I. Historical Perspective

Ill health related to indoor conditions has probably been observed ever since humans moved into huts and other primitive dwellings. Measures for judgment and prevention of indoor conditions were well developed hundreds of years BC, as evidenced by the regulations laid out in the book of Leviticus (for details see chapter “Fungi and Indoor Environment”). During the early days of Roman civilization there were accounts of the effects of bad air indoors, and in the 1500s, Erasmus Rotterdamus in his treatise “The Inn” vividly described the interior air in wayside inns “But nothing is more dangerous than when many people breathe the same air ... and apart from all burps, the smell of garlic and bad breath, many suffer from hidden diseases and every such disease is contagious. ...”

The first scientific account of health and indoor conditions stems from the Dutch physician van Leeuwen, who described the relation between asthma and damp buildings in the Netherlands (van Leeuwen, 1924). Changes in ventilation characteristics to save energy, innovative but unproven building designs, and questionable building practices led to an almost epidemic-like reporting of symptoms and disease indoors during the 1970s and 1980s. Initially the health problems were poorly understood or misdiagnosed, and as always when uncertainty is involved, they were attributed to hearsay, hysteria, or imagination.
It is now quite clear that the symptoms reported under the umbrella of “sick building syndrome” (SBS) are real, that there is an underlying pathophysiology, and that the causative exposure may be multifactorial. The presence of symptoms may be related to the exposure to chemical agents such as ozone, to physical agents such as low frequency noise, and to microbes. The latter contain a number of specific agents in the cell wall, microbial cell wall agents (MCWA) that have important toxicological and immunological properties, some of which occur in a synergistic fashion. The evidence for the importance of MCWA is now well established, and it is gradually becoming possible to make risk assessments based on measured values. This presentation focuses on the particular role of MCWA in sick building syndrome. Allergic reactions to specific allergens or effects caused by mycotoxins will not be discussed.

II. The Nature of SBS

Humidity indoors is a major factor related to symptoms occurring indoors and which have been referred to as “sick building syndrome” (Wieslander and Rylander, 2003). The term is a misnomer, as the phenomenon is a conglomerate of symptoms among sick persons rather than a particular characteristic of a building construction. What, then, is the nature of the symptoms?

The majority of studies on indoor ill health have collected the information on symptoms by using questionnaires. Although this instrument is subject to methodological errors and subjective influence, the similarity in symptom profiles from the different investigations is rather remarkable. The symptom profile is found in Table I.

| TABLE I |
| Symptom Profile for Sick Building Syndrome |

| Mucosal irritation |
| Skin, eyes, airways |
| Skin problems |
| Rash, itching |
| Systemic symptoms |
| Fatigue, headache, lethargy, joint pains |
| Neurological symptoms |
| Loss of sensitivity, pain |
The symptom profile comprises a series of non-specific symptoms that are always present in the population. In studies, it is thus necessary to have a non-exposed control group and compare the prevalence of the different symptoms in the investigated building with those in normal buildings. Another characteristic of the symptom profile is that the symptoms may be widespread, and prevalence figures of 50–60% have been reported.

While it was initially thought that the symptoms reflected an allergic disease and allergic asthma in particular, it is now understood that the underlying pathology is an inflammatory response as depicted in Fig. 1.

It is seen that the inflammatory response in the airways may have two underlying pathologies—an allergic, IgE-driven reaction and a non-specific inflammation caused by a toxic reaction. An inflammatory response in the lungs may also cause systemic effects because of the spread of inflammatory cytokines in the blood from the lung to other organs in the body. Tiredness and headache as examples of such systemic effects occurring as the results of effects in the brain and an

---

**Fig. 1.** Relationship between environmental exposures and different forms of inflammation.
increase in the blood level of C-reactive protein reflects the effect on the liver. There are also data that suggest an effect on the joints, although no clinical markers have yet been identified.

Apart from the symptoms reviewed in Table I, there is evidence that the exposure indoors in humid buildings causes an increased risk for infections (Husman, 1996). Because SBS is predominantly found in buildings with humidity problems, we will in the following examine the presence of microbes in the indoor environment.

III. Indoor Microbial Contamination

There are always a certain number of bacteria and molds in any environment, including the indoor environment. Usually the levels are low and do not cause any harm. This is particularly true for molds, which are present in all environments, and fungal spores can be found in the lungs of normal, healthy persons. Exposure to environmental microbes takes place during normal daily activities such as when cleaning dusty floors, emptying dustbins, or removing objects from dusty bookshelves. Outdoor levels of bacteria and fungi depend on climatic conditions and humid climates. Indeed, particularly when it is windy, a large number of airborne fungal spores are present. These normal exposures are usually not related to medical effects, except for persons sensitized to fungi, who may develop an allergic reaction after high level exposure or when meteorological conditions cause the dose levels to increase very much above normal (Newson et al., 1997).

The problems start when there is an imbalance in the indoor microenvironment. The reason is usually a dramatically increased humidity, either generally or in specific areas of the building. An increase in general humidity may be caused by inadequate ventilation, inhabitant habits such as showering, or storage of humid clothes or furniture. Specific area problems arise when there is water damage such as leaking roofs or windows, burst water pipes, or basement flooding.

Under conditions of increased humidity, there is a very rapid growth of molds. They may grow on surfaces at a material humidity above 12% or when the air humidity exceeds about 80%. Once growth is established, the spores remain even if desiccation procedures are undertaken. Bacteria will grow in stagnant water such as water reservoirs in humidifiers, open cesspits, or in bathrooms. The exposure in humid buildings comprises a mixture of different microbial species depending on local conditions. In some indoor environments, mold may be dominant; in others Gram-negative bacteria from water reservoirs or pets are dominant (Heinrich et al., 2001).
The most well-known effects of microbes are their capacity to settle in the host, multiply, and cause an infection. There are, however, specific agents in the cell wall of microbes that have the capacity to induce an inflammatory response without the need for growth of microbes within the host. A common characteristic for these MCWA is their extreme toxicity; they exert biological effects in concentration of nano- and even picograms.

**TABLE II**

<table>
<thead>
<tr>
<th>Source</th>
<th>Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-negative bacteria</td>
<td>Endotoxin</td>
</tr>
<tr>
<td>Fungi, plants</td>
<td>(1 → 3)-β-D-glucan</td>
</tr>
<tr>
<td>Gram-positive bacteria</td>
<td>Peptidoglycans</td>
</tr>
<tr>
<td>Gram-positive bacteria</td>
<td>Lipoteichoic acid</td>
</tr>
</tbody>
</table>

A number of MCWA have been identified, as illustrated in Table II. In relation to exposure, it is important to realize that MCWA retain their biological activity even when the organism is dead. An estimation of the number of viable microbes may thus be very misleading in terms of dose estimate—the number of dead fungal spores may be millions of times higher than the number of viable spores. In the following, we will review the role of MCWA for the development of inflammation.

**IV. MCWA and Inflammation**

In an evolutionary sense, organisms have always had the need to defend themselves against microbial products, which imply a risk for infection or inflammation. This concept is reflected in the number of ways that man defends himself against exposure to MCWA. This defense involves attachment of MCWA to cells through particular receptors, the triggering of a defense reaction through inflammatory cell activation, and finally the breakdown of the MCWA. These defense reactions may secondarily cause symptoms and under chronic conditions, there may be clinical disease caused by the physiological and cellular changes that take place during the defense response. A swelling of the epithelium caused by neutrophils invading to combat microbes may be experienced as a swelling that hampers normal breathing, and a severe inflammatory reaction in the airways may even lead to suffocation such as in an acute asthmatic attack.
The pathogenic effects caused by microbes are not only infection but also toxic reactions, caused by different molecular structures in the cells. The basic defense against microbes comprises an innate immunity recognition of microbial-line encoded molecules. This represents a strategy of defense not directed against individual, highly specialized antigens, but with a focus on a few, highly conserved structures that are present in larger groups of microorganisms. Such structures from microorganisms are referred to as pathogen-associated molecular patterns (PAMP). The receptors for PAMP are referred to as pattern-recognition receptors (PRRs).

An important group of PRRs is the toll-like receptor (TLR) family (Janssens and Beyaert, 2003). This class of receptors is present even in very primitive organisms and has remained unaltered over the evolution, signifying their relevance for survival. This is in contrast to the adaptive immunity, which developed much later during evolution. TLRs have more than 10 different forms, specific for MCWA (Table III).

### TABLE III

<table>
<thead>
<tr>
<th>MCWA</th>
<th>TLR reactive unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endotoxin</td>
<td>TLR-4</td>
</tr>
<tr>
<td>(1 → 3)-β-D-glucan</td>
<td>TLR-1 + TLR-2?</td>
</tr>
<tr>
<td>Double-stranded (ds) DNA</td>
<td>TLR-3</td>
</tr>
<tr>
<td>Flagellin</td>
<td>TLR-5</td>
</tr>
<tr>
<td>CpG DNA</td>
<td>TLR-9</td>
</tr>
<tr>
<td>Peptidoglycan</td>
<td>TLR-2 + TLR-6</td>
</tr>
</tbody>
</table>

Activation of cells through the TLRs induces a defense response characterized by activation of cellular transcription factors and expression of inflammatory cytokines (Chow et al., 1999; Lien et al., 2000). In the following, we shall examine in detail two important MCWA—endotoxin and (1 → 3)-β-D-glucan—and describe how they induce an inflammation.

### V. Endotoxin

Endotoxin stems from the outer cell wall of Gram-negative bacteria. These are ubiquitous in nature in terms of Enterobacter and Pseudomonas species on vegetation and in soil. Animals and man are important sources in terms of the intestinal load of Escherichia coli, and
indoor contamination is due primarily to fecal contamination, particularly from pets. Gram-negative bacteria grow rapidly in stagnant water such as humidifier reservoirs and also in organic garbage that is kept indoors several days. Endotoxin is a lipopolysaccharide (LPS) compound, where the polysaccharide part is responsible for the antigenic characteristics and lipid A for the toxic effects. Although the chemical composition is LPS, this product is an artificial compound. The LPS molecules in the environment are mostly connected to cells or cell fragments, and it has been suggested to apply the term endotoxin for this form and reserve the LPS denotation to the chemically pure agent.

The cellular mechanisms behind the inflammagenic effects induced by endotoxin are relatively well known (Martin, 2000; Schletter et al., 1995). An important part of this event is the binding of endotoxin to CD14 (Dentener et al., 1993). At the molecular level, CD14 acts by transferring endotoxin and other bacterial ligands from the circulating LPS-binding protein to the TLR-4 receptor, which together with the MD-2 molecule activates innate host defense mechanisms, such as release of inflammatory cytokines, and in upregulation of co-stimulatory molecules (Miyake, 2003).

The inflammagenic properties of endotoxin contribute to lung disease in the occupational as well as the home environment. In occupational environments with high exposure levels to organic dusts, endotoxin has been related to a variety of symptoms and clinical findings such as chest tightness, decrease in lung function, and production of inflammatory cytokines (Rylander, 2002). In the home environment, the amount of endotoxin has been related to the severity of asthma and chest symptoms from children (Michel et al., 1991, 1996).

Several studies have reported the effects of an acute inhalation of pure endotoxin in humans. There are local effects in terms of an increase in the number of neutrophils and levels of the inflammatory marker fibronectin in the airways, a dose-related decrease in pulmonary function, a decreased alveolar-capillary diffusion, and an increased airway responsiveness (Herbert et al., 1992; Rylander et al., 1989; Sandström et al., 1992). There are also systemic effects in terms of an increased number of neutrophils in the blood, an increased amount of tumor necrosis factor-α (TNF-α), myeloperoxidase, and C-reactive protein indicative of a general inflammatory response (Michel et al., 1996, 2001; Thorn and Rylander, 1998a). At high doses, there is also an increase in body temperature. Symptoms are irritation in the throat, chest tightness, tiredness, and headache. This clinical entity is referred to as toxic pneumonitis, caused by the toxic effects of endotoxin.
and distinct from the infectious pneumonitis, which is caused by microorganisms multiplying in the lung.

VI. (1 → 3)-β-D-glucan

The cell wall of fungi, certain bacteria, some plants, and pollen contains a specific polyglucose agent—(1 → 3)-β-D-glucan (Fogelmark and Rylander, 1997; Stone and Clarke, 1992). This has unique biological properties because of the steric nature of the binding between the polyglucose chains.

The role of (1 → 3)-β-D-glucan in inflammation is still unclear. Results from in vitro experiments and intravenous administration demonstrate that it up-regulates inflammmagenic cells, particularly macrophages (Sakurai et al., 1997). In inhalation experiments in animals, (1 → 3)-β-D-glucan does not cause the typical neutrophil response induced by endotoxin, but it dampens the neutrophil invasion caused by endotoxin in a dose-response fashion (Fogelmark et al., 1997). In experiments on mice, exposure to (1 → 3)-β-D-glucan caused an increased expression of messenger RNA (mRNA) encoding interleukin (IL)-10, a T-helper cell (Th)2 driving cytokine, whereas expression of IL-12, promoting interferon (IFN)γ production, was depressed (Wan et al., 1999). Inhalation of (1 → 3)-β-D-glucan also suppressed the formation of antibodies against inhaled ovalbumin (Rylander and Holt, 1998).

In humans, inhalation of pure (1 → 3)-β-D-glucan caused no effects on respiratory function or airway responsiveness (Rylander, 1996). There was a very slight increase in throat irritation and cough. In a following study, inhalation of (1 → 3)-β-D-glucan caused a decrease in the number of blood eosinophils (Thorn et al., 2001). The TNF-α production from blood monocytes stimulated in vitro with endotoxin was decreased as compared to the increase caused by inhalation of saline only. These findings were confirmed in another study suggesting that (1 → 3)-β-D-glucan interferes with the ability of blood monocytes to respond to an inflammmagenic stimulus (Beijer et al., 2003).

In view of the dampening effect of (1 → 3)-β-D-glucan on the inflammatory responses, it may be hypothesized that this depressing effect is also responsible for a decrease in the defense against infectious agents. This could explain the higher incidence of infections among children in humid buildings (Husman, 1996). No experimental data to support this hypothesis are available.

In the following, we will examine the relationships found between SBS and the presence of endotoxin and (1 → 3)-β-D-glucan indoors.
VII. Endotoxin and \((1 \rightarrow 3)-\beta-D\)-glucan in Relation to SBS

As depicted in Fig. 1, exposure in a real-life situation comprises a mixture of agents in different proportions depending on local conditions. It is important to realize that when field studies have measured the amount of endotoxin and/or \((1 \rightarrow 3)-\beta-D\)-glucan, these can only be seen as indicators of the number of (viable or nonviable) Gram-negative bacteria or fungal cells. Conceptually, they may be the causative agent or a surrogate for this. A conclusion concerning causality for different agents can be drawn only if the effect measured is specific for the agent, such as the neutrophil invasion after endotoxin exposure, which is not present after exposure to \((1 \rightarrow 3)-\beta-D\)-glucan, or the Th2-type response induced by \((1 \rightarrow 3)-\beta-D\)-glucan that is not caused by endotoxin. All data on the relation between endotoxin and \((1 \rightarrow 3)-\beta-D\)-glucan and the presence of symptoms in the following must therefore be evaluated in light of the above.

In the home environment, a relation has been found between the amount of endotoxin in the dust and the severity of asthma in terms of medication and clinical scoring (Michel et al., 1996). Among children, a significant relationship has been reported between clinical asthma scores and levels of endotoxin in their homes (Rizzo et al., 1997). In another study, the amount of endotoxin in the house dust was inversely related to the presence of symptoms of shortness of breath, skin rash, and cough (Park et al., 2001).

Some data suggest that the inflammation induced by endotoxin in the home environment may be protective against atopic sensitization. In a study among 61 infants, it was found that the risk for atopic sensitization was inversely related to the amount of endotoxin in house dust (Gereda et al., 2002; Gehring et al., 2002). Among children living on farms where the prevalence of atopic sensitization is known to be low, indoor endotoxin levels were higher than in the control group (von Mutius et al., 2000). A relation has been reported between the levels of endotoxin in children's mattresses and a reduced risk for hay fever, allergic asthma, and atopic sensitization but not for non-atopic wheeze (Braun-Falénder et al., 2002). It was also found that blood cell production of Th1 type inflammatory cytokines was down-regulated.

The present scenario suggests that the level of endotoxin indoors describes the risk for airway inflammation with corresponding symptoms of cough, irritation, and wheeze. On the other hand, endotoxin protects against atopic sensitization and atopic dermatitis. There is thus a dual nature in the endotoxin-induced effects and in real life there will be a balance between what is beneficial against atopy and
what causes symptoms of airway inflammation. There is no information on the levels at which these radically different outcomes occur.

Regarding \((1 \rightarrow 3)\)-\(\beta\)-D-glucan, an early study on SBS showed a dose response between the amount of \((1 \rightarrow 3)\)-\(\beta\)-D-glucan in air samples of agitated floor dust and the extent of eye and throat irritation, dry cough, and itching skin (Rylander et al., 1992). A following study in a day-care center examined the personnel before and after a building renovation (Rylander, 1997). The values of \((1 \rightarrow 3)\)-\(\beta\)-D-glucan in airborne agitated floor dust were 11.4 ng/m\(^3\) before and 1.2 ng/m\(^3\) after the renovation. Among the personnel, the number of persons with increased airway responsiveness, indicative of airway inflammation, decreased after the renovation. In a study on two schools, one with mold problems, the average levels of \((1 \rightarrow 3)\)-\(\beta\)-D-glucan in airborne agitated floor dust was 15.3 vs 2.9 ng/m\(^3\) (Rylander et al., 1998). Among children, the extent of symptoms of dry cough, cough with phlegm, and hoarseness was higher in the school with mold problems. The symptoms were more frequent among atopic pupils. There was also a seasonal variation (Fig. 2).

One study examined 35 persons in homes with suspect or known mold problems (Beijer et al., 2003). When they were divided into those with high and low levels of \((1 \rightarrow 3)\)-\(\beta\)-D-glucan in their homes (6.0 vs 0.9 ng/m\(^3\) airborne agitated floor dust), the secretion of TNF\(\alpha\) from blood mononuclear cells was higher among those with high levels of \((1 \rightarrow 3)\)-\(\beta\)-D-glucan in their homes. Among non-atopics, the ratio

![Fig. 2. Extent of nasal irritation in schools with mold problems (squares) and control schools (circles) during different months (after Rylander et al., 1998).](image)

into high exposure \((1 \rightarrow 3)\)-\(\beta\)-D-glucan was suggested to be an indicator of mold problems in the home.
interferon γ/interleukin-4 (IFN-γ/IFN-4) was also higher in homes with high levels of (1 → 3)-β-D-glucan. These data suggest that the home exposure precipitated a Th1-like cytokine reaction pattern. In a study on children, a relation was found between the levels of endotoxin and (1 → 3)-β-D-glucan and peak flow variability (Douwes et al., 2000).

One investigation explored the relation between different methods to sample dust particles from a house, analyzed for the content of endotoxin and (1 → 3)-β-D-glucan, and related the levels to the presence of clinical markers of inflammation (Beijer et al., 2004). An association was found between endotoxin in the air and the ratio IFN-γ/IL-4, suggesting an inflammatory response of the Th1 type. In contrast, a study on children in moldy houses reported a lower number of IFN-γ producing T-cells during the first year of life, suggesting that inflammatory outcomes may be age dependent (Lehman et al., 2002).

Regarding (1 → 3)-β-D-glucan, a relationship was found for the total amount of IgE in serum (Beijer et al., 2004). This supports data from a previous investigation, where a relationship was found between the number of airborne viable molds and the amount of total IgE (Su et al., 2004). Whether this increase signifies an increased risk for atopy and allergy is not certain, although data from other studies suggest that the proportion of atopic persons is higher in environments with high exposure to molds (Thorn et al., 1998b).

The exposure to (1 → 3)-β-D-glucan in the above field studies should be interpreted as an exposure to mold cells, and it cannot be concluded that (1 → 3)-β-D-glucan is the causative agent. In that sense, the data confirm the findings from many other studies on a relationship between mold exposure and the presence of symptoms that have been reviewed elsewhere in this book (see the Chapter “Fungi and the Indoor Environment: Their Impact on Human Health”).

VIII. Focus on Children

As has been described above, the majority of symptoms in SBS are rather unspecific and relate to inflammation in general. It is thus no surprise that even extended symptoms among children in terms of fatigue, headache, and irritation in the airways are easily explained as “normal,” reflecting the child’s age, the school environment, the child’s behavior in general, etc. This is particularly apparent when the family physician has ruled out the diagnosis of allergic asthma and has no other explanation to offer the worried parents. The following illustrates the severity of suffering that children may experience in humid and moldy houses.
A family with two boys (2 and 4 years old) was living in a villa where moisture problems developed soon after the construction was finished (Rylander et al., 1994). The boys had an irritation in the airways that was initially looked on as “frequent colds” but was only later associated with staying in the house. The symptoms disappeared during summer vacations and when they stayed with their grandparents. When symptoms grew worse, a skin prick test was performed; one boy was negative to all allergens tested and the other was positive only to house dust mite. None of them has a reaction to mold allergens. One boy then developed several episodes of severe dyspnea and bronchoconstriction that had to be treated in the emergency room at the local hospital. Finally, both children moved in with their grandparents and the symptoms disappeared. After an extensive renovation of the house, the boys returned without suffering from any symptoms.

IX. Treatment and Prevention

The presence of symptoms that by the person is related to a particular building must always be taken seriously. An inspection of the building should aim to evaluate the presence of agents that may cause inflammatory responses. Sources of chemical agents should not be forgotten; examples of the many possible agents are solvents in connection with painting, cleaning or hobby work, ozone and particulates from copying machines, and toxic substances remaining from construction materials. Regarding MCWA, focus should be on humidity problems. The question “is there condensation on the inside of windows?” has been used as a simple means to detect general humidity problems. Histories of past or present water penetration from the outside or leaking water pipes are important in the search work. As MCWA from bacteria and molds retain their biological activity in dead microbial cells, water damage problems in the past may be important. Water damage is not always visible; there may be humid spots inside the building construction from which mold spores penetrate into the room with normal air movements inside the building.

It is not adequate to treat the symptoms among affected persons. If the relation between symptoms and a particular building can be established (“I am much better when I stay with my sister,” “Every time I come back from the holidays I feel these symptoms start again,” etc.), there is no choice but to remedy the building. If the symptoms are present among children, they should be moved to avoid the risk for developing atopy and possibly severe allergic disease later on in life (Lehman et al., 2002; Savilahti et al., 2001).
There are at the present no generally accepted methods to measure the indoor contamination with MCWA. Analysis of endotoxin or (1 → 3)-β-D-glucan can be performed on dust from the floor, airborne agitated floor dust, airborne dust, or dust collected on surfaces suspended in the houses. The analysis for endotoxin and (1 → 3)-β-D-glucan is generally made by using the Limulus lysate method (Thorn and Rylander, 1998), although (1 → 3)-β-D-glucan can also be determined by using an ELISA assay against purified (1 → 3)-β-D-glucan from three species of molds (Douwes et al., 1996). Regarding the Limulus assay (Tamura et al., 1994), there is a rather large variation between laboratories, although the reproducibility within a single laboratory is usually quite good (Chun et al., 2002). This variation in the methods for sampling and analysis makes comparisons between studies difficult and constitutes a major obstacle toward the setting of standards for preventive and control purposes. In one study, a threshold value of 25 ng/mg floor dust was suggested for an increase of total IgE in the serum (Beijer et al., 2004). This number needs to be verified in future studies but could in the meantime be used as a hypothesis for further work and risk estimations.

Experience tells us that many of the reasons for humidity problems inside buildings are related to building practices. It might be desirable in the future to appoint a particular person as responsible for humidity control both in terms of building materials and construction details to avoid problems with the finished product. The discovery of extensive medical problems among inhabitants of a building, particularly the children, is a sign that prevention and control has failed and is not acceptable from a public health point of view.

REFERENCES


