2–4% of term pregnancies.[12] Stillbirths as a result of CA may be underestimated. Many studies include foetal-death statistics only after 28 weeks of pregnancy, and infection is not recorded as a frequent cause of stillbirth prior to 28 weeks. However, the placenta is rarely examined in developing countries and the cause is not therefore determined.[1] Common bacterial infections are due to *Mycoplasma*, *Ureaplasma* species and *Streptococcus* group B, which originate in the urogenital tract.[13]

These bacterial infections cause inflammation of the chorion and the amnion (membranes). This results in an inflammatory response of maternal and/or foetal origin.[12] In this chain of events endotoxins and exotoxins are released from bacteria which activate transcription factors and signal transducers and activators of transcription (STAT); these in turn signal the production of cytokines and chemokines (TNF, IL-1, IL-6, IL-8). These cytokines and chemokines generate arachidonic acid through the activation of phospholipases, arachidonic acid – Pg H2 – Pg E2 and Pg F2 alpha. These produce metalloproteases that induce uterine muscle contraction, possibly leading to uterine muscle contraction with consequent preterm labour.

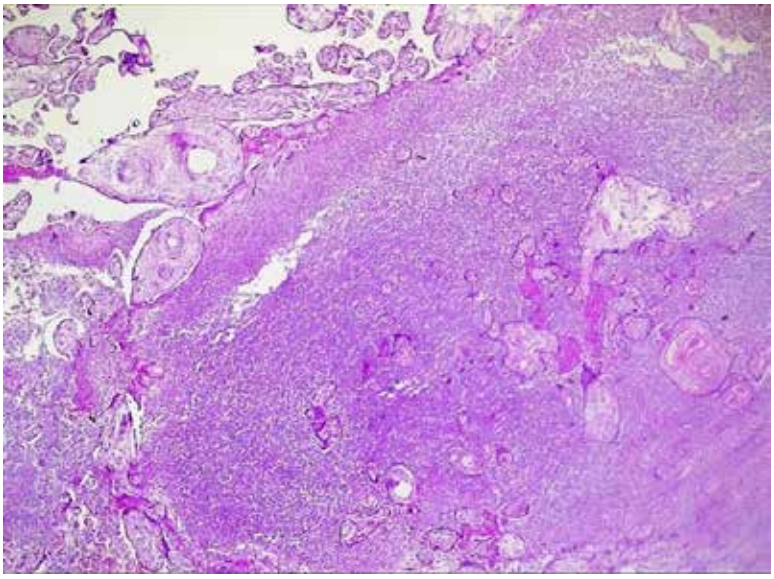

Figure 1: Histological section of placental parenchyma illustrating a destructive micro-abscess formation as a result of Listeria monocytogenes infection (courtesy of CA Wright)

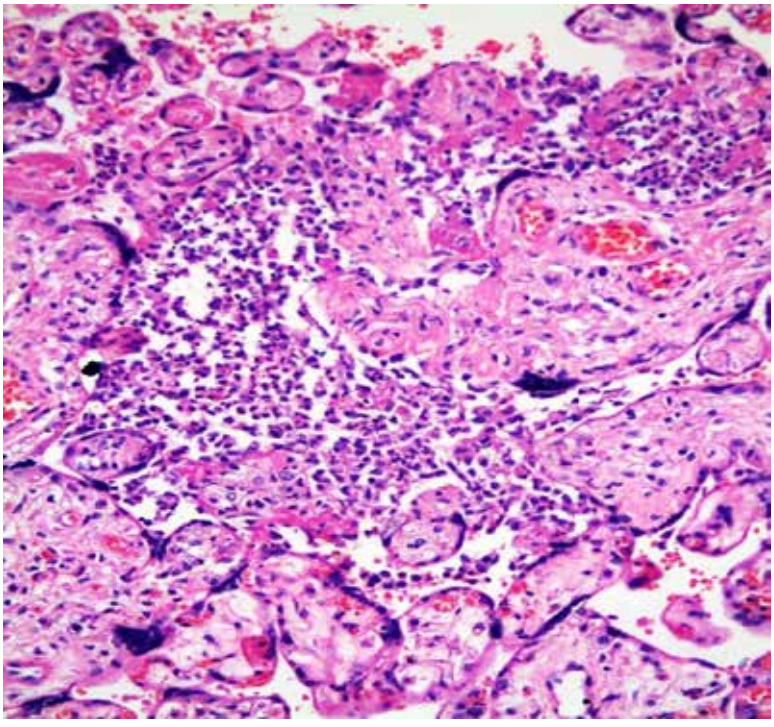

Figure 2: Placental parenchyma, chronic villitis of unknown aetiology (VUE) with villous lysis by lymphocytes. H and E stain (courtesy of CA Wright)

CA can be diagnosed both in a microbiology or histopathology laboratory and clinically.[12] However, there is a poor correlation between clinical and histologic CA. Group B *streptococcus* may show a minimal inflammatory infiltrate but causes perinatal asphyxia and leukomalacia through exotoxin-mediated vascular smooth-muscle contraction of the vessels of the umbilical cord and chorionic plate. Conversely, some bacteria, such as *Listeria monocytogenes* (see Figure 1), may show severe histologic changes with minimal maternal symptoms and present with stillbirth or a severely affected neonate.

In histology, a grading system and a staging system are used in CA where stages 2–3 are recognised to represent a fully developed histological CA and grade 2 is used when there are sub-chorionic abscesses and confluent neutrophils – Amsterdam

classification system.[10] This has prognostic significance for the neurological outcome.

The maternal response (infiltration of the chorion or sub-chorionic fibrin or decidual post-capillary venules by neutrophils) and the foetal response (neutrophils within the walls of large veins and arteries in the umbilical cord and amnion membrane) must be graded and staged.[12]

VILLITIS

Villitis can either be due to an infective agent, usually haematogenously derived. Alternatively, it can be an immunologically mediated phenomenon in which the lymphocytes within the villi are of maternal origin, therefore representing a form of maternal foetal-allograft rejection. It may be associated with intrauterine growth retardation, preterm labour and, in its severe and high-grade form, sudden, unexpected intrauterine death. It may recur in subsequent pregnancies in approximately 30% of cases with increase in severity. Villitis of unknown aetiology (VUE) low-grade and focal may occur in approximately 5–10% of all placentas and be clinically insignificant. A distinction from infective villitis may be suggested on morphology and may require immunohistochemistry and molecular testing for specific organisms, particularly viral infections such as cytomegalovirus. Class II major histocompatibility complex molecules form as the foetal immune system responds and the detrimental effects of high-grade diffuse VUE, particularly when involving stem villous vessels (obliterative foetal vasculopathy), may result in encephalopathy, cerebral palsy or intrauterine death.[12]

PLACENTAL MICROBIOME

The microbiome refers to the genetic component of all the microbes – bacteria, fungi, protozoa and viruses – that live on and inside the human body.[14] Placental microbiomes have been shown to be altered in patients with inflammation. This was assessed in a large American study using whole-genome shotgun sequencing (WGS).[15]

The Human Microbiome project consortium found that one-third of placentas from normal and term pregnancies harboured gram-positive and gram-negative bacteria. They were found to be inside the foetally derived extravillous trophoblast cells in the decidua. In a recent study by Aagaard et al using WGS technology, it was observed that the microbiome of placentas included *Escherichia coli* (the most prevalent), *Prevotella tannerae, Bacteriodes* species, *Fusobacterium* species and *Neisseria lactamica.*[16]

In another study where six nested birth cohorts were investigated, it was shown that the placental microbiome changed during pregnancy, with and without inflammation. However, the severity of CA was influenced by bacterial metabolic processes that played a role in affecting preterm birth.[15]

HOW DOES HIV STATUS INFLUENCE CHORIOAMNIOTIS?

Human immunodeficiency virus (HIV) is an incurable virus which attacks and weakens the immune system.[17] South Africa is undergoing one of the greatest HIV epidemics in the world, with approximately 19% of the general population being affected. Mother-to-child transmission (MTCT) is the biggest cause of neonatal HIV/AIDS infection.[2] In a study performed in KwaZulu-Natal, it was found that all incident HIV-infected mothers are at 2.3 times higher risk of transmitting HIV to their children.[18] An increased incidence of CA is observed in women with AIDS. It has also been reported that women with CA have an increased risk for vertical and perinatal transmission of HIV/AIDS from mother to child.[19] However, a United States study showed that the incidence of CA was similar in women with and without AIDS.[19] More of these studies are currently lacking in HIV-infected women, throughout South Africa.

PRETERM BIRTH AND IMMUNITY

The function of the neonatal immune system is compromised after intrauterine inflammation, which leads to preterm birth in most cases. Innate and adaptive immunity is immature and functions sub-optimally, and this can lead to lifelong morbidity. Owing to decreased lung function, preterm babies may require ventilation, which has adverse effects on neonatal immunity. Further, systemic inflammation (outlined earlier) is observed; this increases cytokine production, which can lead to chronic lung diseases.[20] There is decreased functionality of regulatory T cells (an important component of immune activation) in cases with with CA, and inflammation of the neonate is observed.[21]

CAESAREAN SECTION AND IMMUNITY

The gastrointestinal microbiome of infants seems to be influenced by mode of delivery –Caesarean sections versus vaginal births – at least in the very early neonatal period. The infants delivered vaginally had an increased colonisation of gut bacteria compared to those infants delivered by Caesarean section at three days old. This has been postulated to diversely affect the development and function of the neonate immune system. In addition, the absence of labour causes a reduction in monocyte expression (TLR-2 and TLR-4), which changes the neonate's response to an infectious agent.[20] The results of a study conducted in Denmark over a 14-year period showed that Caesarean section deliveries predisposed neonates to diseases associated with the mucosal immune system. These diseases included asthma, laryngitis, gastroenteritis, ulcerative colitis, celiac disease, lower respiratory tract infection and juvenile idiopathic arthritis.[22]

The colonising bacteria in the foetus obtained from the maternal gut via the placenta continue to colonise in the newborn's intestine after birth, and this significantly improves immune development and protects the newborn from various diseases. During a Caesarean section, however, the colonisation process is disrupted, and this predisposes babies to a higher incidence of allergies, type 1 diabetes and obesity.[4]

CONCLUSION

A review of the placenta highlights the important interface and function between mother and foetus. Clinicians are encouraged to review placental examinations in order to understand the prevalence and cause of infection or immune response. Elucidating the high infant mortality rate is the first step towards achieving this millennial goal.[23] A review of the placenta can assist in characterising the microbiome present in the newborn's gut passed on from the mother and in understanding further the neonatal immune system and a newborn's predisposition to certain diseases.[4]

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest

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Review Article

ALLERGY IMMUNOTHERAPY UPDATE

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ABSTRACT

Allergy immunotherapy has recently been reviewed and the latest recommendations were published by the European Academy of Allergy and Clinical Immunology in 2018. This article summarises these recommendations. This therapy is an effective form of treatment for allergic conditions such as allergic rhinoconjunctivitis, allergic asthma, and bee and wasp hypersensitivity. A formal diagnosis of an allergy (ie the presence of both a convincing clinical picture and confirmation through skin-prick testing or allergen-specific IgE), is necessary for allergy immunotherapy to be initiated. Patients should be considered on a case-by-case basis, with various factors being examined prior to this therapy: their current allergy treatment, compliance, long-term treatment goals and co-morbid health problems. Standardised allergy immunotherapy products administered at regulated doses is the current recommendation and long-term allergy protection is possible with extended treatment. Allergy immunotherapy for food allergies is subject to extensive research, the latest guidelines suggesting that this should be administered only within research settings at this stage. In cases of atopic dermatitis, this therapy has its place in the treatment of aeroallergens. South Africa has a number of standardised allergy immunotherapy products available which are used countrywide. The most common allergens treated in South Africa are grass, house-dust mite and hymenoptera bee stings

INTRODUCTION

In 1911, Englishman Leonard Noon published a paper in *The Lancet* that described the use of prophylactic inoculations for Timothy Grass pollen in patients with hay fever. His results were the first which showed that allergic symptoms could be treated successfully with this regimen.[1] After 108 years, this form of treatment is essentially still being used by allergists throughout the world.

Allergy immunotherapy (AIT) is currently the only form of disease-modifying treatment available for allergic conditions.[2,3] It is largely effective for treating asthma, allergic rhinitis (AR), allergic conjunctivitis and bee and wasp venom allergy.[4] It is available internationally as subcutaneous injections (SCIT) and sublingual immunotherapy (SLIT) in tablet form, perlingual spray and sublingual drops.[5]

The pathophysiologic changes that occur as a result of immunotherapy involve the lymphocyte profile shifting from a Th-2-predominant pathway into an allergen-protective Th-1 pathway. The result of this change is that Th-2 inhibits the production of various cytokines such as interleukins 4, 5 and 13. Interferon gamma and IL-12 also increase. The regulatory T lymphocytes (Treg) are activated with increased production of IL-10 and transforming growth factor beta. Immunoglobulin E (IgE) increases initially following therapy; thereafter, a decrease is observed, with an associated shift of IgG1 into IgG4 and IgA. There is a consequent inhibition of eosinophils, mast cells and basophils. Whether the immunotherapy is administered through a subcutaneous or a sublingual route, a similar immunological picture emerges.[3]

However, many treatment challenges still exist in the allergy world. The variety of products available, definition inconsistencies and conflicting outcomes of research can make the approach to using AIT potentially confusing for the practising allergist.[6] Uncertainty surrounds the long-term benefits of AIT, its safety in young children, the challenge of the polysensitised patient and using AIT as prophylaxis.[6,7] In order to respond to these issues, several guidelines have recently been published which use evidence-based clinical research to provide the current gold standard in AIT.[5,8,9,10]

PATIENT CONSIDERATIONS

Before initiating immunotherapy, these matters should be discussed with the patient and their family: the various available options such as SCIT vs SLIT, the disadvantages, the side-effects of the medication, adherence to treatment and the cost.[5]

Immunotherapy should be considered only in allergic patients who have a clinically significant allergy.[5] This means that the allergy symptoms should present soon after an exposure to the allergen, and the presence of IgE to the allergen must be proved, either through a positive skin-prick test (SPT) or an IgE-specific allergen test.[5] Importantly, the guidelines suggest that only clinically efficacious standardised AIT products at standardised doses should be prescribed.[6]

Immunotherapy is usually instituted only after appropriate measures to reduce allergen exposure and the correct use of pharmacotherapy have been tried.[5] Pharmacotherapy usually works much more quickly than immunotherapy in relieving allergy symptoms and is often very effective at reducing allergic symptoms. The efficacy of immunotherapy, in comparison, is often evident only after several months of treatment, although studies show that both SCIT and SLIT are able to reduce allergy symptoms within the first year of initiating therapy.[5] When counselling the patient and their family, it is important to emphasise that immunotherapy is a commitment, in terms of both time – as it needs to be taken regularly for several years – and cost.[6]

A number of indications for the use of AIT on an allergy patient are possible. After inadequate control with pharmacological means, SCIT or SLIT can be initiated to treat moderate to severe AR in cases of inadequate control using pharmacotherapy. In these cases, a three-year course is suggested.[5] This has been shown to be advantageous for up to two years and, in some cases, even longer after treatment, as illustrated by the Preventive Allergy Treatment (PAT) study.[5] In this study, children were followed up ten years after they had received a three-year course of subcutaneous immunotherapy with a standardised allergen extract for birch pollen or grass extracts.[18] The results confirmed immunotherapy to be a first-line treatment for the cure of allergies.[18] The clinical symptoms of seasonal rhinoconjunctivitis were no longer evident following cessation of immunotherapy after three years, and these benefits persisted long term.[18] Importantly, this study also showed that AIT had a protective effect against the development of asthma in this population of children. Therefore it offers the added benefit as a secondary preventive treatment for respiratory allergic disease.[18]

Other reasons to consider the use of AIT include:
- the cost of the patient's chronic allergy medications, especially if multiple drugs have been required for symptomatic relief;
- the side-effect profiles of these medications;
- non-compliance and the suboptimal use of symptomatic medications or medication-delivering devices such as asthma pumps or nasal sprays.[5]

Immunotherapy might also be considered as an option in cases of moderate to severe AR when other measures are optimally in place but the patients' daily functioning and sleep are still affected.[5]

The current recommendations suggest that SCIT and the initial SLIT dose should be administered by trained medical staff, and that patients should be monitored for 30 minutes post-administration of the AIT drug before being discharged.[5]

SAFETY OF IMMUNOTHERAPY

AIT is generally well tolerated and safe when given to carefully selected patients. however, both local and systemic reactions may occur with this therapy.[10] Following subcutaneous administration of AIT, the area may become swollen and red. Severe reactions include anaphylaxis, the reported incidence being between 1% and 4%.[10] The signs and symptoms of anaphylaxis vary and may involve the skin, the gastrointestinal or respiratory systems, and/or the cardiovascular or neurological systems.[10] The symptoms generally present within 30 minutes of exposure to the immunotherapy.[10] Adrenaline is the medication of choice, and anti-histamines, bronchodilators and corticosteroids may be used as adjunctive therapies.[10] SLIT has had no reported cases of anaphylaxis in an estimated one billion doses that have been administered.[19] Reactions to SLIT that have been reported are often localised, with oromucosal pruritis being the most common.[19] This generally occurs at the initiation of immunotherapy and settles down within a few days of treatment without any further medical management being required.[19]

CONTRAINDICATION FOR IMMUNOTHERAPY

Certain medical conditions such as severe asthma, autoimmune disease, HIV and pregnancy are relative contraindications for immunotherapy.[8,10]

In high-risk venom patients who have cancer or a history of cancer, for example, the guidelines suggest that immunotherapy may be initiated if the cancer is well controlled or in remission.[8] Autoimmune disease may be an absolute or a relative contraindication for hymenoptera immunotherapy.[8] It is a relative contraindication if the autoimmune disease is in remission; however, it is an absolute contraindication if the disease is active.[8] In addition, although data are lacking, a hypothetical risk does exist that immunosuppressive medication may interfere with the AIT.[8] Immunotherapy can be safely used in stable organ-specific autoimmune disease such as Crohn's or Hashimotos thyroiditis.[8] In mastocytosis, venom immunotherapy is recommended; however, these patients will often require a prolonged course.[8] Although immunotherapy is generally well tolerated in people with mastocytosis, these patients may have a greater chance of developing a reaction to immunotherapy.[8]

Severe cardiovascular disease is considered to be a relative contraindication for AIT; however, the guidelines recommend that even in an elderly patient with cardiovascular disease, venom immunotherapy may be advised.[8] The reason for this is that the symptoms following a sting may well be more harmful to the patient than the potential adverse effects of immunotherapy.[5,8]

Beta blockers have been associated with serious and treatment-associated anaphylaxis and so their presence as part of the patient's current treatment regimen would be considered to be an absolute contraindication for aeroallergen immunotherapy.[10] However, in life-threatening stinging-insect hypersensitivity, beta blockers would be considered only a relative contraindication for hymenoptera immunotherapy because the greater risk of a fatal outcome following a sting outweighs the possible side-effects of the immunotherapy.[8,10]

Regarding the age cut-offs for AIT, the age of the patient needs to be assessed in the context of the immunotherapy.[10] The paediatric population requires further well-designed research and so the age at which to initiate immunotherapy has been arbitrarily recommended to commence from six years of age

and onwards.[10] In the paediatric population, severe systemic reactions to stinging insects are not common in very young children. Therefore, there is not a great need for AIT in this age group. However, if necessary, venom immunotherapy can be used, and the recommendations in these cases would then be the same as those for adults.[8] Another reasonable indication for AIT in young children might be in milder cases of allergy, the aim there being the prevention of long-term effects of AR as well as possible prophylaxis to prevent the development of asthma.[5,10]

The use of AIT in elderly patients needs to be considered individually, taking into account various factors, such as age, co-morbid illness and the extent of the allergy.[10]

In pregnancy, immunotherapy appears to be safe; however, empirical data are scarce. The current recommendation, therefore, is not to initiate therapy in pregnancy.[6] However, if a patient is already receiving treatment, the maintenance dose can be continued.[6]

TABLE I: CONTRAINDICATIONS FOR AND SPECIAL CONSIDERATIONS OF AIT USE

Contraindications for AIT use	Uncontrolled/severe asthma Beta blockers for aeroallergen immunotherapy Severe cardiovascular risk for aeroallergen treatment Active autoimmune disease Active malignancies
Special considerations for AIT use	Elderly Children under six years old Pregnancy Beta blockers for venom immunotherapy Cardiovascular disease in venom immunotherapy

ALLERGIC RHINITIS

It would be reasonable to consider the use of immunotherapy in adults, as well as in younger children, for the treatment of symptoms affecting daily functioning and sleep, the prevention of long-term effects of AR, and as a form of prophylaxis to prevent disease-allergic progression.[5] Most patients with AR are polysensitised, and the current guidelines suggest that if a patient is polysensitised to homologous allergens – for example, two different grasses – a single preparation of the two allergens can be given.[11] Current data have suggested that poly-allergic patients with allergens that are unrelated may be given two separate immunotherapy preparations 30 to 60 minutes apart in different locations on the body.[5]

VENOM IMMUNOTHERAPY

Venom immunotherapy is indicated for moderate-to-severe systemic reactions.[8] It can, however, also be used effectively in adults with skin involvement only if this has an impact on their quality of life (QoL).[8] H1 antihistamines as pre-treatment have been found to improve tolerability of the immunotherapy by reducing both large local and severe systemic reactions.[8] The effectiveness of the immunotherapy is unchanged with these medications.[8]

Various protocols exist for venom immunotherapy scheduling.[8] These include up-dosing followed by maintenance schedules (which take several weeks to months to reach maintenance dose) and rush and ultra-rush regimens.[8] Rush immunotherapy is a protocol by which the patient receives daily doses of immunotherapy instead of a dose every few days until maintenance is achieved; ultra-rush protocols take several hours until the maintenance dose is achieved.[8] Considered against side-effect profiles up-dosing and maintenance have been found to be the safest protocols.[8] Immunotherapy given for a minimum of five years has been shown to be superior to shorter courses of immunotherapy; and lifelong therapy should be considered in patients with severe initial systemic reactions, systemic adverse events during venom immunotherapy, and honeybee venom-allergic patients with a high risk of future honeybee stings.[10]

IgE-MEDIATED FOOD ALLERGIES

The current recommendation for food-allergy immunotherapy is that it should be performed only by experienced personnel in research centres or in clinical centres with extensive experience in food-allergy immunotherapy.[6,9] Clinical trials have found immunotherapy to be effective in children from four to five years of age who have experienced clinical allergies to hen's eggs, cow's milk and peanut.[9] Well-designed longitudinal prospective studies are still needed to fill the many knowledge gaps in the use of AIT for IgE-mediated food allergies before Food Allergen Immunotherapy protocols for clinical practice can be finalised and AIT can become standard medical therapy for these conditions.[9]

ALLERGIC CONJUNCTIVITIS

AIT should be considered a treatment option in cases of AR and AR with conjunctivitis, which have proven IgE sensitisation to a single allergen or multiple allergens, and also in the case of moderate-to-severe symptoms despite avoidance measures.[5] Long-term efficacy is commonly achieved after three years of treatment with both SLIT and SCIT.[5]

ECZEMA

The role of immunotherapy in eczema treatment was found to be inconclusive in a number of studies.[12] However, it is effective in the management of house-dust mite (HDM) allergy.[13] A systematic review and meta-analysis of eight studies from Canada showed that immunotherapy decreases symptoms in specific environmental allergies such as birch pollen.[10] From this it appears that immunotherapy is beneficial for AD, specifically those associated with aeroallergens. In addition, despite previous studies, new research seems to suggest that immunotherapy is not associated with eczema flare-ups.[14]

IMMUNOTHERAPY IN SOUTH AFRICA

The kits needed to perform immunotherapy are available in South Africa and enable a variety of allergens to be tested (Immunospec, personal communication). Practitioners using immunotherapy in South Africa include general practitioners, paediatricians and ear, nose and throat (ENT) specialists. Johannesburg has the highest number of patients on immunotherapy, followed by the

TABLE II: PRODUCTS AVAILABLE IN SOUTH AFRICA			
PRODUCT	FORM OF IMMUNOTHERAPY	SCHEDULE	INDICATION
Oraltek	SLIT	No initiation phase	Aeroallergens
Alxoid	SCIT	Initiation and maintenance	Aeroallergens
Venox	SCIT	Initiation and maintenance	Bee sting
Alutek	SCIT	Initiation and maintenance	Alternaria Horse allergy

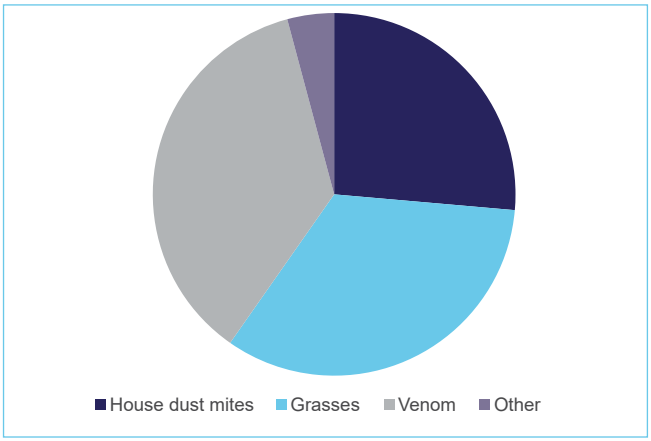

Figure 1: Common allergies treated in South Africa

Western Cape and then KwaZulu-Natal (Immunospec, personal communication).

The most common indications for immunotherapy in South Africa are for grasses, HDM and bee sting. These are followed by cat, dog and mould allergens (Immunospec, personal communication).

THE FUTURE OF ALLERGY IMMUNOTHERAPY

Although we are still using Noon's basic concept of allergen administration to cure allergic disease, some very exciting and promising developments are occurring with allergy immunotherapy.[15] A novel form of immunotherapy – intralymphatic immunotherapy (ILIT) – is currently undergoing investigation. This new method of immunotherapy entails the administration of three ultrasound-guided allergen injections into the inguinal lymph nodes at four-week intervals. The entire course for ILIT is predicted to take only two months. This is in stark contrast to current regimens which estimate that the length of effective treatment is close to three years.[15] Currently, there is limited evidence for its routine use, and no products in this form are available.[15] Epicutaneous immunotherapy (EPIT), another form of AIT which allows allergens to be administered via intact skin, has been used for both milk and peanut allergies. This form of immunotherapy has a favourable side-effect profile and studies are ongoing.[16,17]

These potentially exciting new developments are an indicator that interest and research are alive and well in the field of immunotherapy, and we look forward to what lies ahead. We are hopeful that straightforward and easy-to-administer treatment protocols which will yield effective and long-lasting therapy for the sufferers of these often debilitating conditions are just around the corner. The benefits will be immense.

DECLARATION OF CONFLICT OF INTEREST
The author declares no conflict of interest.

This article has been peer reviewed.

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RESPIRATORY ALLERGIES

WHAT IS ALLERGY?

It is a reaction that occurs when our immune system responds abnormally to substances in the environment (allergens), such as: dust mites, pollen from trees and flowers, animals and fungi.

WHAT CAUSES IT

Substances known as allergens, which our immune system considers foreign and potentially dangerous. To defend itself, it manufactures Immunoglobulin E, which will later bind to the allergens, triggering the symptoms of allergy

WHAT ARE THE MOST FREQUENT ALLERGENS?

Pollen, mites, animal dander and fungi

WHAT ARE THE SYMPTOMS OF AN ALLERGIC REACTION

Eyes: Conjunctivitis with itching, redness and runny eyes
Nose: Rhinitis with nasal itching, sneezing, rhinorrhoea and nasal blockage
Throat: Itching and coughing
Skin: Itching, redness and inflammation
Bronchi: Asthma symptoms: cough, wheezing, choking sensation and chest tightness.

SUBCUTANEOUS AND SUBLINGUAL IMMUNOTHERAPY

HOW CAN ALLERGY BE TREATED?

- Avoiding contact with the allergen .
- Treating the symptoms with appropriate medication.
- Using allergen immunotherapy, also know as allergy vaccines.

WHAT ARE ALLERGY INJECTIONS/ VACCINES OR IMMUNOTHERAPY?

It is a treatment that attacks the immune disorder that gives rise to the allergy. It is the only solution that treats the origin of the disease. It is supplemented with other medications that relieve the symptoms.

HOW DOES IT WORK?

The vaccine causes desensitization, reducing the body's sensitivity to the allergen. It progressively modifies the immune response against the allergen, preventing the occurrence of an allergic reaction.

WHAT CONDITION IS IT INDICATED FOR?

- Rhinitis
- Conjunctivitis
- Allergic asthma.

HOW ARE ALLERGY VACCINES ADMINISTERED?

This type of treatment is administered daily by the patient as a sublingual spray, not requiring a visit to a medical centre.
To guarantee the effectiveness of these treatments, good patient compliance is important.

HOW ARE ALLERGY INJECTIONS ADMINISTERED?

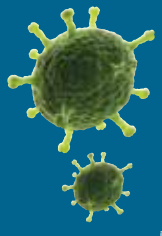
This type of treatment is always administered at a medical centre by a subcutaneous injection in the arm. It is usually administered weekly, during the first few weeks, and monthly, during the rest of the treatment.

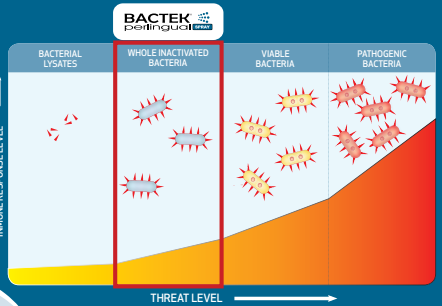
WITH ALLERGY INJECTIONS IT IS ADVISABLE TO:

- Not do physical exercise during the first 3 hours after administration.
- Avoid scratching the skin.
- In case of local reactions, apply a cold compress to the affected area and consult a healthcare professional.

Bacterial immunomodulators[3,4]

- Stimulate the activity of dendritic cells
- Increase cytokine production
- Increase the response of CD4+ lymphocytes





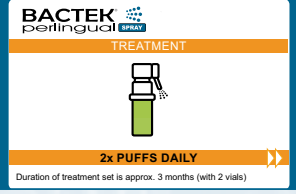
Adapted Blander et al., Beyond pattern recognition: five immune checkpoints for scaling the microbial threat. Nat Rev Immunol, 2012.12(3):p. 215-25.[10]

Selected strains of whole inactivated bacteria by moist heat

Maintains the ability of recognition and activation by dentritic cells

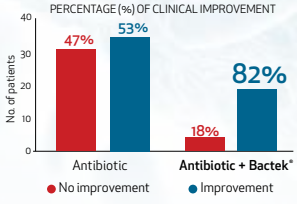
Perlingual spray administration[6]

- Greater absorption surface area in the oral mucosa
- Increased uptake by dendritic cells

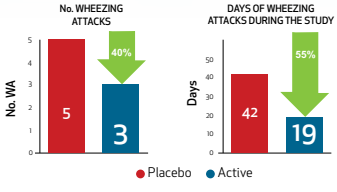


Clinical studies with Bactek® (MV130)

- **Bactek®** significantly **reduces** the number of **recurrent respiratory tract infections**[1,7]
- **Bactek®** reduces **surgery** in patients with **recurrent tonsillitis**[8]



- **Bactek®** decreases the number and duration of repeated wheezing attacks (WA) in children[2]



Available formulations

BACTEK perlingual SPRAY
RESPIRATORY TRACT AND PHARYNGO-TONSILLITIS

BACTERIA (MV130)	%
Streptococcus pneumoniae	60
Staphylococcus aureus	15
Staphylococcus epidermidis	15
Klebsiella pneumoniae	4
Haemophilus influenzae	3
Moraxella catarrhalis	3

BACTEK perlingual SPRAY
OTITIS MEDIA AND SINUSITIS

BACTERIA	%
Streptococcus pneumoniae	35
Haemophilus influenzae	35
Moraxella catarrhalis	15
Streptococcus pyogenes	15

RUNNING SHORT OF BREATH: A CASE STUDY OF HARD-METAL LUNG DISEASE IN AN ASBESTOS-EXPOSED WORKER

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ABSTRACT

In cases of workers previously exposed to asbestos and subjected to screening programmes for asbestos-related lung disease, it is possible that clinicians may not consider investigating them further for other possible aetiologies for their lung disease. This case involves a 58-year-old male fitter and turner employed at a naval dockyard. The patient presented with worsening symptoms of dyspnoea and a change in chest X-ray findings during an asbestos-screening programme. After further investigation that included high-resolution computed tomography and a lung biopsy, the histological picture was in keeping with a diagnosis of hard-metal lung disease. This case highlights the necessity of always considering a differential diagnosis in workers with exposure to multiple respiratory toxins and pathogens. This requires a thorough clinical examination to be performed. Such an examination should elicit a comprehensive occupational history that details all exposures and relevant special investigations in order to ensure that one does not fail to make the correct diagnosis.

Key words: asbestosis, hypersensitivity pneumonitis, hard-metal lung disease, giant cell interstitial pneumonia

INTRODUCTION

Screening programmes for specific occupational lung diseases are of great benefit in the early detection of clinical disease; however, they could result in alternative diagnoses not being made, especially in the early phase of the disease where clinical pictures are not too divergent. In the case described in this article, the employee was initially diagnosed with asbestosis. The clinical presentation of asbestosis has some features in common with that of hard-metal lung disease (HMLD), but the deteriorating clinical status, history of multiple occupational exposures and results of special investigations supported the final diagnosis of HMLD.

CASE PRESENTATION

A 58-year-old male employed as a fitter and turner at a dockyard was referred to the Occupational Medicine Clinic at a tertiary Cape Town hospital by an occupational medical practitioner (OMP) based at the patient's work site. The patient participated in a screening programme for asbestos-related disease due to historical asbestos exposure at the work site. At his latest evaluation, the OMP noticed X-ray changes of progressive pulmonary fibrosis with clear radiological deterioration in the past five years. In addition, the worker reported symptoms of worsening dyspnoea and chest pain.

The patient was a marathon runner and had last completed a marathon two years prior to presentation. One month prior to presentation, he noted that he struggled to complete a ten-kilometre race due to shortness of breath. This decline in effort tolerance appeared to be worsening as he noticed that he had recently became short of breath when walking briskly. The chest pain was felt predominantly on the left anterolateral side of the chest during periods of exertion.

He also reported infrequent chest tightness exacerbated by paint fumes and paraffin; and haemoptysis of small spots of fresh blood about twice a month. In addition, he had a persistent dry cough, had experienced 6 kg of unintentional weight loss in the past year and had experienced intermittent night sweats. His further medical history was unremarkable; he was a smoker with a ten-pack per year history.

The occupational history revealed that the patient had been employed at the same dockyard for his entire career (30 years to date). He was initially employed as a handyman but had held the position of fitter and turner for the past 12 years. His main

work functions were the repair and restoration of the multiple valves found on the vessels undergoing restorative work in the dockyard. The refurbishment involved mechanical stripping of a valve followed by cleaning, mostly with paraffin (and rarely solvents) and refitting of the parts. Where parts required replacing, the machining of both the new and the old metal parts was required in order to ensure a proper fit.

As his work was both on an actual vessel and in a workshop, he was exposed to dust from sandblasting in the dry dock, metal and paint fumes, welding fumes, metal-working fluids (MWF), cleaning materials and bird droppings (from pigeons found in the workshop warehouses).

Prior to 2008, all the workers employed at the dockyard were considered to have had occupational exposure to asbestos. In his case he had worked on vessels where asbestos lagging and insulation sheets were installed, replaced or removed and he would have been at risk of inhaling asbestos fibres.

On clinical examination, the patient was not in respiratory distress at rest and had a resting oxygen saturation of 94% on room air. He had pulse rate of 92 beats per minute and a normal blood pressure of 130/80. He displayed marked clubbing of his fingers (which he reported had only started in the past year) but no cyanosis, lymph adenopathy or oedema. Respiratory examination revealed a barrel-shaped chest, an audible chest rattle at rest, good chest expansion, with auscultation revealing pan-inspiratory crepitations throughout the chest. No other relevant signs were noted on systemic examination. He was referred for pulmonology assessment and the results from the special investigations are summarised in Table I. The chest radiograph images are shown in Figure 1.

WORKPLACE WALKTHROUGH INSPECTION

A workplace assessment was conducted to ascertain and confirm potential causative exposures for his symptoms. He was involved in three main areas of the dockyard site:

- working on board an operational vessel;
- working in a large warehouse where a vessel had been enclosed for refurbishment, and
- working in the workshop where valves would be serviced.

A summary of the workplace exposures and their potential health effects is given in Table II.

The fitters and turners would be in the warehouse for short periods of time to collect items needing repair or refurbishment. During this time, they would be exposed to welding fumes, blasting dust, paint fumes and exposures from other work tasks. One prominent exposure to note in the warehouse was that of the vast amount of bird faeces throughout the area.

The workshop was a large area constructed with a steel-girder system and clad in steel sheeting on the walls and polycarbonate roofing in most areas. Some areas of asbestos-cement roofing remained in the area, but the occupational hygiene assessment deemed this asbestos sheeting to be safe at this stage. The workshop was divided into three areas where the dismantling of valves occurs, the machining of parts happens and the cleaning and reassembly of valves occurs again. The patient was involved in only the first two areas. In the dismantling process, the only agent used to help remove parts was paraffin. Thereafter, in the machining section, parts are kept cool with MWF. Other than this exposure, the patient would also be exposed to small metal particles from the metal-working process and bird faeces from birds in the workshop.

DISCUSSION

Considering his work history and clinical picture, the following differential diagnoses were considered:

- HMLD/giant cell interstitial pneumonia (GIP)
- Asbestosis
- Hypersensitivity Pneumonitis (HP)

TABLE I: DIAGNOSTIC RESULTS

TEST	OUTCOME
Bloods	IgE Isocyanates (-ve), IgE Birds (-ve), ANF (-ve), CTD ENA Screen (-ve), JO1/Mi2 (-ve) Rheum Factor 51 (H), ESR 70 (H), CRP 88 (H), WCC 10.84 (H), Hb 14.4, Plt 189, Lympho 1.71 (H), Na 153 (H), K 4.4, Ur 3.2, Creat 71, Total Prot 90 (H), Conj Bili 8, ALT 16, AST 42, ALP 78, GGT 34 SACE negative
Sputum	GeneXpert –ve, Mixed oral flora
Chest X-ray (CXR)	The CXR showed hyperinflation, with bilateral marked interstitial changes and honeycombing worse in apical and basal regions. He had bilateral mid-and lower-zone pleural thickening (not specific for asbestos related disease, query old TB and thickening secondary to TB) and fissural thickening. Bronchial wall thickening bilateral. Heart not enlarged. No pleural effusion.
Spirometry	FEV1 of 1.68 L (57% predicted), FVC 2.00 L (54%) and FEV1/FVC 84% in keeping with moderate restriction. No significant reversibility was noted post-administration of bronchodilator.
High-resolution computed tomographic (HRCT) scan	The HRCT showed a usual interstitial pneumonitis- (UIP) type pattern with severe bilateral fibrosis and bronchiectasis, which was worse in the lower zones.
Video-assisted thoracic surgery (VATS) open-lung biopsy	Abundant fibrosis with intra-alveolar and interstitial giant cells noted in association with chronic inflammatory cell infiltrates, muco-stasis and peri-bronchiolar metaplasia. Differential diagnosis includes hypersensitivity pneumonitis (HP) from exposure to hard-metal grinding called giant cell interstitial pneumonia (GIP) (favoured aetiology); organisms/antigens in water used to cool hot metal, and birds. No overt evidence of malignancy, granulomas, viral inclusions or features of desquamative interstitial pneumonia (DIP).

TABLE II: WORKPLACE EXPOSURE	
WORKPLACE EXPOSURE	POSSIBLE HEALTH EFFECTS
Welding fumes	Acute: 'metal-fume fever'; ocular-nasal and respiratory irritation.
	Chronic: renal impairment; nervous system damage; bronchitis; emphysema
Blasting dust	Silicosis
Spray paint	Acute: irritant contact dermatitis
	Chronic: occupational asthma; ACD; neurological damage
Bird faeces	HP – 'bird fancier's lung'
Asbestos	Asbestos-related lung diseases
Paraffin	Dermatitis
MWF	Acute: ocular-nasal irritation; skin irritation
	Chronic: ACD; occupational asthma; HP
Metal particles	Occupational asthma; chronic obstructive pulmonary disease (COPD); HMLD

TABLE III: COMPARISON OF DIFFERENCIAL DIAGNOSIS		
DIAGNOSIS	PROMINENT CLINICAL FINDINGS	DIAGNOSTIC WORKUP AND FINDINGS
HMLD[1]	• Fine, end-inspiratory crepitations auscultated mainly at the bases. • Clubbing in advanced stages	• Restrictive lung defect characterised by reduced total lung capacity, vital capacity and lung-diffusing capacity. • CXR: a diffuse micronodular and reticular pattern predominantly in the lower-lung zones. • HRCT: diffuse centriolobular micronodular opacities in the mid- and lower-lung zones and sub-pleural curvilinear densities with ground-glass attenuation.
Asbestosis[2]	• Fine, end-inspiratory crepitations auscultated mainly at the bases. • Clubbing (32–42% of cases)	• Restrictive pattern on pulmonary function studies associated with impaired gas exchange. • CXR (early findings): small, irregular opacities, usually in the mid- and lower-lung zones.[3] • HRCT (early findings): intralobular, small, rounded or branching opacities; thickened interlobular septa; sub-pleural curvilinear lines and parenchymal bands.
Chronic HP[4]	• Muscle wasting and weight loss • Clubbing (50% of cases) • Tachypnea, respiratory distress, and inspiratory crackles over lower-lung fields	• Non-specific markers of inflammation raised. • Positive precipitating antibodies to the offending antigen. • CXR: progressive fibrotic changes with loss of lung volume and coarse linear opacities are common. • HRCT: multiple centrilobular nodules with some ground-glass attenuation, radiolucency or air trapping, extensive fibrosis, traction bronchiectasis and honeycombing.[5]

A comparison of the clinical presentation and findings of special investigations of these diagnoses is given in Table III below.

As can be seen from Table III, the early stages of asbestosis, HMLD and chronic HP are almost indistinguishable on clinical grounds only and the reliance on special investigations for the differentiation of these diagnoses is essential.[6] Furthermore, as with asbestosis, HMLD and chronic HP can have a long latency period.[7] HMLD, however, does not necessarily require a long latency period and, in this case, the rapid progression of symptoms found on clinical history seemed to be unusual for that of asbestosis, which prompted further investigation.

In this particular case, it initially seemed more feasible that pigeon exposure or MWF in the workplace, which were confirmed on a work visit, were the cause of the interstitial lung disease. Both of these exposures usually present as HP, through a delayed immune response to the inhaled antigens of bacterial growth in the MWF and animal proteins in the bird faeces. MWF is increasingly being implicated as a causative agent of HP among machine operators, with mycobacterium immunogenum being identified as the presumed aetiological agent in a number of HP cases due to MWF. But in most instances the causative agent(s) remains uncertain.[8]

On special investigation of this case the patient did not have raised specific antibodies for the pigeon species tested, whereas no testing for potential pathogens found in MWF was done. Considering a presence of specific antibodies in 92%

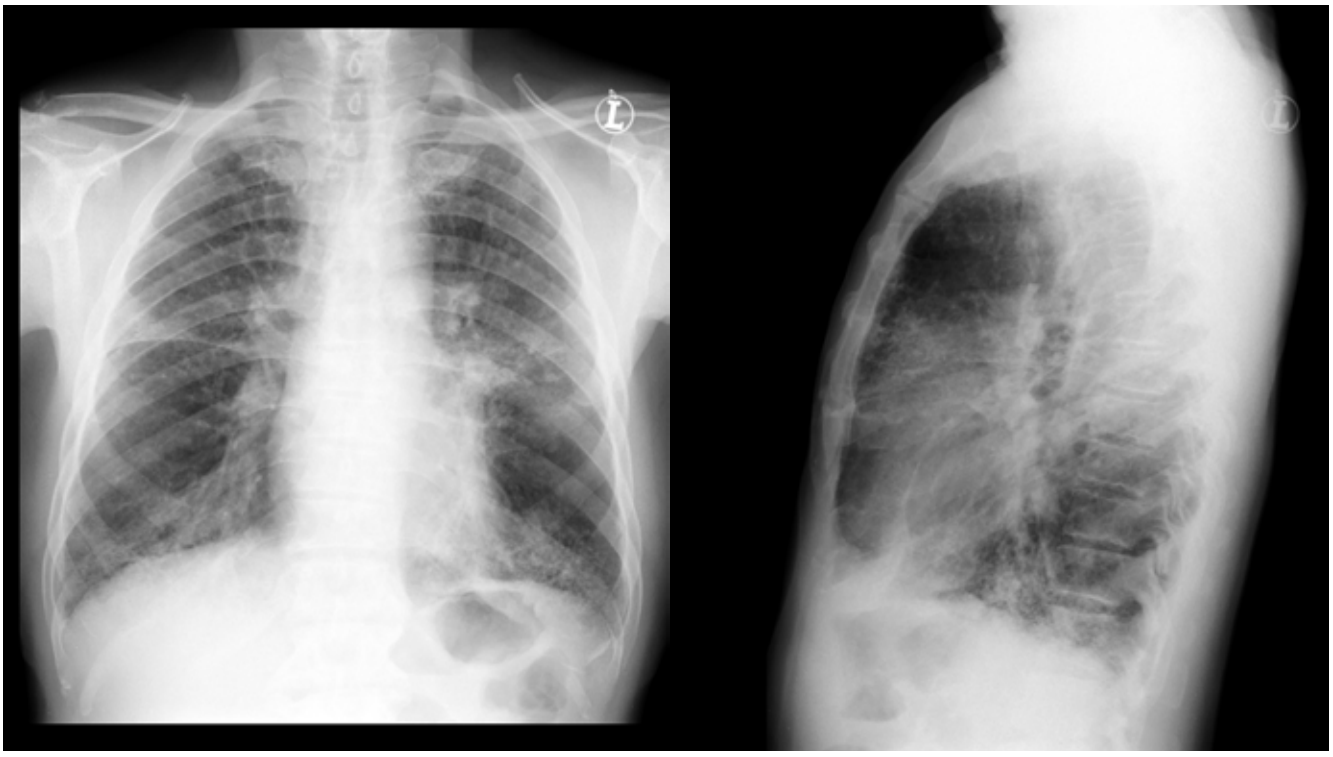

Figure 1: Chest radiograph of patient showing hyperinflation with reticulonodular and pleural changes

of patients in a large case series,[9] a lung biopsy for diagnosis was indicated. In its simplest form, HP is caused by a delayed immune response to an inhaled antigen to which the subject had been previously sensitised. The immune mechanism is a Th1 response, with lymphocytes being the predominant cell found in the lungs of such patients. Regulatory T cells (Treg) lose their normal function in the disease and dendritic cells acquire an enhanced capacity to present antigen to the lymphoid cells. Activated B lymphocytes release IgG antibodies specific to the causative antigen. It is unclear, however, whether these antibodies are a marker of exposure or whether they actively participate in the disease. Many individuals exposed to HP antigens develop these specific antibodies (precipitins) without ever having active HP, and it remains unclear why some patients with HP evolve toward fibrosis or emphysema.[8]

HMLD occurs in persons with regular exposure to tungsten carbide and cobalt. Cobalt is used in the production of tools used for drilling, cutting and grinding.[1] Occupational exposure can also occur during the sharpening and repair of these tools.[10] Cobalt is a well-known skin sensitiser and asthmagen.[11] A hypersensitivity mechanism is postulated to be involved in HMLD.[12] As the incidence of HMLD has been shown to be less than 1% in workers of a tungsten carbide production facility,[13] it is presumed to have an even lower incidence in other workers working intermittently with the same. An alternative hypothesis is that HMLD is caused by varying susceptibility to oxidant injury, because cobalt can increase the production of activated oxygen species. This reaction is enhanced by tungsten carbide, and as a result the combination of cobalt and tungsten carbide may have a greater effect on lung parenchyma than cobalt alone.[14]

The clinical presentation of HMLD is similar to that of HP and is diagnosed by the radiological changes and the pathognomonic presence of giant cells on lung biopsy and, occasionally, bronchoalveolar lavage.[12] The process by which giant cells are produced is not yet fully understood.[6] Nemery has postulated that the stimulation of macrophages by cobalt and tungsten carbide is responsible for the development of the bizarre, cannibalistic, multinucleated giant cells that are so characteristic of GIP/HMLD.[6] While there is no gold standard for the diagnosis of HMLD, the detection of tungsten and/or cobalt by elemental analysis in giant cells or lung specimens lends further support to the diagnosis. It should be noted, however, that cobalt is detected in one-fourth of HMLD cases only and that mineralogical analysis of lung samples is not routinely carried out.[15]

HMLD management is also yet to be clearly defined. Some evidence points to an mprovement of symptoms after removal from exposure. The use of oral prednisone has been described with limited sucess.[5] In the light of the diagnosis of HMLD, the company was strongly advised to remove the employee from tasks involving hard-metal exposure. A trial of oral steroid was administered and the case was reported to the Department of Labour in terms of the Compensation for Occupational Injuries and Diseases Act,[16] which allows for respiratory diseases caused by fibrogenic dust to be reported. The case was therefore considered compensable.

CONCLUSION

This employee was recorded as being historically exposed to asbestos. The focus of the surveillance programme and known exposure may have lowered the level of suspicion in the treating

clinicians for diagnoses other than asbestos-related lung disease. This case of HMLD highlights the importance of being open-minded, taking a comprehensive clinical and occupational history and ensuring that appropriate investigations are used to confirm a diagnosis.

ACKNOWLEDGEMENTS

The authors would like to thank the patient for giving his consent to having his case discussed. We would also like to make special mention of Dr Greg Calligaro in the Department of Medicine at the University of Cape Town for his pulmonology expertise and for assisting with the further investigation of this case.

DECLARATIONS OF CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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Ethics Article

ETHICS AND THE MICROBIOME

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ABSTRACT
The human microbiome and research linked to it have become increasingly important in our discourse about allergy and asthma and numerous other diseases. Accompanying this research and its applications are a host of ethical, legal and social implications. In this article I draw on the work of experts in the field and attempt to address these issues.

INTRODUCTION

This issue of *Current Allergy and Clinical Immunology* is devoted to the microbiome and its influence on the immune system and the development of allergy. Microbial colonies in the nasal, oral and respiratory tracts have been linked to asthma and other allergic diseases. Changes in the microbiome during pregnancy and delivery, and the method of delivery, have also been linked to the health of newborns and older children. The advances in knowledge about the human microbiome and its role in health and disease suggest that deliberately influencing and controlling the microbiome may impact the incidence of disease and may also be therapeutic in some cases. These advances have also made us aware of the potential dangers of exposure to antibiotics in early childhood, with increased risk for allergic and autoimmune diseases.[1]

There are a number of ethical issues accompanying microbiome research, as well as the implications of this research for society. Some of the ethical issues associated with microbiome research are:

- the selection of research participants;
- issues around informed consent and autonomy;
- the protection of participants' privacy;
- sampling methods and associated risks, and
- the return of research findings to participants.

The implications for society include the application of the research findings to the general public, the regulation of new products arising from the research and understanding of the research by the community.[2,3]

The National Institutes of Health (NIH) Human Microbiome Project (HMP) was designed to record the DNA of the human microbiome, with the aim of trying to establish whether all humans have a common microbiome. It also aimed to determine whether the microbiome changes during the course of a person's life and whether this can be correlated with aspects of people's health.[3]

INFORMED CONSENT AND RESPECT FOR AUTONOMY

The greatest challenge in microbiome research is the uncertainty regarding what will be discovered, and therefore the possible risks and benefits cannot be anticipated. This makes the informed consent process fraught with difficulty: How does one fully disclose the risks and benefits of the research to a potential research participant? A similar issue arises to that encountered in biobanking: If one is uncertain as to what future research may be done, is a general, broad consent acceptable, or is re-consenting necessary? An alternative solution has been proposed: 'meta-consent'. This involves asking the research participants to decide how they would like to provide consent for future, as yet undetermined, research.[4] As part of the informed consent process, the participants indicate whether they would like to provide broad or specific consent if their biological material and data are to be used for another purpose in future.[4]

Ma et al propose an alternative approach to informed consent.[5] They argue that microbiome research has the potential to promote human health while posing truly *de minimis* risk, that is 'it entails a degree of risk so low that harms are nominal and unlikely'.[6] Therefore, they believe we should advocate for a shift from concentrating on autonomy of participates (*sic*), to the principle of "solidarity" which entails the obligation of individuals to contribute to the enterprise of biomedicine research.[5]

One of the ways in which participants' privacy and confidentiality can be protected, is the establishment of a multidisciplinary governance board comprising individuals representing medicine, science, ethics and the public, to assess proposals for future research.[3]

What ought researchers to do about informing study participants of individual research results? As an example, what if a participant's intestinal microbiota pattern correlates with a disease that is potentially preventable by behaviour or diet change? Or what ought researchers to do if they discover a participant is colonised with a resistant organism? What are the implications of colonisation versus infection for an individual and the community?[3,5]

DATA-SHARING AND PROTECTION OF PRIVACY

The Human Genome Project (HGP) grappled with protection of participants' privacy in a project that was aimed at international collaboration and publicly accessible databases. Initially, this

was addressed by using de-identified data, but it soon became apparent that this was not sufficient protection, and so access to the databases had to be restricted.[3] The HMP is faced with a similar dilemma: Should the data be publicly accessible so as to maximise the scientific value of the research and remove perverse incentives associated with any discoveries, or should the data-sharing policies be more restrictive? Another contentious ethical issue is the potential linking of the microbial DNA data with human DNA and clinical information.[3,5]

Ma et al point out that human microbiome research and the clinical applications thereof are largely unregulated outside Western countries; this may increase the risk of exploitation of vulnerable research participants.[5]

SAMPLING METHODS AND RISKS

The initial HMP phase planned for sampling of five body sites from adult volunteers: mouth, skin, nose, gastrointestinal tract and vagina, together with peripheral blood samples. Because sampling of some of these areas involves invasive methods, such as endoscopy, the risks to the participants need careful assessment. The initial approach was for minimally invasive sampling only. Emanuel et al emphasise the importance of a favourable risk–benefit ratio when designing research studies.[7] However, as Ma et al point out, many of the risks associated with human microbiome research cannot be anticipated.[5]

SOCIAL JUSTICE CONSIDERATIONS

Social justice considerations require equitable participant selection, including ethnic, geographic and socioeconomic diversity, and minimisation of risks to the participants. One of the requirements for ethically acceptable research proposed by Emanuel et al is the social value of the research.[7] This is as important in human microbiome research as in other forms of research.

MICROBIOME AND STOOL BANKS AND OWNERSHIP ISSUES

Human microbiome biobanks have the potential to become incredibly valuable resources. Similar ethical and legal concerns apply as to biobanks storing human tissue samples. However, problems unique to microbiome biobanks include how to determine and select healthy donors, how to manage and interpret the unprecedented amount of biological data when the relevance to disease is unknown, how to externalise and collaborate with other banks and clinics nationally and internationally, and should volunteers be rewarded for giving stool samples to incentivise stool donation?[5]

The question of ownership of stools also arises, as faeces are usually considered to be waste. Hawkins and O'Doherty posed the question: 'Who owns your poop?'[8] Ownership raises the issue of potential remuneration and profit-sharing in future. The problem with microbiome research is the changing nature of one's microbiota, and whether this can actually be linked to an individual over time.[5,8]

CLINICAL APPLICATIONS OF HUMAN MICROBIOME RESEARCH

Applications of microbiome research include administration of pro- and prebiotics to preterm newborns and people with other diseases, such as irritable bowel syndrome (IBS). Most of these recommendations appear to pose relatively low risk to the recipients.

Two clinical applications of microbiome research that may carry higher risk are those of faecal microbiota transplant (FMT) and vaginal seeding.

FMT is the process whereby large quantities of intestinal microbiota are transferred from a healthy donor into the intestinal tract of a patient.[9] One of the indications for this procedure is recurrent *Clostridium difficile* infection. The evidence for the safety of this procedure is limited and some of the concerns revolve around resultant anxiety and depression.

Vaginal seeding involves swabbing the fluid from a woman's vagina and transferring it into the mouth, eyes and skin of her newborn baby born by caesarean section. The theory is that this will decrease the risk of the infant developing asthma and allergy later in life.[10] The most serious concern is that pathogenic organisms will be transferred from the woman to her infant. Infections that may be transmitted include *C trachomatis*, *N gonorrhoea*, human papilloma virus, group A streptococci and herpes simplex virus.[10] The recommendation is that vaginal seeding should be carried out only as part of a research study that has been reviewed by a Research Ethics Committee, and that it is too dangerous to implement as routine practice.

PUBLIC HEALTH CONCERNS

As Ma and O'Doherty point out, our human microbiomes are inextricably linked to those of others in our family and community.[1,5] Any manipulation of our own microbiome by taking antibiotics or changing our diet may impact others. Ma also discusses the risks of altering the microbiomes of other species, such as chickens and cattle, and the impact that this may have on human health. And finally, an intervention designed to change the microbiome in an attempt to benefit the health of the public, may predispose people to risk from other diseases. The example Ma et al use, is that trying to eradicate *Helicobacter pylori* in an attempt to reduce the incidence of peptic ulcers and gastric cancer, may in fact increase the risk of developing oesophageal diseases such as oesophageal cancer.[5] The incidence of asthma and allergy is also increased in people without *H pylori*.[11]

Public health policy and ethics dictate that large-scale microbiome interventions may be detrimental, and that public opinion needs to be informed regarding the potential risks, harms and benefits.

EVIDENCE-BASED MEDICINE AND THE DANGERS OF HYPE

Human microbiome research is ground-breaking and catches the public interest because it allows for 'natural' cures for disease and disease prevention. Probiotics have become big

business, both in their pure form and as additives to yoghurt and other foods. The 'hype' around the human microbiome has to be tempered with caution, as evidence for many of these interventions is still lacking.[5] The scientific community ought to provide a balanced message that is clearly understandable to the general public. This was not evident in a 'hard-sell' lecture at one of our recent ALLSA congresses, in which a prominent scientist promoted the probiotic supplement of one of the pharmaceutical companies in a pseudo-scientific exposition. As Ma says, 'natural' and 'organic' are often interpreted to mean 'safe', 'healthier' and 'less risky', but these words are not value-free and must be interpreted with caution.[12]

Hanage warned 'microbiomics risks being drowned in a tsunami of its own hype'.[13] He called for the following five questions to be addressed in interpreting microbiome research:[13]

1. Can experiments detect differences that matter?
2. Does the study show causation or just correlation?
3. What is the mechanism?
4. How much do experiments reflect reality?
5. Could anything else explain the results?

CONCLUSION

Human microbiome research focuses on the relationship between human beings, our microbiota and the environment. This research has the potential to explain the relationship between health and certain diseases, including the development of allergy and asthma. Technology resulting from the research offers hope for prevention strategies and interventions. However, as with any other new research, the ethical, legal and social implications (ELSI) require careful consideration and assessment. It is incumbent on us, as healthcare professionals, to interpret the research in a responsible way, and to ensure that the public is kept informed.

DECLARATIONS OF CONFLICT OF INTEREST
The author has no conflict of interest to declare.

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Research Article

THE IMMUNOLOGY OF MIND CONTROL: EXPLORING THE RELATIONSHIP BETWEEN THE MICROBIOME AND THE BRAIN (PART III)

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ABSTRACT

Part three of this four-part series continues to evaluate the relationship between the human species and the human gut microbiome. It focuses on whether their relationship is symbiotic, parasitic or somewhere in between. The possibilities, based on animal studies, are explored and compared to scientific facts proven in human beings. In particular, close attention is paid to the relationship between the gut microbiome and central nervous system, and the effect this has on human behaviour. This relationship is termed the 'microbiome–gut–brain axis'. Through a number of pathways, the gut microbiome has an influence on stress (both acute and chronic), anxiety, loneliness and depression, as well as on odour and attraction. It has also been associated with the development of neurodegenerative diseases such as Alzheimer's and Parkinson's, and with associated cognitive decline. Since the common treatments used for these conditions are not equally effective in all patients, it is vital for clinicians to explore other avenues that could be used as therapeutic targets. The gut microbiome, in particular, requires further research in order to aid the development of future therapies for certain conditions. The concept of the organisms in the microbiome controlling the mind of human beings is relatively new, but such control has been demonstrated through multiple examples in the animal kingdom. Animal studies in this regard have shown promising results, but human studies are infrequent and often present disappointing results. Randomised control trials in human beings are greatly needed, either to prove or to disprove, the effects of the gut microbiome on complex psychiatric diseases.

INTRODUCTION

The brain should no longer be viewed as an immune-privileged organ, and advances in immunology require integration into the pathogenic pathways of a variety of neurodegenerative conditions.[1] Neurodegenerative diseases are characterised by a slow but progressive loss of neurons in the central nervous system (CNS), resulting in specific brain-function deficits.[2] Since the common treatments for these conditions are not equally effective in all patients, clinicians must explore other avenues as therapeutic targets. Part three of this series will evaluate the relationship between the development of neurodegenerative diseases – specifically Alzheimer's disease (AD) and Parkinson's disease (PD) – the cognitive decline associated with them, and the gut microbiome.

COGNITION AND COGNITIVE DECLINE

Cognition – a general term that encompasses thought processes which contribute to memory and learning – is affected by numerous disorders of the CNS such as autism, depression, schizophrenia and AD. Furthermore, it is widely known that the neuronal pathways involved in cognition are strongly influenced by chronic stress experiences early in life, experiences that have a negative impact on cognitive function later in life. The potential contributions of gut microbiota to cognitive development (and decline) also need to be considered.[3] This article focuses on the connection between the microbiome, cognitive decline and age-related changes in physiology: ageing is associated with progressive changes in the motility of the gastrointestinal tract, immune function weakness, defects in the gut–blood barrier, and defective protein folding. These changes have a marked impact on gut microbiome diversity and therefore may be linked to age-related neurodegeneration.[4]

The decreased microbial diversity in the elderly can be observed in two phenomena: phyla levels and short-chain fatty-acid (SCFA) production. The two dominant phyla in younger age groups, *Firmicutes* and *Bacteriodes*, do remain prominent in older age, but possible changes in their ratios occur. An age-related decrease in SCFA production also occurs. The fatty-acid butyrate is especially important in maintaining colonic epithelial

integrity and inflammation.[5] These changes in phyla ratio and SCFA production are consistent with the theory of 'inflamm-aging', which associates chronic low-grade inflammation with various age-related pathologies, including cognitive decline.[5,6] This theory further suggests that ageing is associated with a global reduction in one's capacity to cope with a variety of stressors, and a concomitant progressive heightening in pro-inflammatory status.[6]

Fatty acids in the brain (namely, arachadonic and docosahexaenoic acids) have been shown to have an influence on depression, anxiety, and learning and memory, as they play a vital role in neurogenesis, protect against oxidative stress and may even alter neurotransmission. Recent evidence has shown that *Bifidobacterium breve* supplementation in mice increases brain fatty-acid levels.[7] Further research is required to ascertain how microbiota control fatty-acid production.

Studies on germ-free mice have shown that commensal gut microbiota are essential to membrane electrical activity, namely, the excitability of the sensory neurons in the gastrointestinal tract, ion fluxes and action potentials. Therefore, gut microbiota are involved in bidirectional signalling exchange between the gut microbiome and the CNS, in the enteric nervous system, in the autonomic nervous system and also in the neuroendocrine and neuroimmune systems.[8] The varying combinations and strains of species of bacteria among human populations may contribute to human biochemical individuality or genetic vulnerability and on an impact on resistance to disease.[9]

Miklossy published a review in 2011 demonstrating that oral bacteria, particularly oral spirochetes (obligate anaerobes), were present at seven times higher density and variety in the brains of patients with AD, when compared to cognitively normal controls.[10,11] Furthermore, one longitudinal study conducted at the University of Kentucky explored the potential for using oral bacteria in blood as a predictive tool for cognitive decline. The study found that increased baseline serum-antibody levels, particularly for the oral anaerobes *Prevotella intermedia* and *Fusobacterium nucleatum*, correlated with cognitive deficits.[10,12] Also of interest in relation to oral hygiene and cognitive decline, are a Swedish study of monozygotic twins as well as a study of North American nuns, both of which found a statistically significant correlation between tooth loss and the development of dementia risk.[13,14] An American study corroborated the assumption of these studies that tooth loss is a rough indicator for poor oral hygiene; it did so by showing that dentate individuals who did not brush their teeth daily had a 22–65% increased risk of developing dementia compared to those who brushed their teeth three times daily.[10,15]

Inadequate sleep also increases the risk of age-related cognitive decline: preliminary data suggest a possible relationship between sleep quality, gut-microbiome composition and cognitive flexibility in healthy older adults. Better sleep quality was associated not only with increased proportions of *Verrucomicrobia* and *Lentisphaerae* in the gut microbiome, but also with better cognitive scores – suggesting that improved microbiome health may buffer against sleep-related cognitive decline in older adults.[16]

Studies of Western-style diets with a high energy intake have also shown an association between such diets and cognitive impairment, increased anxiety-like behaviour and hippocampal-dependent memory inhibition.[17,18] Diets high in animal fat and protein are associated with a higher number of *Bacteroides*; high-fibre diets are associated with *Prevotella*, and plant-based diets have higher numbers of *Bacteriodes* and *Firmicutes*.[19] Bacteria and a high-fat diet interact to promote pro-inflammatory changes in the gut.[17]

High-fat diets change the gut microbiome composition and result in significantly increased concentrations of lipopolysaccharide and endotoxin. These increased levels result in increased intestinal permeability. This chronic gut inflammation is characterised by the infiltration by macrophages of adipose tissue during obesity. This results in increased TNF-α secretion from hypertrophied adipocytes, which leads to immune-cell alteration (such as TH1 cells, B cells, neutrophils and mast cells), with subsequent M1 activation of macrophages. The additional associated secretion of chemo-attractants (MCP-1, MIF) and cytokines (TNF-α, IL-1β, IL-6) drive immune cells, namely, T cells, dendritic cells and macrophages, into adipose tissue. This results in a chronic low-level systemic inflammation, which in turn leads to neuroinflammation. Persistent systemic inflammation triggers and sustains neuroinflammation.[17]

Neuroinflammation is associated with a variety of neurodegenerative diseases in several brain regions, including the hippocampus and the cerebellum.[1,17] This results in the up-regulation of amyloid beta and neurofibrillary tangles, synapse/neuronal degeneration, atrophy of the grey-matter volume and resultant progressive cognitive decline with memory and learning impairment.

It is important to note that the hippocampus is strongly linked to food-related behaviour. It controls feeding behaviour via the detection and integration of energy-state signals through memory and encodes information regarding food experiences. The hippocampal-dependent memory inhibition therefore vitally

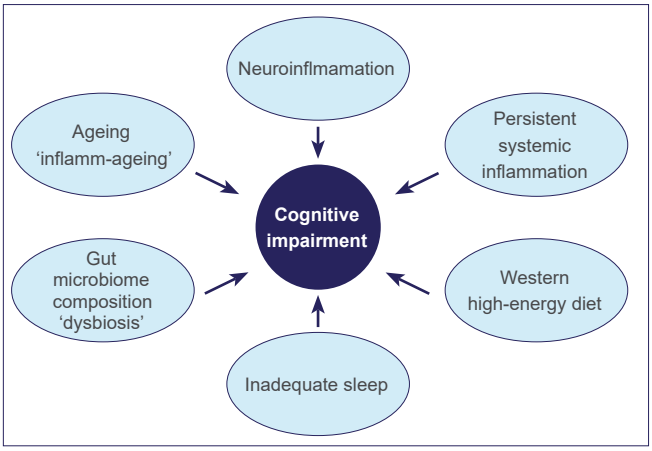

Figure 1: Factors affecting risk for cognitive decline

limits the response to environmental food-related cues, which results in excess energy intake. A dysfunctional hippocampus is a risk factor for obesity, leading to a vicious cycle.[17]

Researchers have proposed using anti-inflammatory drugs to combat the chronic neuroinflammatory process. However, limited studies have been conducted and clinical trials on the use of non-steroidal anti-inflammatory drugs have shown disappointing results.[1]

PARKINSON'S DISEASE

Treatments for neurodegenerative diseases in adults are delivering mixed results worldwide. This has led researchers to turn towards the gut microbiome for answers; a marked interest has developed in understanding its role in the context of PD.[19] Studies of mice have shown that enteric bacteria can regulate movement disorders through the deposits of unfolded protein in certain parts of the brain. These deposits are insoluble and promote neuronal apoptosis. AD and PD are types of amyloid disorder that are characterised by fluctuating manifestations. These diseases are cause for a growing health concern in an ageing population.[20]

The concept that patients with PD have low-grade gut inflammation has existed for some time: the colonic biopsies of PD patients reveal an increased mRNA expression of pro-inflammatory cytokines. This chronic low-grade inflammation may trigger blood–brain barrier leakiness, the activation of immune cells and subsequent neuroinflammation.[19]

The classic motor symptoms of PD (tremors, muscle rigidity, bradykinesia and impaired gait) are caused by the deposition of α-synuclein (αsyn) in dopaminergic neurons of the substantia nigra, leading to the subsequent death of these dopamine-generating cells.[20,21] Pathogenic bacteria release SCFAs into the peripheral circulation; they cross the blood–brain barrier and generate a pro-inflammatory environment in the brain through the activation of microglia. This dysregulated inflammation prevents the unfolding of certain proteins and accelerates their turnover and deposition in neurons.[20]

A wide variety of non-motor manifestations are also associated with PD: these involve the olfactory, cardiovascular, urogenital and gastrointestinal systems. Gastrointestinal manifestations (constipation, reflux, dysphagia, nausea and hypersalivation) often precede motor symptoms in the course of the disease.[21] In fact, gastrointestinal pathology precedes motor symptoms by years, which indicates that the peripheral nervous system (PNS) is involved before the CNS, and that pathology spreads from the gut to the brain via the vagal nerve.[22] Subsequently, αsyn aggregates have been found in the enteric and parasympathetic neurons of the gut.[21] This revelation has sparked much debate. The dual-hit hypothesis indicates that αsyn pathology is transmitted to the midbrain via two separate pathways: nasal (from the olfactory bulb to the temporal lobe) and gastric (through the enteric nervous system (ENS), secondary to the swallowing of nasal secretions via saliva). The disease may begin in the ENS and subsequently spread retrogradely to the CNS, or vice versa. The lesions in the ENS are not only thought

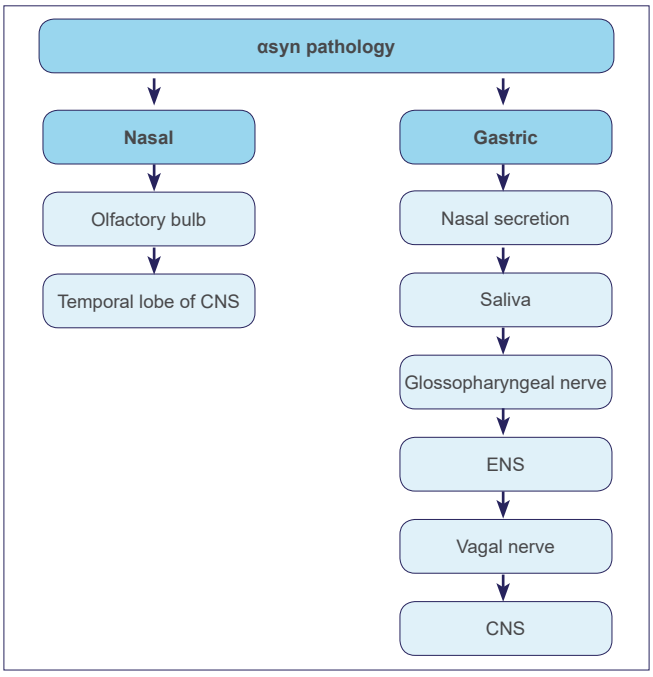

Figure 2: Alpha-synucleinopathy spread along the brain–gut axis (adapted from Sampson, Debelius & Thron and Mulak & Bonaz)

to affect the dopaminergic neurons, but are also suspected of being responsible for the digestive symptoms of PD. However, data available on the neurochemical and structural changes of enteric neurons are not yet complete.[21]

Prevotella is known to break down complex carbohydrates into SCFAs, with folate and thiamine as by-products. Patients with PD have decreased *Prevotella* numbers and have therefore reduced the production of these essential micronutrients. This results in both a decreased production of important vitamins and impaired gut-hormone secretion. These gut-microbiome changes could therefore have a direct effect on the CNS, although further studies are required before definitive conclusions may be made in this regard.[19]

PD has a defined clinical pattern, with gastrointestinal symptoms being followed by impaired olfaction and abnormal salivation. This step-wise march can be replicated by the Braak staging system, which shows increasing deposits of αsyn in the enteric nervous system, the motor nucleus of the vagus nerve, the olfactory bulb and the submandibular gland. All of these sites are exposed to the environment.[23] The analysis of urinary products of bacterial metabolism provides further evidence to support bacterial dysbiosis in the aetiology of PD.[24,25] Urinary indoxyl sulphate reflects small intestinal bacterial overgrowth (SIBO) and is consistently elevated in PD patients.[24] Similarly, microbial communities from faecal and mucosal specimens in PD patients are significantly different from those in control subjects.[26] Several studies have also shown a close relationship between nutritional status and neurodegenerative disease, reaffirming the interdependency between the brain and gastrointestinal function.[27] Nutritional assessment should, therefore, be included in the work-up of patients with PD. There is, however, inadequate

clinical evidence to make appropriate suggestions regarding nutritional advice for symptom reduction and improved health-related quality of life in this group of patients.[27]

SIBO is defined as a syndrome of malabsorption associated with an increased density of bacteria of more than 10^5 colony-forming units/mL of small intestinal aspirate and/or the presence of colonic-type species.[28,29] Predisposing factors for the development of SIBO are related to impaired gastrointestinal motility (caused by systemic diseases such as hypothyroidism and diabetes mellitus). Fasano et al found an increased prevalence of SIBO in PD patients without finding an increased prevalence of *Helicobacter pylori* when compared to healthy controls. *Helicobacter pylori* infection did, however, play a synergistic role with SIBO in the pathogenesis of motor fluctuations, with worsening unpredictable motor fluctuations that improved with *Helicobacter pylori* eradication.[28]

SIBO contributes towards the development and severity of motor complications in numerous ways. SIBO may impair drug absorption by changing chyme composition. Mucosal injury may lead to malabsorption secondary to loss of brush-border disaccharidases activity. Detrimental inflammatory effects on enterochromaffin-like cells further reduce gastric emptying. SIBO may impair levodopa absorption due to jejunal mucosal inflammation or as a result of partial metabolism of levodopa by bacteria.[28] The fact that bacterial overgrowth is significantly higher in the small intestine of PD patients, together with the predictable clinical pattern, suggests that dysbiosis may be central to CNS disease and that future treatment strategies may need to be adjusted accordingly.[28] Additional studies are required in this regard.

Gut microbiota have also been shown to modulate behaviour via fermentation products. One of these fermentation products – namely, D-lactic acid – has cognitive effects, with cognitive impairment associated with rising levels of D-lactic acid. SIBO is associated with increased D-lactic acid production and therefore subsequent cognitive decline.[7]

Various systemic diseases, including obesity and diabetes, show significant changes in both resident gut-microbiome composition and the host's gut homeostasis and metabolic processes.[19] Type 1 and type 2 diabetes mellitus, obesity and PD have been associated with dysbiosis of the gut.[25] Individuals with type 2 diabetes mellitus have been shown to have higher proportions of *Betaproteobacteria* than healthy individuals. This correlated with higher plasma glucose levels more than with body mass index (BMI), implying that *Betaproteobacteria* may be involved in glucose metabolism. Preliminary studies have also shown an increased association with type 2 diabetes mellitus and the development of PD. Although the mechanistic link between these conditions is unknown, both share pathophysiological mechanisms: chronic inflammation, oxidative stress and mitochondrial dysfunction.[19]

Since individuals with type 2 diabetes mellitus have a higher risk of developing PD, the effect of anti-diabetic drugs on PD has also been evaluated. An individual's risk of developing PD increases 2.2-fold when they are diagnosed with type 2 diabetes mellitus. However, with oral anti-hyperglycaemic agents, the risk is decreased to 1.3-fold. This suggests that oral anti-hyperglycaemic agents may have a protective effect against the development of neurodegenerative diseases such as PD.[19]

ALZHEIMER'S DISEASE

The gut microbiome has been shown to condition the host's immune system to foreign microbes while regulating autoimmune responses that can have an effect on neural-signalling and homeostatic metabolic functions.[8,9] The particular contribution of the gut microbiome and bacterial amyloid to misfolding and amyloidogenic diseases needs to be researched further.[8] AD has been associated with dysregulation of innate immune and auto-immune responses.[8] Some studies have suggested that differences in exposure or 'human biochemical individuality' and genetic vulnerability towards autoimmunity, mediated by the gut microbiome, may have a significant impact on the course of age-related neurological disease.[8,9]

The distinctive neuropathological features associated with AD include amyloid plaques and neurofibrillary tangles.[1,10] In many areas of the brain – namely, the hippocampus, the frontal, temporal and parietal cortices, and the cholinergic basal forebrain – these neurofibrillary tangles form amyloid-β peptides that are deposited as amyloid plaques. This results in the withdrawal of neurites, lost synapses and subsequent cell death.[10] The 'amyloid-cascade' hypothesis of AD suggests that amyloid-beta peptide accumulation is the primary influence on the inflammatory degeneration of neurons in the CNS and is possibly the driving factor for the pathogenesis of AD. The presence of bacterial lipopolysaccharides or endotoxin-mediated inflammation contributes greatly to amyloid neurotoxicity and, once formed, may induce a self-perpetuating sequence resulting in the amplification, aggregation and spreading of pathological protein assemblies.[30]

A wide variety of gut microbiome-resident species, including bacteria, viruses and fungi, generate significant quantities of functional lipopolysaccharides, amyloids and related microbial exudates. Therefore the human physiology is chronically exposed to a marked systemic burden of a wide variety of microbial amyloids.[30] This exposure becomes exceedingly important during the ageing process, during which the blood–brain barriers and gastrointestinal tract epitheliums become significantly more restructured and permeable.[25,30,31] This results in the CNS being more susceptible to microbes and/or their neurotoxins as well as to neurotoxins produced by environmental pathogens.[31]

There is a direct communication between the brain and the oronasal cavity via many nerves, particularly the trigeminal and olfactory nerves. The trigeminal nerve may act as a route of entry for oral bacteria into the brain in AD, because it has been shown to harbour Treponema. The 'olfactory hypothesis' introduced by Mann et al suggested that the olfactory tract is a possible entry route for pathogens which are able to trigger production of neurofibrillary tangles and amyloid plaques.[10]

The neurotrophins, the brain-derived neurotrophic factor (BDNF) and the nerve-growth factor are essential to maintaining cell viability and synapse connectivity, both of which are diminished in AD.[10] Amyloid protein is thought to possibly also be produced by human microbiota. This raises concern about the role of the gut microbiome in the induction of AD.[4]

Some microorganisms in the gut microbiome, such as *Lactobacillus brevis* and *Bifidobacterium dentium*, are able to metabolise glutamate to produce gamma-amino butyric acid (GABA). GABA is the major inhibitory neurotransmitter of the CNS. Increased GABA in the gut is associated with increased CNS levels. CNS dysfunctions in GABA-mediated neurotransmission and neuromodulatory functions have been linked to the development of behavioural deficits, depression, anxiety, epilepsy and cognitive impairment, including AD.[8]

BDNF is also known to be essential in neuronal maintenance and survival and also has pleiotrophic effects on neuronal development, differentiation and synaptogenesis.[8,9] BDNF plays an important role in the synaptic plasticity that underlies neuronal circuit formation and cognition.[8,9] It has been found to be reduced in the serum and brain of patients with behavioural defects, anxiety, schizophrenia and AD.[8,9]

Interestingly, β-N-methylamino-L-alanine (BMAA), a neurotoxic amino acid not typically incorporated into polypeptide chains that form brain proteins, has been linked with intra-neuronal protein misfolding.[8] This is a hallmark feature of the resultant inflammatory neurodegeneration that is secondary to amyloid peptide-enriched senile plaque lesions which are characteristic of AD, prion disease, PD and amyotrophic-lateral sclerosis. BMAA has been shown to be a neurotoxin that depletes glutathione, targets NMDA glutamate and induces oxidative stress.[8,9] It has been postulated that BMAA is produced by the cyanobacteria of the gut microbiome. Gastrointestinal disease, malnutrition and stress are further associated with increased BMAA production.[9]

Approximately 95 per cent of human beings harbour the intensely neurotropic herpes simplex-1 (HSV-1) in their trigeminal ganglia. Whether this relationship between host and virus is symbiotic, neutral or detrimental remains controversial. The activation or re-activation of HSV-1, as well as other forms of endogenous virus, are stress-related; however, it is still unclear whether gut microbiome metabolites play a role in pathogenic activation mechanisms. Recent studies have linked the activation of endogenous HSV-1, or other neurotropic microorganisms, to neurological stressors that are further linked to inflammatory neurodegeneration, amyloidogenesis and progressive cognitive impairment.[9] It may also play a contributory role to the predisposition to, or the early development of, AD and schizophrenia.[9,31]

Chronic fungal infections, *Toxoplasma gondii* (*T gondii*), *Chlamydophila pneumoniae*, hepatitis C virus, cytomegalovirus and prion diseases have been shown to increase the risk of AD.[31] Cytomegalovirus has also been linked to schizophrenia,

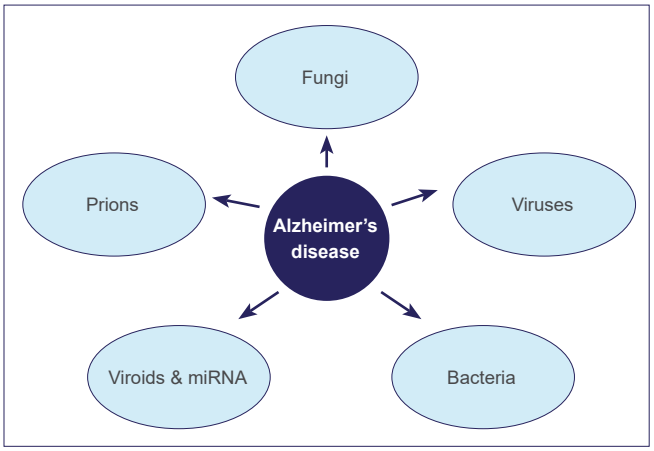

Figure 3: Potential contributing pathogenic microbes to the development of AD (adapted from Hill, Clement, Pogue, Bhattacharjee, et al)

depression and anxiety.[32] Prion diseases are self-replicating 'microbes' and amyloid-beta peptide 'prion-like' deposits induce widespread amyloidogenesis after inoculation into susceptible animal hosts.[31] *T gondii* has been shown to have strange effects on mice.[32,33] It causes them to lose their wariness of cats and cat urine, become more exploratory and spend increased time in daylight, therefore increasing their exposure to cats.[33] In human beings, *T gondii* causes males to be more aggressive and more jealous, and to indulge in riskier behaviour, while causing females to be more likely to commit suicide. *T gondii* has been associated with dementia, but also bipolar mood disorder, obsessive-compulsive disorder, autism and schizophrenia.[33]

Dietary manipulation of the gut microbiome and future personalisation of medicine may improve the clinical management and outcomes of brain disorders such as AD.[1] Future AD disease therapies may also involve probiotic approaches, particularly as a prophylactic tactic prior to the diagnosis of mild cognitive impairment or early AD.[9] Many antibacterial dietary components have been associated with reducing the risk of AD. Foodstuffs with proven antibacterial activity include garlic, olive oil, curcumin and honey. Many of these 'protective' foodstuffs also have anti-inflammatory properties that are thought to help in the prevention of AD progression, though clinical trials have shown disappointing results in this regard.[10]

The brain is an organ with a high metabolic rate, and therefore oxidative stress is a common phenomenon in its neural tissue. Antioxidant enzymes are endogenous but need exogenous nutrients for proper functioning.[5] Epidemiological studies researching the association between the dietary intake of antioxidants – particularly vitamins A, C and E (and trace minerals copper, selenium, zinc and manganese) and polyphenols (found in vegetables, fruit and plant-derived foods and beverages) – and cognitive decline, have shown inconsistent results.[5] One large randomised control trial (RCT) on long-term supplementation with extra-virgin olive oil revealed a significant improvement in episodic memory and verbal fluency and a decreased incidence of mild cognitive impairment.[5]

The relationship between the gut microbiome and the immune system is a fundamental aspect of the pathogenesis of obesity and obesity-related disorders. Increasing evidence has linked obesity to an increased risk of AD, as a result of more structural brain changes such as altered white matter and increased age-related brain atrophy. These findings have been shown in longitudinal data from the Framingham Heart Study. Significant literature has emphasised the vital role that the hippocampus plays in obesity-associated cognitive dysfunction. The metabolic syndrome results in neurocognitive impairment secondary to the long-term effects of poor metabolism. Mean grey-matter cerebral blood flow was shown to be 15 per cent reduced in individuals with metabolic syndrome when compared to that of controls. However, even short-term metabolic impairments have been associated with smaller hippocampal volumes and cognitive decline. Obesity has also been associated with increased levels of reactive oxygen species that promote cognitive impairment.[17]

For future clinical management of AD and related neurodegenerative disorders with inflammatory and immune components, therapeutic approaches should include anti-inflammatory, anti-bacterial and/or antiviral medication directed towards the health and homeostasis of the microbiome.[1,8,31]

CONCLUSION

Gut microbiota play a crucial role in the bi-directional interaction between the CNS and the intestines.[34] There is a long history of co-evolution between human beings and the microorganisms in our gut microbiome. This co-evolution may have effects on mental and physical health. The effects of imbalances in gut microbiota composition at different stages of life, and their subsequent short- and long-term impact on behaviour and brain modulation, require extensive consideration.[7] However, premature conclusions in extrapolating these findings to human beings should be avoided.

The extrapolation of findings in studies based on laboratory animal models to human beings is unwise because laboratory animals live under abnormally hygienic conditions. Animals living in less hygienic conditions, and human beings, encounter different immune responses. Human beings also exhibit cognition and adaptive behaviour that is different from other species.

More studies (including carefully designed translational and clinical studies) need to be conducted to investigate the aftermath of imbalances in gut microbiota composition. Investigations also need to be launched into possible avenues of prevention and treatment in the clinical sector to avoid long-term or permanent

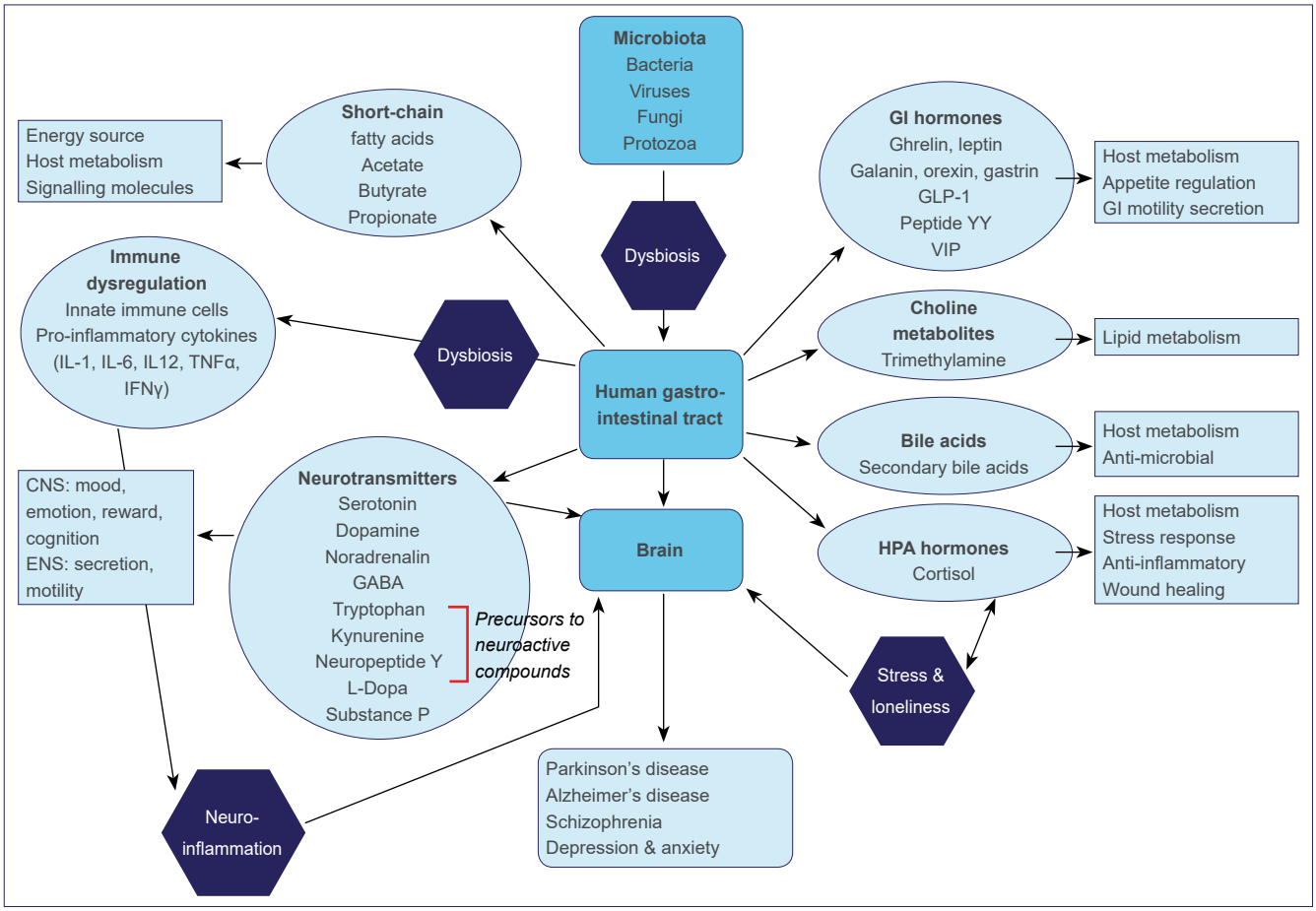

Figure 4: The microbiome–gut–brain axis and its effects (adapted from Forsythe & Kunze; Panduro, Rivera-Iniguez, Sepulveda-Villegas & Roman; O'Callaghan, Ross, Stanton & Clarke and Ramezani & Raj DS)

effects.[7] Infant studies are of particular importance, first, to ascertain the effects of alterations in the gut microbiome early in life on brain development and gut–brain interactions and, secondly, to assess whether interventions aimed at reducing gut dysbiosis can alter such effects.[35]

Several other areas need further consideration. These include obesity, the hygiene or microbiota hypothesis for allergic disease and the possible association between gut microbiota and psychiatric conditions. Of further relevance is the role of probiotic or commensal strain exposure to certain inflammatory conditions – such as rheumatoid arthritis, asthma, inflammatory bowel disease (IBS) and chronic obstructive pulmonary disease – all of which have strong associations with mood disorders and depression.[36] Further studies in human beings are therefore essential before any conclusive remarks, regarding the effect of the gut microbiome on complex psychiatric disorders, can be made.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

This article has been peer reviewed.

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CPD QUESTIONNAIRE

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ALLERGY AND THE MICROBIOME

1. *True or false:* A healthy community of microbes has the potential to resist colonisation and invasion by a pathogenic organism.
2. *True or false:* Exposure to all microbes is protective in terms of allergy development later in life.
3. *True or false:* Exposure to microbes in early life may affect the development and maturation of the immune system.
4. *Choose the correct answer:* Which one of the following factors has an effect on the human microbiota:
   a. Diet
   b. Topical emollients
   c. Smoking
   d. Vaginal delivery
   e. All of the above

ALLERGY IMMUNOTHERAPY UPDATE

5. *True or false:* In South Africa we have availability of both sublingual (SLIT) and subcutaneous (SCIT) immunotherapy preparations.
6. *True or false:* Immunotherapy should only be advised if a patient has both a) the symptoms of an allergy, and b) a positive IgE result, either by a positive skin prick test or an elevated allergen-specific IgE.
7. *True or false:* Allergy Immunotherapy may be considered in young children with allergic rhinitis to grass pollen to prevent asthma from developing.
8. *Choose the correct statement:*
   a. Cancer is always a contra-indication for immunotherapy.
   b. The elderly should not be offered immunotherapy as they may have co-morbid disease.
   c. Patients with mastocytosis may be considered candidates for immunotherapy.
   d. Crohn's Disease is a contra-indication for hymenoptera venom immunotherapy.

RESPIRATORY INFECTIONS AND THEIR EFFECT ON THE PAEDIATRIC LUNG MICROBIOME

9. *True or false:* Rhinovirus is the most common virus detected in respiratory samples from patients with respiratory tract infections in a private South African laboratory.
10. *True or false:* Unimmunised adults are most at risk for severe *Bordetella pertussis* infections.
11. *True or false:* The make-up of the microbiome is largely shaped during infancy.
12. *Choose the correct answer:* The following microbial profile predisposes infants to acute severe respiratory tract infections:
    a. An abundance of candida species;
    b. A diverse mix of bacteria, viruses and fungi;
    c. An abundance of *Pneumococcus* and *Haemophilus* species;
    d. An abundance of *Corynebacterium* species;
    e. A persistence of respiratory syncytial virus.

THE INFLUENCE OF PLACENTAL MICROBIOMES ON IMMUNOLOGY AND ALLERGY AND THE EFFECT OF PLACENTAL INFLAMMATION ON IMMUNITY IN NEWBORNS

13. *True or false:* Inflammation of the villi is referred to as chorioamnionitis.
14. *True or false:* The placental microbiome refers to the genetic component of bacteria, viruses, protozoa and viruses.
15. *True or false:* The absence of labour results in an increase in the TLR-2 and TLR-4 in neonates.
16. *Choose the correct answer:* The most common cause of placental inflammation is:
    a. Infection
    b. Host immune response
    c. Unknown agent
    d. All the above

ETHICS AND THE MICROBIOME

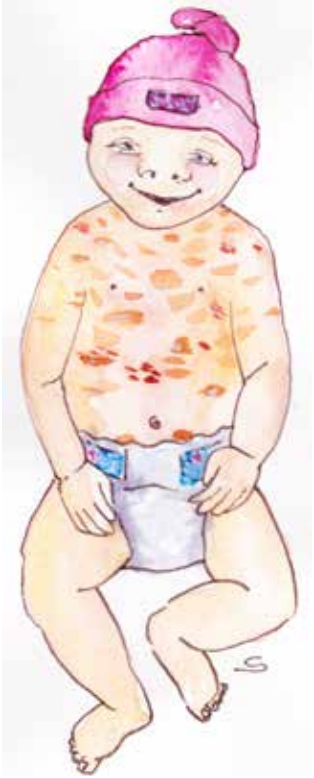
17. *True or false:* The informed consent process in microbiome research is difficult because of the uncertainty of future risks.
18. *True or false:* Meta-consent involves asking a research participant to indicate how they would like to provide consent for future, as yet undetermined, research.
19. *True or false:* Vaginal seeding of newborn babies to reduce later development of allergic disease is safe.
20. *True or false:* Manipulation of an individual's microbiota has potential public health consequences and may predispose others in the community to disease.
21. *True or false:* Human microbiome biobanks pose the same ethical concerns as other human tissue biobanks and so the same regulatory frameworks would apply.

MASTOCYTOSIS

Shaunagh Emanuel and Di Hawarden

Dr Do-a-lot presents some clinical cases to her students:

1. A four-month-old baby girl who, over the past few weeks, has developed pale-brown marks on her torso.
2. A 52-year-old man with intermittent headaches, flushing, abdominal pain, bloating, flatulence, nausea and dermatographism.
3. A 28-year-old footballer with an unexpected vertebral fracture following a fall on the sports field.
4. A 40-year-old woman who recently developed hypersensitivity to aspirin and a variety of foods, especially tomatoes, guavas and dried fruits.
5. A 38-year-old businessman under immense stress at work who has been struggling to concentrate for a few months, has difficulty sleeping, has been feeling intermittently light-headed and describes a recent unexplained episode of flushing and collapse after his Thursday night squash game.
6. A 25-year-old girl on a clinical trial hoping for a potential cure for an aggressive haematological cancer.
7. A 60-year-old woman who is otherwise well but reports being especially sensitive to heat and alcohol.
8. A teenager who will swim in the sea with his friends for the first time this summer as the weird marks on his skin have finally faded and almost disappeared.



Four-month-old baby with cutaneous mastocytosis

Dr Do-a-lot explains that these are all cases of *mastocytosis*. 'How can mastocytosis manifest in so many different ways?' she asks. It has everything to do with the *mast cell.* Understanding the mast cell is key to understanding the possible plethora of clinical presentations of mastocytosis.

The mast cell

Produced in the bone marrow and derived from the myeloid cell line, this cell has formed part of our primary defence system from the early stages of the evolution of our human immune system. Being a sentinel cell, it is stationed in all parts of the body where we interface with the environment. This means that mast cells are found in a great many tissues, especially the skin, the upper and lower respiratory tracts, the gastrointestinal tract and the genitourinary tract. Although they spend only a short time in the blood once they leave the bone marrow, mast cells have a relatively long life, surviving for about 2–4 years in the peripheral tissues. Mast cells do not usually form aggregates. They are typically found distributed fairly evenly in tissues.

The mast cell can produce a vast variety of substances. When we consider inflammation that results from mast-cell degranulation, we tend to think in terms of histamine and a few other inflammatory mediators such as tryptase, leukotrienes and prostaglandins. However, mast cells are capable of producing many more substances including cytokines, chemokines, proteases, growth factors, vasodilators, platelet-aggregation factor, heparin, serotonin, antimicrobial interferons and acid hydrolases.

Many mechanisms may activate the mast cell. We are familiar with immunological mast-cell activation and degranulation via the mechanism of immunoglobulin E crosslinking in response to exposure to a specific allergen, but a great many other mechanisms can activate mast cells. These include physical injury, microbial infection and exposure to medications and chemicals.

Therefore, if we consider that mast cells reside in any tissue where blood vessels may be found, that they can produce a large variety of active substances, that they can be stimulated to do so via many immunological and non-immunological mechanisms, that inflammation is the outcome, and that inflammation is expressed in different organs and tissues in myriad ways, it is hardly surprising that mastocytosis can have an extraordinary number of possible clinical presentations. To top it all, mastocytosis is rare, so clinicians seldom see (and may fail to recognise) the condition.

What is mastocytosis?

Mastocytosis is a group of disorders in which *an excess number of mast cells* accumulates in the tissues of the body. The disease affects males and females in equal numbers and can begin in childhood or adulthood. There are **two main types** of mastocytosis:

1. *Cutaneous mastocytosis*, which usually affects children- where mast cells gather in the skin forming typical lesions, but are not found in large numbers in other tissue in the body.
2. *Systemic mastocytosis*, which mainly affects adults- where mast cells infiltrate internal organs like the bone marrow, gut, liver and spleen. The skin may or may not be involved in the systemic form of the disease.

Cutaneous mastocytosis is divided into:

- maculopapular cutaneous mastocytosis (previously known as 'urticaria pigmentosa')
- diffuse cutaneous mastocytosis (in which the skin may have a thick leathery appearance)
- localised mastocytoma of the skin (cutaneous mastocytoma).

Systemic mastocytosis is classified in the following sub-types:

- Indolent systemic mastocytosis: the most common systemic form, with mild to moderate variable symptoms. The skin is often involved.
- Smouldering systemic mastocytosis: symptoms are more severe than in the indolent form, but prognosis is better than in the aggressive form.
- Systemic mastocytosis with associated haematologic non-mast-cell clonal disease: in which the over-production of mast cells affects the production of other blood cells.
- Aggressive systemic mastocytosis: mast cells multiply in organs (such as the bone marrow, gut, liver and spleen) and symptoms are more severe than in the indolent form. There is typically no skin involvement.
- Mast-cell leukaemia.

Localised mast cell tumours e.g. Mast cell sarcoma.

History

Mastocytosis was first described as a skin disease by Nettleship and Tay in 1869. The first case of systemic mastocytosis was described 70 years later in 1949. Since then the classification has expanded to include several sub-variants.

What causes mastocytosis?

The cause of this condition is poorly understood. It is seldom inherited. Genetic mutations in the genes that produce KIT proteins play a role. The kit mutation makes mast cells more sensitive to a signalling protein called 'stem-cell factor', which plays a role in the production of cells in the bone marrow.

Diagnosing mastocytosis:

In children with *cutaneous mastocytosis* the skin lesions provide a helpful clue to diagnosis. In maculopapular cutaneous mastocytosis yellowish-tan to red-brown marks are typical, usually found on the torso and areas of the body that are not usually sun-exposed. The marks may be flat macules or raised papules and may blister to form vesicles. They may be as small as a millimetre wide, or up to several centimetres in diameter. There could be just one lesion, or as many as a thousand.

If rubbed, the lesions may become red, raised and itchy due to mast-cell activation – this reaction is referred to as Darier's sign.

A biopsy of a skin lesion showing increased numbers of mast cells confirms the diagnosis. Dermatographism is a common sign.

60-year-old woman reacting to ingestion of alcohol

The diagnosis is more difficult in *systemic mastocytosis* when skin lesions are not present.

The variety of possible presentations of this disease is vast. Typical symptoms may include hives, itchy rash, flushing, diarrhoea, abdominal pain, nausea, vomiting, gastro-oesophageal reflux, weight loss, fainting, tachycardia, shortness of breath, wheezing, bone pain, bone fracture due to osteoporosis, psychological changes such as difficulty in concentrating and irritability, and anaphylaxis.

'Red flags' for considering this diagnosis include; a history suggestive of inflammation in more than one organ system, poor response to treatment, recurrent unexplained anaphylaxis, a well-defined diagnosis such as lymphoma coupled (unusually) with a condition such as anaphylaxis, and sudden seemingly inexplicable ill health in a previously well adult.

Testing for systemic mastocytosis includes the following:

- serum tryptase levels
- urinary histamine or mediator levels
- full blood count
- abdominal ultrasound to identify enlargement of the spleen or liver
- gastrointestinal workup in patients with gastric symptoms
- dual-energy X-ray absorptiometry (DEXA) scan to measure bone density
- bone-marrow biopsy to identify increased numbers of mast cells
- skin biopsy if lesions are present
- molecular testing.

Diagnostic criteria

The following *criteria* are helpful in confirming a diagnosis of systemic mastocytosis:

- *Major criterion*: multifocal dense infiltrates of mast cells (>15 mast cells in aggregates) in bone-marrow biopsies or other organs (not skin).
- *Minor criteria*:
  a. Atypical mast cells on blood smear or spindle-shaped mast cells in organ sections
  b. KIT point mutation on genetic testing
  c. Mast cells exhibit CD2 and/or CD25 on flow-cytometry testing
  d. Baseline serum-tryptase level is greater than 20 ng/mL.

If at least one major and one minor, or three minor criteria are fulfilled, the diagnosis of systemic mastocytosis can be established.

Treatment:

There is no cure for mastocytosis. Treatment is aimed at management of symptoms and preventing anaphylaxis.

Advise patients regarding the avoidance of agents that precipitate mast-cell mediator release such as non-steroidal anti-inflammatory medications, aspirin, opiates, alcohol, thiamine, quinine, anaesthetic agents, radiographic contrast media, dextran, some antibiotics, muscle relaxants and anti-emetics.

Cutaneous mastocytosis has a good prognosis

Dietary modification may be helpful in some patients. Foods high in salicylates, shellfish, alcohol, spicy foods, hot beverages and cheese, for example, may trigger symptoms in certain individuals.

Physical stimuli that trigger symptoms should be avoided if possible, for example, emotional stress, extreme temperatures, physical exertion and rubbing or scratching of lesions.

Treatment options depend on the type and severity of the disease.

The following strategies may be adopted:

Managing skin manifestations:

- Topical glucocorticosteroids
- Ultra-violet light treatment with oral psoralen (PUVA)
- Surgery (e.g. to remove a mastocytoma).

Stabilising the mast cell and curbing the effects of inflammatory mediators:

- Sodium chromoglycate
- H1 and H2 antihistamines and hydroxyzine
- Leukotriene antagonists
- Systemic glucocorticosteroids in specific situations.

Targeted therapy:

- Biologic medications
- Some chemotherapeutic agents
- Immune modulators.

Management of anaphylaxis:

- Adrenaline auto-injector.

Prognosis

Children who have cutaneous mastocytosis typically have an excellent long-term prognosis if well managed during childhood. There is often full resolution of the disease by puberty.

Adults who have the most common (indolent) form of systemic mastocytosis typically have the same life expectancy as the general population but, depending on the levels of mediator release from their many mast cells and the organs involved, may have significant impairment of quality of life, even on treatment.

Other forms of the disease may have a poor prognosis.

Anaphylaxis

When mast cells degranulate they release inflammatory mediators that cause rapid vasodilatation and bronchospasm, which may result in anaphylaxis. It is important for people with mastocytosis to carry adrenaline auto-injectors at all times and to be aware of potential triggers. They must wear MedicAlert jewellery and have a written anaphylaxis action plan.

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PID123

Primary Immunodeficiency

'GO WITH THE FLOW' – A VISIT TO THE FLOW CYTOMETRY LABORATORY

For the past couple of weeks, Dr Goodheart has been seeing a 15-month-old boy, Nathan, who had a history of recurrent infections requiring longer than usual courses of intravenous antibiotics and also two intensive care unit admissions. Based on previous laboratory results, Nathan had low to absent IgG and IgA but normal to raised IgM antibody levels. B and T lymphocyte counts were normal. Dr Goodheart's suspicion of a possible **X-Linked Hyper IgM Syndrome** (due to defects in CD40Ligand-CD40 interaction)[1,2] as underlying immune defect increased when she found out that two of Nathan's uncles had died in early infancy due to severe infections.

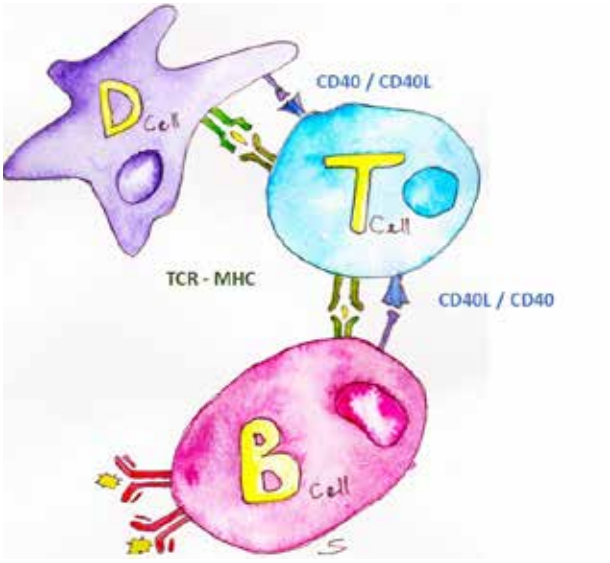


Figure 1: CD40/CD40 Ligand interaction on T cells

CD40/CD40L interaction is needed both for the activation of T cells (through interaction of dendritic cells with CD4 T cells) as well as for class-switch activation of B cells (interaction of T helper cells with B cells; this happens sequentially, not in one step, as shown in the picture). ***Hyper IgM syndrome*** *(HIGM) is characterised by defective antibody class-switching, where IgM antibodies are still produced but IgG and IgA production is defective. Diagnostically, the lack of CD40L expression can be measured by flow cytometry on 'artificially' activated T cells of patients with HIGM who lack CD40L.[1] (TCR = T cell receptor; MHC = Major Histocompatibility Complex)*

After reviewing the case with her colleague, the immunology specialist Dr Bala, the decision was made to test Nathan for a possible CD40L-deficiency (see Figure 1). Dr Goodheart suggested visiting Dr Bala's laboratory to observe how the test would be conducted. She knew that flow cytometry was used and she was keen to see how this was carried out. In the past, she had struggled to interpret flow cytometry results and she had never before seen a flow cytometer.

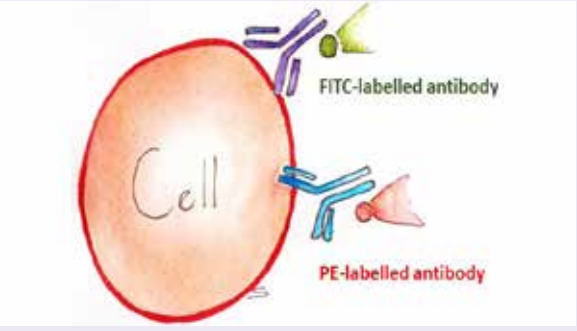
When she arrived at the laboratory, Dr Bala was already waiting and saw that she was provided with the obligatory lab coat and a visitor's badge. Dr Goodheart was then introduced to chief technologist Andile. Andile explained that the 'prepping' of some samples for flow cytometry is quite straightforward and includes the lysis of red blood cells (in whole-blood samples) and labelling of the white cells with specific fluorescent-dye-coupled monoclonal antibodies (see 'CD' – Box 1). Nathan's case (a possible CD40L-deficiency) was rather more complicated. It included dividing the sample and stimulating one part while leaving another part unstimulated. Andile had done the analysis already on the previous day with a multicolour flow cytometry instrument.

Box 1: 'CD' – Clusters of differentiation

Monoclonal antibodies identify specific cell surface molecules, called 'Clusters of differentiation' (CD).

Common CD markers include:

- **CD3**: Associated with the TCR; **all T cells**
- **CD4**: Co-receptor for MHC II; **T helper cells, monocytes, macrophages**
- **CD8**: Co-receptor for MHC I; **cytotoxic T cells**
- **CD14**: LPS/LBP receptor; **monocytes**
- **CD16**: FcγRIII; **neutrophils, NK cells, macrophages**
- **CD19**: Co-receptor for B cells; **B cells**
- **CD20**: A membrane protein; **B cells**
- **CD45**: Leukocyte common antigen; **leukocytes**
- **CD56**: Neural Cell Adhesion Molecule (NCAM); **NK cells**



In flow cytometry, cells are typically pre-treated with CD-specific, fluorescent monoclonal antibodies [Fluorescein isothyocianate (FITC) and Phycoerythrin (PE) are two common fluorochromes used.]

When they walked over to the flow cytometer, Dr Goodheart was a bit disappointed to see 'only' a white cupboard-sized 'box' (see Figure 2) connected to a computer. Various tubes appeared to feed liquids into the 'box'. Andile explained that flow cytometry is a system for analysing individual cells. The cells to be analysed are taken in through the sample injection port in the front. He continued to explain that in a flow cytometer such as this one, cells are forced through a nozzle in a single-cell stream that passes a laser beam (see Figure 2). Photomultiplier tubes inside the instrument detect light-scattering (cell size and granularity) as well as emissions from the fluorescent dyes for each cell. This information is then analysed in a central processing unit which is linked to the computer. Whereas some flow cytometers are equipped with a cell-sorting mechanism at the exit nozzle, the flow cytometer in Dr Bala's laboratory only analyses samples, without subsequent cell sorting (and is therefore strictly not a fluorescence-activated cell sorter, or FACS). Older flow cytometers could analyse only four different fluorescent labels simultaneously, whereas newer ones can analyse many more.

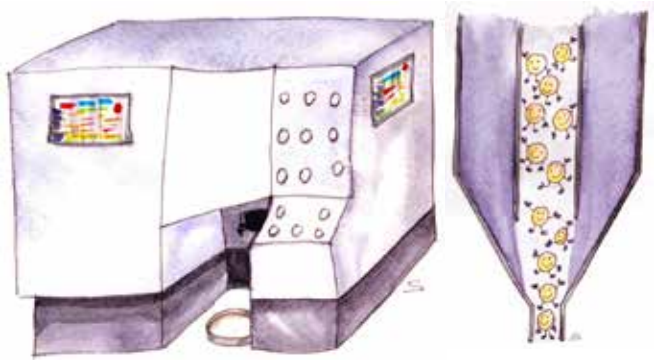


Figure 2: Flow cytometer

In a flow cytometer, cells are forced through a nozzle and pass through laser beams in 'single file' to be analysed individually.

Andile then called up yesterday's results on the computer screen. He explained that the 'little dots' on the computer screen represented single 'events', essentially positively fluorescent-stained single cells (see Box 2). As an operator, he had to 'gate', that is, define limits for data acquisition of cell populations. While there are different ways to show results, he used a so-called 'dual parameter plot' here (Box 2), which shows four quadrants. In this case, the right half represented T cells. The bottom half represented CD40L-negative cells. Only the stimulated control sample showed dots in the top right quadrant, that is, CD40L-positive T cells. The unstimulated sample as well as Nathan's stimulated sample showed only a few dots in this quadrant, representing less than 1% of total events gathered. This indicated that Nathan was missing CD40L on stimulated T cells.

Dr Bala and Dr Goodheart thanked Andile and walked back to Dr Bala's office. Dr Bala showed Dr Goodheart the report she created based on the flow cytometry results. This report omitted all the complicated details about the stimulation assay and indicated only that Nathan was negative for CD40L expression on stimulated T cells (<1 %). The results were entered into the laboratory information system (LIS) with authorisation by the senior technologist and comment by the pathologist before release and then stored on the hard-drive/database of the laboratory.

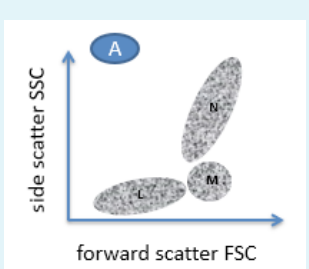
Box 2: Display and analysis of flow cytometry data (not specifically for HIGM)

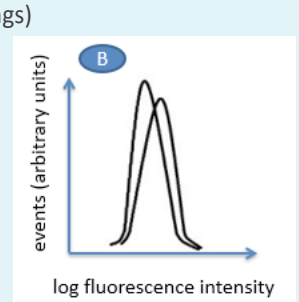
A flow cytometer collects data (parameters) for light scatter and fluorescence intensity, registering as '**events**'. The **forward scatter** FSC measurement is related to cell size, whereas the **side scatter** SSC is related to the internal granularity or complexity of a cell.

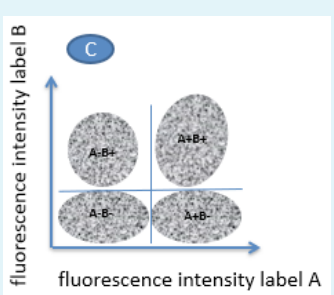
Cells can be separated from each other and from debris based on forward/side scatter alone (**A**). (L = lymphoctes; M = monocytes; N = neutrophils). If **fluorescence intensity** or light scatter is plotted in a single dimension against relative cell number, a **single parameter histogram** is obtained (**B**).

Modern flow cytometers allow **multi-parameter analysis**. (**C**) shows a **dual parameter plot** with four **quadrants** defining four distinct cell populations of interest.

(The figures A–C are schematic drawings)







Box 3: Flow cytometry in the analysis of primary immune deficiencies (PID)[3]

Antibody (humoral) defects

- B cell numbers CD19/CD20 (lack of CD19- and/or CD20-expressing cells in agammaglobulinemia [also transient effect of rituximab treatment – not a PID])
- Memory B cells (switched memory B cells in Common Variable Immunodeficiency, CVID)
- Bruton's Tyrosine Kinase (BTK) (X-Linked agammaglobulinemia)
- CD40-L (X-Linked Hyper-IgM Syndrome – this case!)

T cell defects

- T cell numbers (Absent or low T cells as in Severe Combined Immunodeficiency -SCID, combined immunodeficiencies, secondary immunodeficiencies)
- Naïve T cells and recent thymic emigrants - screening for SCID (together with TREC - T cell receptor excision circle testing on PCR)
- Memory T cells (SCID patients: not fully expressed (leaky) SCID, chimerism, engraftment as in transplantation; LOCID (Late-onset combined immunodeficiency) and 22q deletion syndromes (Di George/Catch-22 syndromes)
- T regulatory cells (T_{regs}) CD4+CD25+FOXP3+ - in autoimmune states and autoimmune syndromes with PID, for example IPEX (Immunodeficiency, Polyendocrinopathy, X-Linked disease)
- αβ- and γδ-T cells (Leaky SCID, Omenn's syndrome; definition of separate T cell lineages in severe immune deficiency states)
- HLA-DR (-) (Bare lymphocyte syndrome)
- Common γ-chain (X-Linked Severe Combined Immunodeficiency [XL-SCID])
- TH17 T cells (decreased TH17 in Hyper IgE Syndrome, HIES)

Lymphocyte proliferation tests

- to mitogens and recall antigens to test responsiveness to these

Neutrophil defects

- Neutrophil and monocyte oxidative burst (Chronic Granulomatous Disease- CGD)
- Leukocyte adhesion (CD11, CD18, one of the more common LAD defects)

Dr Bala elaborated that while only a genetic analysis could provide a definite diagnosis, flow cytometry still plays an important cost-effective role in the primary evaluation of immune deficiencies (see Box 3). The flow cytometry tests that are most often requested by independent practitioners are lymphocyte subsets/immunophenotyping, class-switched memory B cells and neutrophil oxidative burst. In Nathan's case, the absence of CD40L on T cells evaluated by flow cytometry makes a strong case for a diagnosis of X-Linked Hyper IgM Syndrome. CD40 expression defects on antigen-presenting cells can give a similar picture in females - with an autosomal recessive mode of inheritance.

Dr Goodheart was glad to have seen a real flow cytometer, although she still found the science behind it and the technical jargon quite confusing. She was also very relieved that the report she would receive later condensed all that into a simple result: CD40L expression-negative, indicative of HIGM syndrome.

With the diagnosis of X-Linked Hyper IgM Syndrome now made, she could plan specific recommendations for Nathan's management.

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Shaunagh Emanuel (illustrations Figures 1, 2 and 3)

The input of **Sylvia van den Berg** and **Cathy van Rooyen** (AMPATH) (Box 3) is kindly acknowledged.

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Special precautions: Pharmacokinetics of intranasal fluticasone furoate in patients with severe liver disease may be altered. Systemic effects of nasal corticosteroid may occur, particularly at high doses prescribed for prolonged periods. These effects vary between patients and different corticosteroids. The dose of intranasal fluticasone furoate should be reduced to the lowest dose at which effective control of the symptoms of rhinitis are maintained.[2]

References: 1. Berger WE, Godfrey JW, Slater AL. Intranasal corticosteroids: the development of a drug delivery device for fluticasone furoate as a potential step toward improved compliance. *Expert Opin Drug Deliv* 2007;**4**(6):689-701. **2.** AVAMYS Nasal Spray (Suspension) Approved Package Insert. GlaxoSmithKline South Africa (Pty) Ltd. 4 December 2008. **3.** Fokkens WJ, Jogi R, Reinartz S, *et al.* Once daily fluticasone furoate nasal spray is effective in seasonal allergic rhinitis caused by grass pollen. *Allergy.* 2007;**62**:1078-1084. **4.** Vasar M, Houle PA, Douglass JA, *et al.* Fluticasone furoate nasal spray: Effective monotherapy for symptoms of perennial allergic rhinitis in adults/adolescents. *Allergy Asthma Proc.* 2008; **29**(3): 313-321. **5.** Medical Design Excellence Awards. 2008 Winners and their Suppliers. Available from: http://www.devicelink.com/expo/awards/awards/index.php?catId=-1&year=2008&view=View. Cited 2017 March 26.

Trade marks are owned by or licensed to the GSK group of companies. S4 **AVAMYS** Nasal Spray (Suspension). **REGISTRATION NUMBER:** 41/21.5.1/0968. Each 50 µl spray contains 27,5 µg of fluticasone furoate. Preservative: Benzalkonium chloride 0,015 % m/m. **PHARMACOLOGICAL CLASSIFICATION:** A.21.5.1 Corticosteroids and analogues. **INDICATIONS: Adults/Adolescents (12 years and over): Treatment of the symptoms of seasonal allergic rhinitis:** Indicated in the treatment of nasal symptoms and ocular symptoms. **Treatment of the symptoms of perennial allergic rhinitis:** Indicated in the treatment of nasal symptoms. **Children (2 to 11 years):** Treatment of the symptoms of seasonal allergic rhinitis: Indicated in the treatment of nasal symptoms. Treatment of the symptoms of perennial allergic rhinitis: Indicated in the treatment of nasal symptoms. **CONTRA-INDICATIONS:** Hypersensitivity to the active substance or to any of the excipients of AVAMYS nasal spray. **WARNINGS:** If there is any reason to suppose that adrenal function is impaired, care must be taken when transferring patients from systemic steroid treatment to fluticasone furoate. **PREGNANCY AND LACTATION:** Safety and efficacy in pregnancy and lactation has not been established. **DOSAGE AND DIRECTIONS FOR USE:** For administration by the intranasal route only. Shake the nasal spray before use. For full therapeutic benefit regular scheduled usage is recommended. Adults/Adolescents (12 years and over): Recommended starting dosage is two sprays in each nostril once daily (total daily dose: 110 µg). Once adequate control of symptoms is achieved, dose reduction to one spray in each nostril (total daily dose: 55 µg) may be effective for maintenance. Children (2 to 11 years of age): The recommended starting dosage is one spray in each nostril once daily (total daily dose: 55 µg). Patients not adequately responding to one spray in each nostril once daily (total daily dose: 55 µg) may use two sprays in each nostril once daily (total daily dose: 110 µg). Once adequate control of symptoms is achieved, dose reduction to one spray in each nostril once daily (total daily dose: 55 µg) is recommended. Children under 2 years of age: There is no experience in **children under the age of 2 years. Hepatic Patients:** The normal adult dosage is applicable in mild to moderate hepatic impairment. There are no data in patients with severe hepatic impairment. **SIDE-EFFECTS AND SPECIAL PRECAUTIONS:** ***Side-effects:*** Clinical Trial Data: ***Respiratory, thoracic and mediastinal disorders:*** Very common: Epistaxis. Common: Nasal ulceration. **Special precautions:** Pharmacokinetics of intranasal fluticasone furoate in patients with severe liver disease may be altered. Fluticasone furoate has a negligible (0,50 %) systemic bioavailability at intranasal doses of up to 24 times the recommended adult daily dose. Systemic effects of nasal corticosteroid may occur, particularly at high doses prescribed for prolonged periods. These effects vary between patients and different corticosteroids. Treatment with higher than recommended doses of nasal corticosteroids may result in clinically significant adrenal suppression. If there is evidence for higher than recommended doses being used, then additional systemic corticosteroid cover should be considered during periods of stress or elective surgery. Fluticasone furoate 110 µg once daily was not associated with HPA axis suppression in adult, adolescent or paediatric subjects. However the dose of intranasal fluticasone furoate should be reduced to the lowest dose at which effective control of the symptoms of rhinitis are maintained. It is recommended that the height of children receiving prolonged treatment with nasal corticosteroids is regularly monitored. **KNOWN SYMPTOMS OF OVERDOSAGE AND PARTICULARS OF ITS TREATMENT:** Intranasal doses of up to 2 640 µg per day were administered over three days with no adverse systemic effects observed. Acute overdose is unlikely to require any therapy other than observation. All adverse events should be reported by calling the Aspen Medical Hotline number or directly to GlaxoSmithKline on +27117456000. For full prescribing information refer to the package insert approved by the medicines regulatory authority. © 2018 Aspen Group of companies or its licensor. All rights reserved. **HCR:** GlaxoSmithKline South Africa (Pty) Ltd. (Co. reg. no. 1948/030135/07). 39 Hawkins Avenue, Epping Industria 1, Cape Town, 7460. ZAF/FF/0001/15a(1)a 07/18

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