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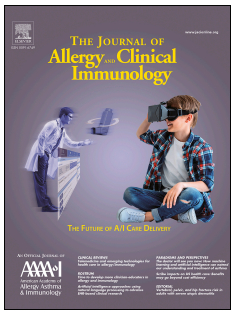

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Microbial exposures that establish immunoregulation are compatible with Targeted Hygiene

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Immunoregulation, microbiota, microbial exposures, hygiene, evolution, Th2-adjuvant, vaccine, hygiene hypothesis

Abbreviations
GI: gastrointestinal
RT: respiratory tract
Th2: T helper type-2 CD4+ T cell
LPS: lipopolysaccharide
BCG: Bacillus Calmette-Guérin
MHC: Major Histocompatibility Complex

Abstract

It is often suggested that hygiene is not compatible with the microbial exposures that are necessary for the establishment of the immune system in early life. However, when we analyse the microbial exposures of modern humans in the context of human evolution and history, it becomes evident that, whilst children need exposure to the microbiotas of mothers, other family members and the natural environment, exposure to the unnatural microbiota of the modern home is less relevant. In addition, any benefits of exposure to the infections of childhood within their household setting are at least partly replaced by the recently revealed non-specific effects of vaccines. This paper shows how targeting hygiene practices at key risk moments and sites can maximize protection against infection whilst minimizing any impact on essential microbial exposures. Moreover this targeting must aim to reduce direct exposure of children to cleaning agents since these probably exert Th2 adjuvant effects which trigger allergic responses to normally innocuous antigens. Finally, we need to halt the flow of publications in the scientific literature and the media that blame hygiene for the increases in immunoregulatory disorders. Appropriately targeted hygiene behaviour is compatible with a healthy lifestyle that promotes exposure to essential microorganisms.

Introduction

Microorganisms encountered in early life populate the microbiota, and provide data to expand and select lymphocyte clones, and molecular signals such as some forms of endotoxin and muramic acid derivatives that drive development of the innate and adaptive immune systems together with their crucial immunoregulatory control mechanisms (1-3). Faulty immunoregulation is at least partly responsible for the increased prevalence of chronic inflammatory disorders, such as allergies, autoimmunity and inflammatory bowel diseases that emerge as societies adopt Western lifestyles (4). It has been suggested that this faulty immunoregulation is attributable to distortion of early life microbial inputs by domestic hygiene practices (5). However hygiene in our homes and everyday lives is a life-saving strategy. In this paper we use the word hygiene to refer to practices which are used to prevent the spread of infection. The term cleaning will be used to refer to practices which are used to remove soil and dirt to produce a surface which is visibly/aesthetically clean using products containing materials such as surfactants, soaps, enzymes, oxidizing agents, acids or ammonia. This paper shows how the development of Targeted Hygiene enables us to modify hygiene behaviour so that it preserves essential microbial exposures while continuing to protect against infection. We reach this conclusion by combining an evolutionary approach with recent advances in our understanding of the roles of nonspecific effects of vaccines, and of a Th2 adjuvant effect of direct exposure to cleaning agents.

Evolution of homes and their microbiota

Which microbial inputs are necessary for health? Some of the organisms in the home are derived from the occupants, and others from the building itself. We can approach the latter by considering human evolution. Early humans lived in caves or shelters built with natural products such as stones, mud, branches and leaves. These shelters later evolved into houses constructed with the same natural products reorganised for human convenience. Walls were built with straw, timber, mud or stone and rendered with mixtures of straw, soil, clay and animal dung, while roofs were covered with thatch or turf. The microbiota of such a home would not differ greatly from that of the natural environment, and even when damp and deteriorating, the organisms present would be those with which humans co-evolved. In contrast, modern homes, built with synthetic products including biocide-treated timber, plywood, and synthetic gypsum board develop an unusual microbiota that bears little resemblance to that of the natural environment (6, 7). This difference is exacerbated if the

home is urban and remote from nature (8). Moreover when a modern home is damp and deteriorating, as homes low of Socioeconomic Status frequently are, its bacterial and fungal microbiota can produce secondary metabolites that are toxic to humans, resulting in various degrees of "Sick Building Syndrome" (9-11). It is therefore unlikely that this unnatural microbiota of the modern home is an optimal, or even a desirable microbial exposure for infants (Figure 1).

Microbiota of the natural environment that enters the home

When the unnatural microbiota of the home becomes more natural, and resembles that of farms and the natural environment, it is beneficial, at least where asthma and other disorders associated with faulty immunoregulation are concerned (2, 12, 13) (Figure 1). In support of this view, exposing children to biodiversity from the natural environment in their school playgrounds resulted in increases in peripheral blood biomarkers of immunoregulation (14). So evolutionary and epidemiological considerations point to the view that children need exposure to the microbiota of the natural environment, rather than to the unnatural microbiota of modern buildings (15).

Microbial molecular components in the home

At least some of the establishment of immunoregulatory mechanisms is driven by exposure to microbial components such as some forms of LPS or muramic acid derivatives (Figure 1), rather than to specific organisms (1). For example, LPS entering the airways drives expression of *TNFAIP3*, the gene that encodes A20, an immunoregulatory protein that limits several inflammatory pathways (1, 16). Interestingly, a detailed study of the impact of cleaning and hygiene practices in the home found that exposure to endotoxin and muramic acid was associated with protection from allergies in children, and that this exposure was not reduced to ineffective levels by cleaning. In fact, in this study, it was found that neither hygiene interventions (such handwashing and laundering of personal towels) nor home and personal cleanliness had any impact on the development of the allergic disorders (17).

Microbiota of human origin in the home

The microbiota of the modern home is also enriched in microbiota of human origin (6). Mother-to-infant (and sibling-to-infant) transfer of microbiota is crucial for the development of the infant's microbiota, as well as for development of the immune and metabolic systems (18) (Figure 1). But the major lifestyle factors that reduce this transfer and correlate with increased immunoregulatory disorders are caesarean deliveries, lack of breast feeding, and lack of mother-baby intimacy (18-20), (together with antibiotic use and poor diet which fall outside the scope of this discussion). Some components of the child's microbiota appear later in infancy and are still accumulating at 5 years of age (21). These organisms must be picked up from the father and other family members, and from children and personnel at day-care centres as well as from the natural environment. Studies of social networks have demonstrated person-to-person transmission of microbial strains both within and outside the home (22, 23). These findings suggest that the transfer occurs mostly via normal social and mother-infant interactions, rather than via exposure to human-derived strains which are shed into the home environment.

"Crowd infections" in the home do not protect against allergies

But what about pathogens, rather than microbiota? The 1989 hygiene hypothesis suggested that mothers and siblings help to expose the infant to the common infections of childhood and that lack of such exposures due to improved household amenities and cleanliness contributes to the increase in allergic disorders (5). However, the common infections of childhood are mostly "crowd infections" that were not present during most of human evolution (24). Therefore it is unlikely that humans are in a state of evolved dependence on such infections. In support of this, epidemiological studies have failed to find evidence that they protect against allergies (25-27). A possible exception to this is *Helicobacter pylori* which has been endemic in human populations for millennia. There is some evidence that this infection primes immunoregulatory pathways and protects against allergic disorders but its incidence has fallen dramatically so that exposure to *H. pylori* is no longer a relevant variable (28). Thus, hygiene measures that protect against the common infections of childhood have little to do with the immunoregulatory disorders responsible for the massive clinical problem that we are discussing here.

Could exposure to pathogens induce non-specific cross protection against other infections?

Some members of the public believe that we need exposure to infections to "keep our immune systems strong". This concept may have some validity. It has been known since the 1930s that some pathogens (if you survive them) induce protection against other unrelated infections (29). So although the common infections of childhood do not protect from the immunoregulatory disorders that are a major theme of this essay, they might prime non-specific resistance to other infections. However exposure to potentially lethal infections such as measles must be regarded as a very risky strategy for obtaining protection from other infections. Moreover new data outlined below suggest that this function of non-specific priming of the immune system is now exerted safely by vaccines.

Vaccines can replace nonspecific effects of infections

In the 1980s it began to be reported that vaccination with a live measles vaccine in Africa reduced overall childhood mortality to a degree that could not be explained by the incidence of measles itself. By the early 2000s the same claim was being made for BCG vaccination, and multiple studies have led to the conclusion that several live vaccines (measles, polio, smallpox, BCG) enhance resistance to unrelated infections in children (30, 31), but similar effects may be seen in adults. A recent clinical trial confirmed that BCG vaccination protects the elderly from probable virus infections (32). This may explain why treating latent tuberculosis in non-HIV-infected individuals reduces the incidence of tuberculosis, but fails to provide an overall survival benefit because of increased mortality from other causes (33). These non-specific and cross-protective effects are mediated by components of the innate immune system including natural killer (NK) cells and monocytes (34), and involve epigenetic changes in haematopoietic stem cells (34, 35). The non-specific effects of vaccines are similar to the non-specific survival benefits seen after recovery from the corresponding infections (36). Such recovery is more likely following low dose infection, so good ventilation to keep the infectious dose low should be encouraged. Thus vaccines might replace non-specific benefits of clinical infections, and if they do, this obviates any justification for relaxing hygiene standards to provide this protective effect (Figure 1). These non-specific protective effects of vaccines are seen in low income countries, but also in wealthy countries such as Denmark, Italy, The Netherlands and the USA (30, 31).

Direct effects of cleaning products on human health?

Over the years the amounts of cleaning agents purchased for home cleaning have risen steadily (37). Studies carried out to determine whether use of these products in the home correlates with an increase in chronic inflammatory disorders have yielded conflicting results. We provide two typical examples. A longitudinal study of 14,541 pregnancies and the resulting offspring ongoing since 1990 found that exposure to high levels of personal hygiene (high frequency of hands and face washing, and bathing and showering) at 15 months of age was associated with wheeze and atopic eczema between 30 and 42 months (38). By contrast, the detailed study quoted above found that neither hygiene interventions nor home and personal cleanliness had any impact on the development of allergies in children (17). Conflicting data such as these may be attributable to the fact that cleaning products are relevant for two entirely separate reasons, one of which has nothing to do with microbial exposures (Figure 2). The cleaning products might indeed act by reducing human exposure to the microbiota of the home, but recent findings suggest that they might also exert a Th2 adjuvant effect that predisposes the immune system to an allergic response. Repeated exposures to cleaning and disinfectant agents such as detergents and quaternary ammonium compounds, as experienced every working day by cleaning personnel, are linked epidemiologically to asthma in adults, especially when used as sprays (39). These agents are not only toxic to cells (40), but also increase epithelial permeability (41). Moreover many products contain potential allergens such as enzymes, so that exposure to these agents may increase the risk of allergic responses to extraneous allergens, but also to the allergens contained within the product itself. Could inhalation of these agents be affecting children? Interestingly the UK cohort quoted above (38), where personal hygiene was associated with wheeze and atopic eczema, also revealed that use of chemical household products was inversely associated with socioeconomic status and correlated with low educational level, smoking, and poor, crowded housing (42). In such households infants, especially if crawling on floors, might inhale sufficient toxic cleaning agents to exert physiological effects, including Th2 adjuvanticity (Figure 2).

Cleaning products as Th2 adjuvants

Mild cytotoxicity can lead to Th2 adjuvant properties. Eight different commercially available adjuvants were combined with an influenza vaccine and administered to mice by intranasal injection. Then, within 24 hours of this challenge, levels of double-stranded DNA in bronchoalveolar lavage were measured as a correlate of host cell death. Interestingly, 3 of the vaccines tested (Alum, AddaVax [an oil in water emulsion] and SiO2 nanoparticles) caused very significant release of host DNA and elicited potent Th2 responses but little Th1 (43). Previous work had shown that DNA released by cell death in response to aluminium adjuvant enhances MHC Class II mediated antigen presentation, and prolongs interaction of dendritic cells with CD4 T cells (44), suggesting that local cytotoxicity initiated by the adjuvant and release of DNA are an integral part of the Th2 adjuvant's mode of action. Interestingly this notion that mild local cell damage might exert Th2 adjuvant effects has been suggested in relation to both airway and gut allergies (40, 45). For example, antigens in food usually evoke tolerance, but if detected by the immune system in the gut in the context of a cytotoxin, an allergic Th2 response may be generated (Figure 2) (40). In effect, the food antigen is being used as a proxy for recognition of the cytotoxic molecule (which might not itself be immunogenic), and will evoke an allergic reaction in the future even if the cytotoxin is not present. Thus the conflicting data on the effects of exposure to cleaning agents on the incidence of allergic disorders might be explained if these agents exert two entirely unrelated influences on the developing immune system (restricting microbial exposures, and Th2 adjuvanticity).

Targeted hygiene: preventing infection whilst allowing essential microbial exposures.

By summarising the arguments in the previous sections (as in Figure 1) it can be seen that the microbiotas to which a modern infant needs to be exposed are the microbiota of the mother, and the microbiota of the natural environment, supplemented by vaccines. Home hygiene therefore should, as far as possible, avoid reducing human contact with these organisms, while targeting key moments and sites that are most likely to cause transmission of infections, and other microorganisms such as toxic fungi that sometimes contaminate deteriorating modern homes (Figure 1). It also shows why we need to restrict exposure of children to the cleaning agents themselves because they may act as Th2 adjuvants.

At what human activities should hygiene measures be targeted?

Since 1997 the International Scientific Forum on Home Hygiene and partners have exploited evidence on how infections are transmitted to develop the concept of Targeted Hygiene that is focused on the times and places that matter most (Table 1) (46, 47). This is based on risk management approaches developed and used by the food and pharmaceutical industries since the 1960s to control microbial risks. By observing behaviour and using microbiological data it is possible to identify 9 key moments during our daily lives when hygiene can break the chain of infection (47, 48). Although these are not the only moments when hygiene practices are needed, it is argued that focussing on these moments will deal with most of the risk of spread of infection in our homes, other than that which is airborne.

At what surfaces should hygiene practices be targeted?

During these 9 moments, hygiene measures need to focus on the surfaces most likely to spread infection (Table 1). Risk assessments suggest that the surfaces most often involved at key moments (called critical control points) are the hands, together with hand and food contact surfaces, and the cleaning utensils used to decontaminate surfaces. Other surfaces which can be involved in spread of infection are clothing, towels and household linens, together with contact surfaces of sinks, baths, showers and toilets (47). In the last 20 years increasing access to quantitative data on transmission of infections in living environments together with the development of Quantitative Microbial Risk Assessment have enabled us to combine cleaning (dry wiping or cleaning with detergent and rinsing with clean water) and microbicidal processes (heat, disinfection) more precisely to produce a sufficient reduction in level of contamination on risk surfaces (49). Tailoring hygiene procedures in this way minimises both the impact on necessary microbial exposures and the use of cleaning products.

Hygiene practices that are not useful and do not involve the 9 critical moments

Based on Risk assessment, floors and other general environmental surfaces in home settings are generally regarded as low risk when it comes to infection transmission, because they are rarely contaminated with harmful microbes and they are not “critical contact points” in close contact with household members at the key moments (Table 1). (There are of course

exceptions to this, for example when the floor becomes contaminated with vomit or faeces, or when a crawling child is playing in the same floor area with a family pet). Studies in home settings show that cleaning and disinfection reduce the microbial load on treated surfaces, but the microbial levels are restored within a couple of hours (50). Non-targeted routine daily cleaning carried out in the mistaken belief that it gives protection against infection may have adverse impacts on the immune regulatory system (Table 2), and increase exposure of crawling infants to cleaning products that may have Th2 adjuvant properties.

Halting the flow of misinformation

As suggested in a previous 2016 review (51), if we are to get the public to adopt targeted hygiene behaviour we need to halt the misrepresentation of "hygiene" as an inevitable cause of immunoregulatory disorders. Such misrepresentation is widespread in the media and in the medical literature (52). We must discourage suggestions in the media or published articles that we should relax hygiene standards, and ensure that such statements are replaced by instructions for intelligent use of Targeted Hygiene (53). Similarly we must stop the flow of research publications which refer to intensified non targeted cleaning strategies as "intensified *hygiene* measures". Microbial risk assessment shows that intensified strategies i.e involving cleaning and disinfection of floors etc is a valid part of hygiene strategies in controlled environments such as hospital intensive care units and isolation rooms (54). However when applied in public open spaces these are not seen as hygiene measures at all because they contribute little to preventing the spread from the major sources of infection which are people, food and domestic animals.

The response to the 2020 COVID-19 pandemic has illustrated the failure to distinguish between cleanliness and hygiene. Despite attempts to promote a Targeted Hygiene approach (hands, face, space), people still practice untargeted "deep" or "intensified" cleaning (Table 2) as do facility managers of public spaces with the belief that this will make the space "COVID secure". In Table 2 we list several examples of what can only be described as "Hygiene Theatre" (55, 56). These are ostentatious measures aimed at publicity and at giving peace of mind. In reality, facility managers need to concentrate on targeted measures such as organising how the public is moved about, seated, and provided with easy access to hand sanitisers in situations where there is not ready access to handwashing facilities to encourage

315 them to practise Targeted Hygiene not only in their homes but also in their daily lives in
316 public spaces.
317

318 ## Conclusions

319 We conclude that if we are guided by evolutionary and historical knowledge we can identify
320 the microbial exposures that are most essential to human physiology. We also conclude that
321 this understanding, in the context of 21st century reality, is increased further when the
322 recently revealed non-specific benefits of vaccines, and probable Th2 adjuvanticity of
323 cleaning agents are taken into consideration. Using this understanding we can be guided by
324 modern microbiological risk assessments that identify critical moments and we can reconcile
325 these physiological needs for microbial exposures with appropriate hygiene practices (which
326 may involve not only targeted cleaning of hands and surfaces but also social distancing and
327 mask wearing to prevent airborne transmission) that minimise the risks of infection, and
328 minimise unnecessary exposure to cleaning agents.
329 We are fully aware that there is an element of speculation in these conclusions. We cannot
330 be sure that vaccines fully replace the nonspecific immune-system boosting effects of
331 infections, and we do not know the relative importance of the Th2 adjuvant effects of
332 cleaning agents. However we hope that we provide, as summarised in Figure 1, a framework
333 for a more nuanced discussion of how we can reconcile hygiene with healthy immune
334 systems.
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Table 1. The key moments for hygiene that are essential components of Targeted Hygiene

Situations: The 9 moments when hygiene really matters	**Sources:** Determine types of microbes	**Organisms** most likely to be spread from these sources at these moments	**Surfaces** most likely to spread infections at key moments such that people become exposed and infected
• Food handling	Food People	GI pathogens from food GI pathogens from gut: Faecal/oral transmission via hands and surfaces RT pathogens from gut (unlikely but not impossible; e.g SARS found in sewage & faeces)	Hands Surfaces contacted by hands and food
• Eating with fingers	People	GI pathogens Faecal/oral via hands to food	
• Using the toilet	People	GI pathogens: Faecal/oral via hands and hand contact surfaces RTs via hands and hand contact surfaces in toilet areas	Contact surfaces of sinks, baths, showers
• Changing a baby's nappy/diaper	Baby	GI pathogens from babies gut	Clothing, towels, household linen
• Coughing, sneezing, nose blowing	People	RT pathogens via hands and surfaces and airborne routes	
• Touching surfaces frequently touched by other people	People	GI pathogens: faecal oral via hand contact surfaces and hands RT pathogens: person to person via hands and hand contact surfaces	Cleaning utensils used to decontaminate surfaces
• Handling clothing, towels, bed linen	People	GI pathogens, RT and skin pathogens	
• Caring for domestic animals	Domestic animals	Zoonotic pathogens: Salmonella, Campylobacter, Cryptosporidium, Toxoplasma, Toxocara	
• Handling and disposing of rubbish	People, food, animals	GI and RT infections via hand contact surfaces and hands	
Caring for infected family members	People	The same 9 moments for hygiene apply, the difference is that, failure to comply with hygiene practices carries a higher risk of spreading infection to others	

Typical gastrointestinal (GI) pathogens: Salmonella, Campylobacter, Listeria, norovirus
Typical respiratory tract (RT) pathogens: cold and influenza viruses, coronaviruses, Legionella
Typical skin and mucous membrane pathogens: *Staphylococcus aureus* (including methicillin resistant *S. aureus*), Tinea, *Candida albicans*

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555 **Table 2. Strategies that are not useful – and could be harmful**
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"Hygiene Theatre" - Attempts to "sterilise" floors & other general environmental surfaces - Deep cleaning, and fogging of entire premises - "Disinfecting tunnel" which claims to disinfect people entering facilities such as sports stadia - In many countries, spraying and fogging of open spaces such as streets & metro stations	
Harmful microbes likely to be present	
Harmful microbes (GI, RT, skin) are sometimes found on these surfaces – but low frequency, and low numbers Most harmful microbes do not survive for long time periods (exceptions e.g Multi-resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile*, norovirus, cold viruses) so infectious numbers usually low	Exposure and infection are unlikely. We rarely touch these surfaces with hands. There is no good vector

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Figure 1. Microbial communities to which hygiene should, and should not be targeted. Appropriate development of the immune system and its immunoregulatory mechanisms can be driven by the microbiota from mother (and siblings) and from the natural environment, supplemented by the non-specific effects of vaccines. Targeted Hygiene avoids reducing these exposures, and also avoids exposing the child to the cleaning agents which may have Th2 adjuvant properties (explained and referenced in Figure 2), while reducing exposure to infections and to harmful contaminants of deteriorating modern homes. There is, of course, some overlap between the microbial communities.

Figure 2. Antigens presented to mucosal surfaces in the presence of toxic molecules may become allergens. Antigens entering the gut or airways usually induce tolerance. However in the presence of a toxin they can be associated with cell death, DNA release, and Damage-associated molecular patterns (DAMPs) that activate the immune system. Adjuvants that activate Th2 responses often cause cell death (40, 43-45).