Epithelial Barrier Hypothesis and Its Comparison with the Hygiene Hypothesis

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Abstract: Chronic inflammatory conditions including allergic, autoimmune, metabolic, and neuropsychiatric disorders are constantly increasing and leading to a high burden, especially in more industrialized countries. The prevalence is still on the rise in developing countries. The start of the steep increase in asthma, atopic dermatitis, and allergic rhinitis dates to the 1960s, whereas a second wave with an increase in eosinophilic gastrointestinal disease, food allergy, and drug hypersensitivity started after the 2000s. These diseases also started to appear more with neuropsychiatric and autoimmune conditions during the last few decades. Many theories have been proposed to explain this outbreak. The hygiene hypothesis was consolidated by ”old friends” and biodiversity, although some gaps remained unresolved. The introduction of the epithelial barrier hypothesis gave us a new perspective to explain the effects of industrialization without environment control and health concerns creeping into our daily lives. The present review touches on the possible explanations of why epithelial barrier hypothesis covers all previous ones, which are not contradictory but mostly complementary.

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REVIEW
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Epithelial Barrier Hypothesis and Its Comparison with the Hygiene Hypothesis

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ABSTRACT

Chronic inflammatory conditions including allergic, autoimmune, metabolic, and neuropsychiatric disorders are constantly increasing and leading to a high burden, especially in more industrialized countries. The prevalence is still on the rise in developing countries. The start of the steep increase in asthma, atopic dermatitis, and allergic rhinitis dates to the 1960s, whereas a second wave with an increase in eosinophilic gastrointestinal disease, food allergy, and drug hypersensitivity started after the 2000s. These diseases also started to appear more with neuropsychiatric and autoimmune conditions during the last few decades. Many theories have been proposed to explain this outbreak. The hygiene hypothesis was consolidated by "old friends" and biodiversity, although some gaps remained unresolved. The introduction of the epithelial barrier hypothesis gave us a new perspective to explain the effects of industrialization without environment control and health concerns creeping into our daily lives. The present review touches on the possible explanations of why epithelial barrier hypothesis covers all previous ones, which are not contradictory but mostly complementary.

Keywords: Allergy, autoimmune disorders, epithelial barrier hypothesis, hygiene hypothesis, industrialization

INTRODUCTION

Allergic diseases have been increasing for decades and represent an enormous psychosocial and economic burden. Development of novel medical techniques, in addition to therapeutic advances, has broadened our perspectives and diagnostic capabilities. There is still lack of clear knowledge about the exact pathogenesis of allergic, autoimmune, metabolic, and neuropsychiatric diseases, particularly the steep increases in their prevalence. Since the emergence of allergic diseases, many explanations have been proposed which are described below in comparison.

THE MATURATION OF THE ALLERGY IDEA

At the end of the nineteenth century, the "immune system" was first described by scientists such as Louis Pasteur, Paul Erhlich, Elie Metchnikoff, Jules Bordet, and Emil Von Behring.[1] The fundamental definitions had focused on the protection of the body, and no one could imagine that it could hurt itself. However, with the discovery of antitoxin treatments and vaccines, physicians documented some "reactions" due to these treatments. Clemens von Pirquet was one of the few who had suspected a connection between the immune system and these adverse reactions[2] and came up with the idea that the immune system could damage the host itself.

In the same year, the French immunologist Nicolas Maurice Arthus published his experiment describing local reactions after the injection of horse serum, which became more and more

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severe when repeated.[3] With the description of serum sickness and the precipitating antibodies, knowledge of these reactions was expanded. Meanwhile, the first terminology for "allergy" was proposed by von Pirquet in 1906; it was defined as altered reactions of the body to foreign substances that get severe upon subsequent exposures.[4]

THE ALLERGY EPIDEMICS

Besides the death of Pharaoh Menes due to bee sting, the first "realistic" description of an allergic disease was made by Charles Blackley in 1873 as hay fever.[5] With the arrival of "hay fever," the seasonal association with different pollens in different locations was defined, and this has been proposed to be linked to extensive lawn making. In addition, the increase in arable farming in the USA and many other countries led to a large-scale spread of ragweed.[6]

Furthermore, public hygiene gained momentum soon after sewage and intestinal diseases became known. At the end of the nineteenth century, drinking water was separated from sewage. By 1920, chlorination of water became widespread.[6]

The increase in hay fever in the 1940s led to the first attempts at immunotherapy against grass pollen. With regard to asthma, increased numbers of reports showing high numbers of cases point to the 1960s. Many of these children were found to be allergic to house dust mites.[7] Increasingly, denser and warmer homes with more carpeting and indoor activities were blamed.[8] During the 1960s, 2 studies from Sweden[9] and Canada emphasized that living in urban sites was more frequently associated with allergic disorders than populations living in rural sites.[10] In the early 1960s, it was noticed that asthma suddenly increased in almost every age group in England.[7] In 1961, asthma increased in Finnish soldiers.[11] From 1965 to 1980, there was a 10-fold increase in the number of hospitalized children with asthma in Australia, England, New Zealand, Canada, and the USA.[12] Between 1971 and 1981, it was found that asthma increased by 3-fold in Swedish soldiers, especially in those who came from the cities.[13] When East and West Germany were united, the prevalence of asthma and atopic dermatitis (AD) was very low in the East in 1990. They have caught up with the West within 10 years (5-10 times increase).[14,15] An increase in AD was similarly reported after the 1960s. The frequency differed from 1.1% to 3.1% among the population born before 1960,[16,17] whereas an increasing trend was suggested by tabulating individual year groups after the 1960s, reaching 12% in 1974.[18]

HYGIENE HYPOTHESIS

In 1989, David Strachan came up with the idea of "hygiene hypothesis" to explain the increase in allergic disorders. He suggested that the changes in the microbial environment would shape the development of the immune system. It was hypothesized that fewer infections would cause a shift toward allergic responses. Recurrent microbial exposure would initiate T-helper 1 (Th1) response rather than a Th2-mediated immune response associated with elevated interleukin-4 (IL-4) and IL-5 levels and eosinophilia.[19] Rook's "old friends hypothesis" almost supported this idea by arguing that infectious diseases evolve with human body, and adequate exposure is necessary for prompt development of the immune system.[20] Further "Alpine farm studies" reflected the allergo-protective effects of traditional farming habits such as close contact with farm animals and unprocessed milk consumption.[21,22] All in all, it was convincing that the more diverse the microbial environment, the better the immune system functions. The study "Prevention of Allergy Risk factors for Sensitization In children related to Farming and Anthroposophic Lifestyle (PARSIFAL)" then showed that this link is already established during pregnancy.[23] Maternal exposure to a diverse microbial environment, such as is present in farming activities, was associated with lower atopic sensitization in the offspring. This kind of exposure modulated allergen-specific responses toward a Th1 pattern.[24] The concomitant increase in autoimmune disorders in Westernized populations has been explained by the need for a microbial environment to fine-tune the Th1 and Th2 responses.[25]

The mechanism underlying the hygiene hypothesis consists of elements of the innate system such as Toll-like receptors (TLRs). After encountering bacterial products such as muramic acid and endotoxin, TLRs relay the microbial signals to the immune system and also to regulatory T cells. In short, it was clear that TLR2 and TLR4 ligands were protective in allergen-induced lung inflammation. The same protective functions were also attributed to TLR9 in mouse models.[26] After recognition of bacterial endotoxins, Th1 cells can exert their protective function in several ways: inhibiting respiratory tract damage by antiviral defenses and reducing the abnormal repair mechanisms responsible for mucosal and smooth muscle hyperplasia. Excessive endotoxin exposure could also be harmful, as is the case with occupational asthma. In this context, it appears that the dose and timing of exposure are critical to the subsequent response.[27] The biodiversity hypothesis endorsed the hygiene hypothesis. Briefly, the greater the diversity of microbial species in a given space, the less dominant their existence, and consequently the immune system balance is maintained. Similar to hygiene hypothesis, it supports the idea that more contact with natural environments would enrich the microbiota.[28]

In recent decades, several shortcomings of the hygiene hypothesis, the old friends hypothesis, and the biodiversity hypothesis have been discussed, suggesting that these hypotheses do not fully explain the rise in allergic and autoimmune diseases. These include the fact that water sanitation was introduced in many Western cities in the 1920s, but allergy and asthma epidemics did not begin until the 1960s. The protective role of parasite infections in increasing biodiversity has been questioned for the same reason. Moreover, allergic asthma is still increasing in some Asian and African cities with low hygiene standards.[29] Another pitfall of the hygiene hypothesis is that it does not seem to protect against allergic diseases, despite the increase in respiratory diseases and measles.[30] Moreover, allergic diseases are not uncommon in rural Africa, where children are exposed to a traditional, unhygienic lifestyle.[30]

Another limitation of the hygiene hypothesis and the biodiversity hypothesis is that probiotics are not viable alternatives for the prevention or treatment of allergies.[31] Moreover, studies of migrants moving from developing countries to affluent regions show a rapid increase in asthma and allergic diseases, as well as autoimmune diseases such as type 1 diabetes and multiple sclerosis.[32-34] It appears that home living conditions, the

increase in births by cesarean section, antibiotic use, dietary habits, urbanization, and indoor air pollution are more important factors than general public hygiene.[35-37]

The protective role of growing up on a farm on the development of asthma and allergies has received the most attention in this context, and a substantial number of studies have supported the initial findings.[38] For example, children in Amish communities in the USA where traditional dairy farming is practiced have been found to be highly protected from asthma and allergies.[39] In contrast, Hutterite communities practice industrialized agriculture with extensive cleaning practices and have a significantly higher prevalence of asthma and allergies in children.[39] The Amish community uses homemade detergents and cleaning products whose main ingredient is washing soda (Na_2CO_3) and does not use commercial cleaning products that may contain barrier surfactants and enzymes.

EPITHELIAL BARRIER HYPOTHESIS

The first links between the epithelial barrier and inflammatory diseases were established in the early 1990s with the description of a disrupted intestinal barrier in celiac disease and inflammatory bowel disease.[40] Later, this was also demonstrated for other diseases such as asthma, AD, chronic rhinosinusitis, and eosinophilic esophagitis. The mechanism underlying epithelial barrier hypothesis is that disrupted epithelia are prone to bacterial leakage and dysbiosis. Therefore, bacterial translocation leads to inflammation in the adjacent tissue.[41-43] It has been suggested that this could have different consequences: either local pathologies as in AD or triggering chronic metabolic or autoimmune diseases such as diabetes or obesity and neurodegenerative disorders.[41-43]

In addition to known allergens and pathogens, various toxins we encounter daily can also cause epithelial damage. Air pollutants such as smoke and diesel exhaust are well described, but substances we use for hygiene measures can also hide in cleaning products or even in personal hygiene products (Table 1).

The hygiene and epithelial barrier hypotheses overlap with the increase in cleaning products such as detergents and also air pollution as a result of industrialization. Even exposure to highly diluted laundry and dishwasher detergents has been shown to upregulate genes involved in oxidative stress and cell survival.[44,45] On the contrary, genes involved in wound healing appear to have been downregulated in response to laundry detergents.[44] It is now clear that some of the most harmful toxins, namely surfactants and emulsifiers, in detergents and processed foods, respectively, are part of our daily lives (Table 2). These chemicals have been overused in parallel with the increase in allergic and autoimmune diseases.[46,47] Besides toxins, it is well known that proteolytic allergens such as house dust mites may cause epithelial barrier defects by cleaving the tight junctions.[48]

The epithelial barrier, with its physical, chemical, and immunological properties, is the first line of defense of the innate immune system. It mainly lines the intestine, skin, urogenital system, and respiratory tract. The epithelial cells are tightly bound to each other with tight junctions and are well organized with the contribution of mucus and microbiota. Their immunological functions include the clearance of particles and the activation of the immune cells by the production of antimicrobial peptides and cytokines. In addition to its antimicrobial action, it is also essential for a prompt tissue repair. Once the epithelial barrier is impaired, in addition to tissue injury, an inflammatory state occurs and exacerbates epithelial damage.[49] Healthy tight junctions prevent the entrance of foreign substances, while a disrupted barrier allows passage from both sides, either by the outflow of immune cells from the subepithelium to the surface or by the translocation of microbiota to deeper tissues. The latter can lead to inflammation due to colonization by opportunistic pathogens (Figure 1). Consequently, an inflammatory microenvironment disrupts epithelial barrier and regeneration from epithelial stem cells. These sequential

Table 1. Experimental Models of Barrier Disruption

Substance	Evidence
Anionic surfactants and commercial detergents	Cultures of human skin keratinocytes show that anionic surfactants and commercial detergents reduce the integrity of the tight junction barrier[54,74]
Cigarette smoke	Mouse models show that cigarette smoke causes acute lung damage[46]
Detergent residue	Cultures of human bronchial epithelial cells at the air–liquid interface show that detergent residues disrupt the integrity of the tight junction barrier in human bronchial epithelial cells even at low concentrations[44]
Diesel exhaust particulates	Human and rat alveolar epithelial cells exposed to diesel exhaust particles exhibit low occludin expression and a leaky barrier[53]
Emulsifiers in processed food	Emulsifiers increased damage to hamster small intestine structure in vivo and the translocation of *Escherichia coli* across M-cells in vitro.[47,75] It has been shown in rat models that food emulsifier polysorbate 80 decreased the expression of proteins related to mucus barrier and mucosal barrier in the intestine, changed the integrity of intestinal epithelial cell, and increased the permeability of intestinal epithelial mucosa[76]
Nanoparticles	Human cell cultures show that nanoparticles disrupt gut barrier homeostasis[77]
Ozone	Mouse models show damage to the airway barrier by ozone[78]
Particulate matter	Ex vivo experiments with human and rat alveolar epithelial cells show that particulate matter affects occludin distribution and the alveolar barrier. Particulate matter 2.5 causes defects in the nasal epithelial barrier in noninflamed nasal biopsies from patients with sinusitis. Particulate matter 10 stimulates myeloid dendritic cells to induce Th17 cells in vitro with the property of migrating to the brain.[53-55] Mice exposed to particulate matter showed epithelial barrier dysfunction and an increase in eosinophilic inflammation in the sinonasal airways.[79]
Polystyrene microplastic	Mouse models show the effect of polystyrene microplastics on the intestinal barrier[80,81]

Table 2. Comparison of Hygiene and Epithelial Barrier Hypotheses

Hygiene Hypothesis	Epithelial Barrier Hypothesis
Water sanitation started in the 1920s but allergy epidemics only started in the 1960s	This correlates with the general use of everyday substances
Lack of rationale for increased Th1 response	Disruption of the barrier may contribute to both Th2 and Th1 responses
Increased allergy prevalence even in countries with low hygiene conditions	Allergic diseases are less pronounced in communities using less toxic substances
Probiotics does not prevent allergic disorders	Evidence is shown by in vitro models

Th1, T-helper 1, Th2, T-helper 2.

events can lead to a local or systemic inflammatory state that may be causative for many immune-related disorders.[50] Local epithelial damage in the skin and mucosa can lead to type 2 inflammation, which manifests as AD, asthma, allergic rhinitis, and eosinophilic esophagitis. The changes in the microbiome due to leakage of the epithelial barrier can trigger autoimmune processes in the gut.[51]

In several autoimmune diseases, a link between disruption of the epithelial barrier in the intestine or lung and inflammation in distant organs has been demonstrated. For example, an association between intestinal barrier disruption and inflammation in distant organs was recently found in a mouse model of arthritis. In this study, Th1 and Th17 effector T cells accumulated in the lamina propria of leaky gut and migrated to affected joints, where they triggered pathology.[52] Similarly, barrier disruption due to environmental exposures, such as particulate matter in the lungs, can trigger inflammation in distant organs in multiple sclerosis.[53-55] In a mouse model of multiple sclerosis, the disease was induced by intra-tracheal administration of the autoantigen myelin basic protein in combination with a barrier-damaging adjuvant. Autoantigen-specific effector T cells were shown to be "licenced" in the airways to migrate to the brain, where they caused multiple sclerosis-like inflammation.[56]

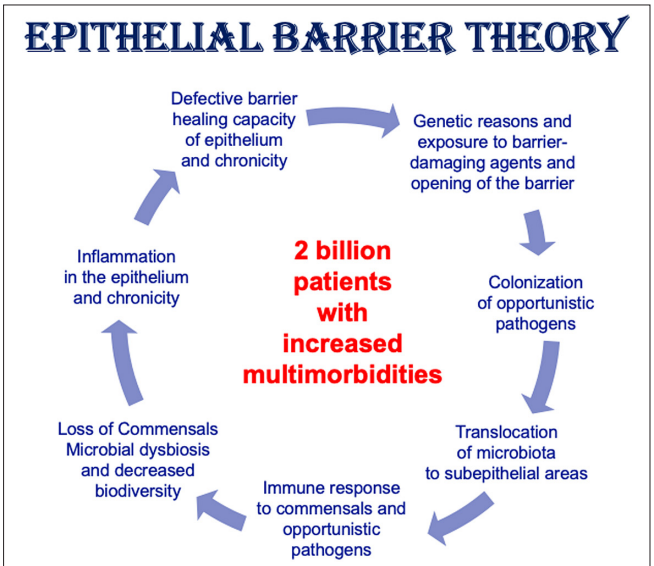

Figure 1. Exposure to barrier degraders or genetic deficiency of barrier molecules leads to colonization by opportunistic pathogens and epithelial inflammation.

Dendritic cells, macrophages, innate lymphoid cells (ILCs), T cells, and their cytokines interact with stem cells in the chronic inflammatory environment and are critical for both damage and regeneration of mucosal epithelial barriers.[57-58] B cells, mast cells, eosinophils, type 2 ILCs, and Th2 cells are also usually involved in the response to the translocated microbiome.

Impaired epithelial barriers are in parallel to the development of extensive immune response against harmless environmental agents. Increased immunoglobulin G (IgG) and IgE responses to allergens, documented from the 1970s to the present, link disrupted epithelial barriers and demonstrate that antigens are reaching deeper tissues: In the 1970s and 1980s, healthy individuals occasionally showed IgG responses to environmental antigens.[59,60] However, after the 1970s, there was an increase in allergen-specific IgE and IgG levels to environmental antigens.[60-63] Comparing frozen serum samples from 1998 with those from 1990, the 1998 samples had more allergen-specific IgE, even when analyzed with the same assay.[63] In 2015, 49.8% of Norwegian children aged 10 to 16 years were found to be IgE sensitized to at least 1 environmental allergenic protein.[64] In 2017, most adults had IgG antibodies to grass pollen, olive/ash pollen, birch pollen, and house dust mites.[65] A year later, almost every baby aged 1 year had IgG antibodies to cow's milk and hen's egg.[66] In 2019, more than 90% of individuals with asthma, rhinitis, and conjunctivitis had elevated IgE levels to at least 1 allergen in a panel of 64 aeroallergen components.[67] Similarly, IgE response to *Staphylococcus aureus* increased in the 1980s. In 1985, serum-specific IgE to *S. aureus* was not detected in individuals colonized only in the skin.[68] In individuals with infected skin pustules with *S. aureus*, only 12% showed *S. aureus*-specific IgE.[68] However, in 2019, *S. aureus*-specific IgE was found in 39% of healthy controls, 58% of patients with mild asthma, and 76% of patients with severe asthma.[69] Today, nearly 90% of patients with AD and chronic rhinosinusitis have *S. aureus* colonization and *S. aureus*-specific IgE.[70,71]

CONCLUSION

Soon after the hygiene hypothesis was first put forward, many others followed with similar ideas such as the old friends and biodiversity. However, the main gap in hygiene hypothesis was that less exposure to pathogens in childhood would be protective for allergies. Today, we know that the type, dose, and nature of microorganisms are important for this type of protection. Additionally, recent studies have shown that our homes may not be as clean as we first thought. It is also almost impossible to reduce the microbial load only by daily cleaning routines.[72] In a German birth cohort study, although personal cleanliness was associated with decreased endotoxin levels, the same could not be demonstrated for household cleanliness.[73] So far, no direct connection in the sense of the hygiene hypothesis with an increase in allergic diseases could be confirmed. Hygiene measures are not sufficient to change the microbiota in the sense we understand it. Microbial interactions are undoubtedly necessary for adequate development of the immune system, but dietary habits and foreign exposure

should not be excluded.[73,82] Nevertheless, the parallel increase of westernization and allergic diseases seems quite convincing. From this point of view, epithelial barrier hypothesis seems more reasonable regarding the environmental exposures that we encounter every day which also have an impact on biodiversity.

Microbial dysbiosis and translocation of commensals and opportunistic pathogens across the epithelial barrier is usually followed by a type 2 immune response characterized by a predominance of Th2 cells, ILC2, and eosinophils. Mast cells, macrophages, and antibody-producing B cells may also be involved in this response. The epithelium cannot fully repair and close the barrier, setting in motion a vicious cycle of leaky barriers, microbial dysbiosis, and chronic inflammation.

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