Disconnection of man and the soil: Reason for the asthma and atopy epidemic?

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Intense search has been going on to find factors responsible for the asthma and atopy epidemic in Western societies. Attention has increasingly been devoted to environmental saprophytes, which, in addition to gut commensals, might be the major players in the development and fine tuning of immunologic homeostasis. This review outlines current evidence for the role of environmental saprophytes in the development of atopic disease and considers the consequences of urbanization in reducing contacts with soil microorganisms. The major microbial components that have been shown to possess immunomodulatory capacity and their respective Toll-like receptors are also discussed, as are the possible mechanisms underlying the ability of saprophytes to confer protection against atopic disease. (J Allergy Clin Immunol 2006;117: 334-44.)

Key words: *Allergy, asthma, atopy, hygiene hypothesis, saprophytes, urbanization*

The current asthma and atopy epidemic in Western societies has raised a common concern and questions of factors involved. Although in some countries prevalence rates in atopic diseases appear to have leveled off,[1-4] trends are still on the increase in many other countries.[5,6]

Numerous studies have consistently shown that high asthma and atopy rates are associated with urbanization and Western lifestyle.[7,8] Accumulating data suggest that something that is necessary for the normal maturation of the immune system might be lacking in our affluence.[9] Conversely, farm environment and a more traditional lifestyle in nonaffluent countries appear to confer protection against atopic disease.[8,10,11] Although the ultimate factors responsible for the asthma and atopy epidemic have remained unidentified, a common denominator for both living on a farm and in a nonaffluent environment is the heavy exposure to microorganisms in soil and vegetation.

Most of the microorganisms we encounter do not cause any overt infection but are still recognized by the innate immune system. Microbes in this respect need not be alive because even nonviable microbial components interact with the innate immune system. Persistent and moderate environmental exposure to microbial components might play a decisive role in the normal maturation of the immune system in childhood.[12] It has been proposed that certain microorganisms that have been present throughout the mammalian evolutionary history are recognized by the innate immune system as ''no danger'' signals and thus do not trigger inflammatory responses but instead have the ability to induce tolerance through rapid regulatory T (Treg) cell responses.[13] These organisms include saprophytic mycobacteria, lactobacilli, and some intestinal parasites that are able to elicit Treg cell responses *in vivo*[14-17] and *in vitro*.[18] The list of such microbes will certainly grow in the next few years.

The focus of the research in the context of the hygiene hypothesis has largely shifted from overt infections and the T_H1/T_H2 paradigm to noninfectious organisms, Treg cells, and Toll-like receptors (TLRs), as new data have been accumulated and the paradigm was found to be unable to unambiguously explain some important epidemiologic findings.[19,20] Indeed, diseases of immune dysregulation, including atopic diseases, are now considered to develop, more or less, as a result of failure in Treg cell function.[13] Immune defense mechanisms that evolved during the long history of humankind in a hostile environment appear now to be less appropriate when living in a clean environment.[21]

Abbreviations used
TLR: Toll-like receptor
Treg cell: Regulatory T cell

HYPOTHESIS

In this review we propose a hypothesis that one major factor in the current asthma and atopy epidemic might be the disconnection of man and the soil.

EVIDENCE TO SUPPORT THE HYPOTHESIS

There is abundant literature on adverse respiratory health effects attributable to exposure to environmental

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bioparticles (eg, bacteria, molds, and fugal spores).[22] Paradoxically, data are now accumulating to suggest that exposure to microbes in soil and vegetation might be beneficial, even necessary, for the normal maturation of the immune system.

Several lines of evidence indicate that settings associated with high-level exposure to microorganisms in soil are associated with reduced risk for asthma and atopy. Such settings include farm environments, environments in nonaffluent Eastern countries, and rural areas, particularly in developing countries.

Farm environment and atopic disease

More than 30 studies from the last 6 years have consistently shown that children who have lived or are living on a farm are less likely to have atopic disease than their counterparts not living on a farm. The issue of farming and atopic disease has been thoroughly reviewed elsewhere and is not reiterated here.[8,10,23] In many of these studies, the effect of parental farming on the development of atopic disease in the child has been found to be dose dependent,[24-26] and many of these studies have also revealed ''frequent contacts with farm animals'' as one of the major factors responsible for this effect.[25,27,28] However, frequent contacts with farm animals can also be a surrogate marker for exposure to microorganisms in soil and vegetation because farm animals (and pets) are likely to serve as a secondary source of exposure to such microorganisms. In addition, frequent contacts with farm animals could also reflect general activity of the child on the farm. The effect of farming on conferring protection against asthma and atopy might not be restricted to early life only because current parental farming has been found to be an even stronger protective factor than that in early life.[28]

Environments in nonaffluent societies: The effect of traditional lifestyle

Frequent compost and waste handling, wood handling, and animal excreta and manure handling are examples of high-level microbial exposure[22] associated with a traditional lifestyle. Unchlorinated surface water from lakes and rivers might be used as domestic water, untreated waste water might be used for irrigation, and animal excreta might be used as manure. Traditional lifestyle might also be associated with a microbe-rich diet (eg, frequent use of fermented vegetables).[20] We found recently that occurrences of atopy (determined by means of skin prick tests) and atopic diseases were substantially lower among schoolchildren and their mothers in Russian Karelia compared with that seen in their counterparts in North Karelia, Finland, irrespective of the geographic proximity of the areas and similar geoclimatic and vegetative conditions (see Fig E1 in the Online Repository at www.jacionline.org). Analysis of generational differences revealed that in Finland children had higher atopy rates than their mothers, whereas in Russia the opposite trend, children having lower atopy rates than their mothers, emerged. No signs of westernization, with atopy prevalence as a proxy, were yet discernible in Russian Karelia, which was part of the Soviet Union until 1991.[29] The results are in line with those reported earlier from other Eastern countries in transition crisis.[11] The East-West gradient in light of the occurrence of atopic diseases has been thoroughly reviewed.[8]

Rural areas and atopic disease: Evidence from relocation studies

Data both from Western and particularly from developing countries, in which great differences in lifestyle still exist between urban and rural areas, have shown that living in rural areas might confer protection against atopic disease, even in a dose-dependent manner.[30-34] A recent study in Mongolia that compared the occurrence of atopy and allergic disorders in 3 different environments of various degree of urbanization—a city area, rural towns, and villages—found significant increasing trends in the prevalence of allergic rhinitis and atopy, as determined by using skin prick tests, with increasing degree of urbanization.[30] Analysis of the effect of relocation revealed that continuous living in a village since birth was most protective against atopy and allergic rhinitis, whereas those who relocated from villages to towns in adolescence or adulthood acquired allergic conditions at rates approaching those found in subjects who had always lived in towns.[35] The results are in line with those of other migrant studies showing that sensitizaton rates and profiles among immigrants shift along with time, resembling finally those in natives,[36,37] thus supporting the view that there might not be any strictly limited window period in early life during which the individual is susceptible to immunomodulatory effects of the environment; rather, susceptibility to immunomodulation probably continues to adolescence, even to adulthood.[38-40] However, it must be borne in mind that disparities between asthma and atopic conditions in this respect might exist.[10]

Indicators of urbanization and atopic disease

Before urbanization, humans have lived in close contact with soil, either directly or indirectly through food, water, and air,[41] and heavy exposure to environmental microorganisms has occurred through inhalation, ingestion, and skin contact.[20] Inhalation of bioaerosols (composed of microbes and their components, such as products of plants and fecal material from animals) has been considered to represent the major route of exposure.[22] This natural exposure to microbes, particularly in soil, has been dramatically reduced along with urbanization characterized by living in environments covered with asphalt and concrete.

There are no unambiguous and commonly accepted criteria for urbanization. Many of the suggested criteria are based on population density and are not relevant for sparsely populated countries, such as Finland.

We performed time-series analyses of occurrence of atopic diseases and urbanization using the asphalt index (use of asphalt, tons per inhabitant per year, years 1960-1990; The Road Administration, the Ministry of Traffic and Communication, and The Finnish Asphalt

Association. Census statistics; Statistics Finland; http://statfin.stat.fi) and the decreasing proportion of farmers among the population (years 1966-2000, Statistics Finland; http://statfin.stat.fi) as indicators of urbanization here because both are closely related to reduced contacts with soil.

Prevalences of asthma and allergic rhinitis were based on our recent data on occurrence of atopic disease among military conscripts.[6] The database here covered the years 1966 through 2000 and comprised more than 1 million military conscripts aged 18 to 19 years. The men had been examined to establish their fitness for service at the call up. Similar diagnostic codes for asthma and allergic rhinitis have been used throughout the study period on the basis of ICD-8 and ICD-9 in 1966 through 1996 and ICD-10 in 1997 through 2000.

We found that the use of asphalt, which in Finland started at the end of the 1950s and was very modest still in the early 1960s (The Road Administration and The Finnish Asphalt Association, unpublished data), increased 10-fold in 3 decades. A nearly similar increase was also found in asthma prevalence among military conscripts, from 0.3% in 1966 to 2.6% in 1995 during a 30-year period, and the trend was upward for the whole study period (Fig 1, *A*).[6]

Along with urbanization, heavy structural changes have occurred in agriculture and forestry. In Finland, the proportion of farmers among the population has decreased from 17.3% in 1970 to 4.9% in 2000. During the same time, the occurrence of allergic rhinitis, as assessed among young Finnish men, increased almost exponentially, from 0.1% in 1966 to 8.9% in 2000 (Fig 1, *B*).[6] The proportion of population that is continuously in natural connection with soil has thus diminished since the 1960s and will evidently still diminish, whereas the opposite has occurred for the prevalence of allergic rhinitis.

Urbanization can also be characterized by living in apartment houses, which is, similarly to the use of asphalt and decrease in farming occupation, likely to reduce contact with soil. Dwelling type has indeed been shown to affect the magnitude of exposure to microorganisms in the environment. A study among 81 randomly selected teachers showed that both personal exposure to microorganisms (assessed with transportable inhalable aerosol samplers) and microbial concentrations in their homes were higher among persons living in family (single) houses compared with those in apartment houses, and this was considered partly to be due to increased outdoor activities among those living in family houses.[42] A sedentary lifestyle with little outdoor activity might not only be involved in the association between asthma and obesity[43] but can also increase the risk of atopy through reduced exposure to saprophytes in the environment.

Although exposure to pathogens has been found to be inversely associated with atopic diseases[44] and undoubtedly is able to exert immunomodulatory effects in early life, infectious agents might represent only a minimal part of our total exposure to microorganisms. The largely neglected group of saprophytes in the environment might play a decisive role, in addition to gut microbiota,[45] in the development and maintenance of immunologic homeostasis.

Source of drinking water and atopic disease

An important issue closely related to soil is the runoff of soil microorganisms into natural waters[46] and the use of such waters as drinking water. We found that in Russian Karelia, where atopy and atopic diseases are uncommon,[29] surface water bodies, lakes and rivers, are used as domestic water, frequently without any chemical or other treatment. Previous data have shown that consumption of unpasteurized milk in early life is associated with reduced risk of asthma and atopy in later life independently from other determinants.[26] It is reasonable to assume that consumption of untreated surface water could have similar effects and could be involved in the low atopy prevalence in Russian Karelia. Indeed, this view is supported by recent data from Ethiopia showing that consumption of river water in rural areas, as contrasted with consumption of pipe water in urban areas, conferred protection against atopic eczema.[47] In another study among schoolchildren in a rural area of Latin America, consumption of river water was found to be weakly protective against atopy.[48]

SOIL MICROBIOTA

Soil is considered the most complicated biomaterial and at the same time the most diverse and important ecosystem on the planet.[49] The definition of the microbial composition of a typical soil has proved to be problematic because of this diversity of soil types and the complexity and variability of the physicochemical circumstances. Nonetheless, the majority of soil bacteria are considered to belong to the lineage of gram-positive bacteria,[50] and members of the phylum Actinobacter have been found to predominate in the soil.[51] This phylum includes genera such as *Mycobacterium* species, *Streptomyces* species, *Actinomyces* species, *Corynebacterium* species, and *Bifidobacterium* species.[52] Fungi are often dominant in soils in terms of their biomass, particularly fungi dominate in acid temperate or polar soils that are oligotrophic,[53,54] whereas bacteria predominate in near-neutral or moderately alkaline soils.[54] Some estimates of the density of microorganisms in a normal near-neutral organic soil obtained by means of cultivation and microscopy have been reported: Actinobacter, for example, might occur at the concentrations of $10^{9\text{-}13}$ bacteria/dm3 soil and other bacteria at the level of 10^{8}/dm3 soil.[51] Cultivation, which has traditionally been used to measure bacterial densities in soil samples, probably greatly underestimates the true values. The more modern methods, such as PCR tests and fatty acid analyses, have revealed that a considerable proportion of all bacteria in soil is in a dormant (metabolically inactive) stage.

Mycobacteria, one of the major bacterial groups in soil and natural waters, including more than 80 saprophytic species,[13] has received considerable attention during the last decade as a potential immunomodulatory agent in

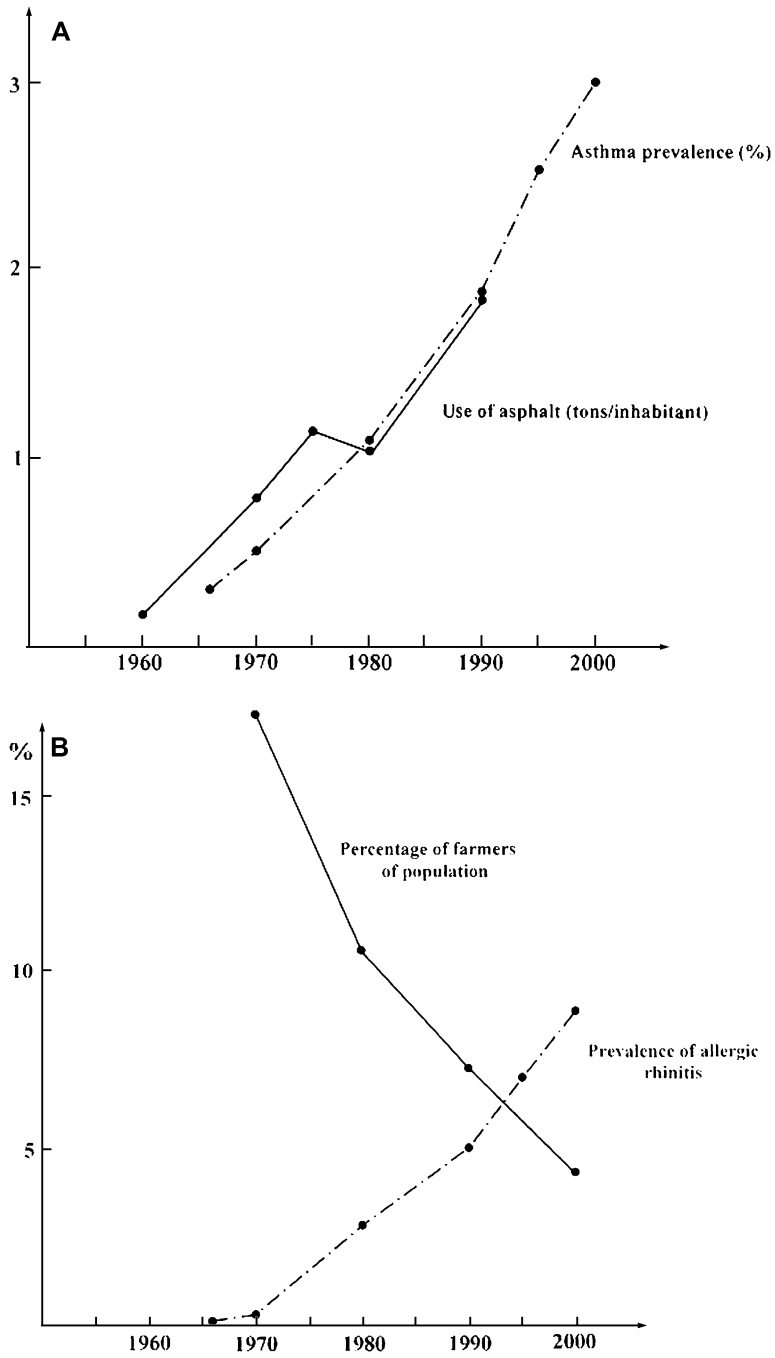

FIG 1. A, Use of asphalt (tons per inhabitant per year) from 1960 through 1990 and occurrence of diagnosed asthma among military conscripts from 1966 through 2000 in Finland.[6] **B**, Proportion of farmers among the whole population from 1970 through 2000 and occurrence of diagnosed allergic rhinitis among military conscripts from 1966 through 2000 in Finland.[6] Supplemental information is available in the Online Repository at www.jacionline.org.

alleviating symptoms of atopic disease[55] and even conferring protection against them.[14,15] In addition, much of the current research of atopic diseases has been focused on lactobacilli, which are considered one of the potential groups of immunomodulatory agents with prophylactic and therapeutic potential.[56-58] Notably, lactobacilli are originally common inhabitants of plants and grow at the expense of the nutrients liberated from decomposing plant material.[59]

Results from our laboratory indicate that, indeed, gram-positive bacteria represent the great majority (90%) of all bacteria in settled barn dust measured by means of

exact fatty acid analyses (Saris et al, unpublished data). However, the proportions of gram-negative and gram-positive bacteria might vary to some extent according to the season,[60] particularly in northern boreal latitudes, where the soil is frozen and covered with snow for months.

MAJOR COMPONENTS OF MICROORGANISMS WITH IMMUNOMODULATORY POTENTIAL AND THEIR INTERACTION WITH THE INNATE IMMUNITY

The idea that microbial products have immunomodulatory potential and could be used as immunotherapeutic agents in asthma and allergies dates back to the 1950s.[61] Bacterial extracts obtained mainly from species associated with upper respiratory tract and urinary tract infections administered subcutaneously were earlier used for such purposes but are not in use today because several double-blind studies showed no efficacy in asthma, possibly because of overly low concentrations of bacterial material in these extracts, nonoptimal route of administration, and overly long intervals between the doses (1 week or longer). No studies on the preventive effect of oral bacterial extracts are available.[62]

Because both viable and nonviable bacterial components have been found to be immunobiologically active, a renewed interest in bacterial cell-wall components has been raised, although the literature on immunologic effects of cell-wall components other than LPS (endotoxin) is relatively scarce. However, in addition to LPS, 2 other ubiquitous bacterial components, lipoteichoic acids and peptidoglycans, might be of importance in this respect, and the cell-wall components of fungi (eg, β-glucans) have also been found to have immunomodulatory potential. In addition, a potent immunomodulator appears to be bacterial DNA, the unmethylated CpG oligonucleotide.

TLRs

TLRs, the receptors that recognize conserved microbial structures, represent an ancient system of host defense but were not discovered until 1989.[63,64] This discovery brought the formerly underappreciated innate immunity into the focus of research. TLRs have been extensively reviewed during the last few years.[64-67] To date, at least 10 different TLRs have been identified in mammals, and they have been found to play a decisive role in recognizing microbes and bridging the innate and acquired immune responses.[66] Although the role of TLRs has been mostly shown in infectious systems, there are good reasons to believe that TLRs are equally important in repeated exposure to saprophytic bacteria in the environment and commensals in the gut and are involved in the induction of tolerance. TLR2, TLR4, and TLR9 are briefly considered here in the light of exposure to environmental saprophytes. TLR2 is the principal receptor and signaling molecule for gram-positive bacteria, lipoteichoic acid, mycobacteria, mycobacterial lipoarabinomannan, bacterial lipoproteins, and fungal β-glucan. TLR4 is the primary receptor for LPS from gram-negative bacteria, and TLR9 recognizes bacterial unmethylated CpG oligonucleotides.[64]

Major cell-wall components of bacteria and fungi

LPS (endotoxin). The chemical composition of LPS, the major cell-wall component of gram-negative bacteria, has been known for more than 50 years, and its physicochemical properties, stability and heat resistance, are also well established.[68] Abundant literature exists concerning the biologic effects of this macromolecule. In several studies the relative lack of exposure to endotoxin has been suggested to be one major reason for the asthma and atopy epidemic.[33,69,70] The relationship between LPS and the occurrence of asthma and atopy has also been thoroughly reviewed elsewhere.[68,71,72]

It has also been known since the 1950s that the biologic activity of LPS resides in the lipid A moiety (the theory of the ''endotoxic principle''[73]). Moreover, it is now well established that the immune response to LPS is dependent on the chemical structure and molecular conformation of the lipid A moiety. Lipid A consists of a phosphorylated glucosamine disaccharide (the backbone), to which fatty acids are attached. It has been found that biologic effects of LPS from different gram-negative bacteria are not similar.[74] The critical determinants here are the length and number of acyl (fatty acid) chains, the asymmetry of these chains, and the number and distribution of negative charges.[74,75] Interestingly, the widely used test to detect LPS in various samples, the Limulus amebocyte lysate test, has been found to be specific for the LPS glucosamine backbone and is thus not a measure of biologic activity. Fatty acid analyses might better correlate with biologic activity because the conformation and number of acyl (fatty acid) chains in lipid A appear to be central to the capacity of LPS to interact with TLRs and to induce cytokine production.[76]

Peptidoglycan and teichoic acid. With the exception of a few groups of some minor bacteria, such as *Mycoplasma* and *Chlamydia* species, all members in the domain Bacteria have one common denominator, the presence of peptidoglycan as the main strengthening and shape-determining constituent of the cell wall. In gram-positive bacteria, peptidoglycan accounts for at least 50%, and often more, of the total dry weight of the wall, but in gram-negative bacteria, it might comprise less than 10% of the dry weight of the wall.[59] In gram-negative bacteria peptidoglycan is localized in the innermost layer of the wall and, although extremely thin, is still capable of retaining the shape of the cell.[59] Peptidoglycan is composed of 2 amino sugars, N-acetylglucosamine and N-acetylmuramic acid, and a side chain of 4 amino acids that can vary from species to species.[77] The other of these amino sugars, N-acetylmuramic acid, is a molecule specific to the domain Bacteria and can thus be used for laboratory diagnostic purposes. In a recent study, van Strien et al[78] showed that muramic acid can be found in dust from children's mattresses and in higher concentrations in those of farmer's children.

Wheezing and asthma were inversely associated with the muramic acid concentration, independently from LPS, whereas no association was found for atopic sensitization. Increased muramic acid concentrations were found in homes heated with wood or coal, independent of whether it was a farm home, which suggests that settings associated with traditional lifestyle and increased exposure to microorganisms, rather than farming per se, is responsible for this effect.

The other characteristic cell-wall components of most gram-positive bacteria are teichoic acids (up to 50% of the dry weight of the wall). Teichoic acids are water-soluble polymers containing ribitol or glycerol residues joined through phosphodiester linkages.[77] In lipoteichoic acids there is a single lipid side chain anchored to the ribitol or glycerol backbone.[79] Teichoic and lipoteichoic acids have been found to exert potent inflammatory responses.[80-82] As stated above, teichoic acids are recognized by TLR2. Peptidoglycan has similarly been considered to be recognized by TLR2,[64,67] but this view has recently been challenged by the identification of intracellular proteins, the nucleotide-binding oligomerization domain 1 and 2, as the principal receptors for peptidoglycan fragments.[83,84]

Fungal cell-wall components. Fungal cell walls differ from those of bacteria by lacking peptidoglycan, teichoic acids, and LPS. In their place are the external and antigenic peptidomannans embedded in matrices of α- and β-glucans, and structural rigidity is provided by chitin.[85] The principal sterol in fungal cell membranes is ergosterol (corresponding cholesterol in mammalian cells), which has been used, in addition to β-glucans, in laboratory diagnostics of environmental samples. The immunobiology of β-glucans has been reviewed earlier.[86] Most of the recent studies on fungal recognition by TLRs have been centered on a few potentially pathogenic fungi, such as *Candida albicans* and *Aspergillus fumigatus*. It has been found that both TLR2 and TLR4 can be important for their recognition[87] and that TLR2 might be involved in maintaining prolonged candidasis by mediating anti-inflammatory signals leading to IL-10 production and generation of regulatory T cells.[88] In addition, a new coreceptor for β-glucan, dectin-1, expressed on macrophages, dendritic cells, and monocytes, has been found to be involved in mediating proinflammatory responses to fungi together with TLR2.[89,90] The recognition of fungi by the innate immune system appears to be more complex than that of bacteria because fungi can exist in 2 forms, as hyphae or conidiae.

Bacterial CpG oligonucleotides

Since the late 1980s, bacterial DNA has been known to possess immunostimulatory properties[91] that are not found in vertebrate DNA.[92] The activating element was identified as an unmethylated CpG oligonucleotide that was found to be 20-fold more abundant in bacterial than in vertebrate DNA, and when present in vertebrate DNA, about 70% of it was found to be methylated.[93]

Unmethylated CpG oligonucleotides have the ability to elicit a multifaceted innate immune response characterized by the production of IL-12, IL-18, and IFN-γ and the upregulation of costimulatory molecules by antigen-presenting cells, B cells, and natural killer cells.[94] They have both direct and indirect effects on the commitment of $CD4^+$ cells to a T_H1 phenotype and are thus able to downregulate or reverse T_H2 responses.[94-96] Mammalian DNA or methylated bacterial DNA, in contrast, does not induce these responses.[97] In addition, CpG oligonucleotides have been found to strongly induce IL-10 release, which inhibits both T_H1 and T_H2 responses in a dose-dependent manner.[98] This IL-10 is the key cytokine in the development of adaptive regulatory T (Treg) cells, which in turn are able to downregulate antigen-specific IgE responses and promote tolerance to allergens.[99,100] Data on synthetic CpG oligonucleotides in murine models of atopic diseases and as vaccine adjuvants and therapeutic agents in human subjects with allergic disorders are promising.[94-96,101]

In a study by Roy et al,[102] bacterial DNA and LPS contents in dust from urban, rural, and farm homes and from farm barns were quantified (by means of PCR specific for bacterial ribosomal DNA and the Limulus test, respectively) to determine whether there are differences in the immune stimulatory capacity between different dust samples. The highest bacterial DNA levels were found in farm barns, followed by rural homes, farm homes, and urban homes. Farm barn DNA significantly potentiated LPS-induced IL-10 and IL-12p40 release from PBMCs, whereas DNA from urban homes did not show this effect, probably because of the low content of bacterial DNA in urban home dust; only approximately 3% of the total DNA content in urban samples was bacterial in origin. Increased IL-10 and IL-12 release shown after stimulation of PBMCs with barn dust DNA and LPS might be crucial in the context of environments conferring protection against atopic diseases.[102] Furthermore, we found a 3.5-fold higher bacterial DNA content (measured by means of bacterial ribosome-specific PCR) in barn dust compared with urban-suburban home dust (5127 vs 1479.5 ng bacterial DNA/g dust; Saris et al, unpublished data). It is known that vertebrate DNA does not possess immunostimulatory capacity but might neutralize or even inhibit the immunostimulatory effects of bacterial CpG motifs.[103,104] The only known TLR for unmethylated CpG oligonucleotides is TLR9.

The innate immunity recognizes saprophytic bacteria, which results in release of proinflammatory cytokines. We have shown *in vitro* that robust responses are elicited in murine macrophages when they are stimulated by common soil microorganisms isolated from barn dust, such as *Streptomyces* species, *Sphingomonas* species, and *Macrococcus* species (Pylkkänen et al, unpublished data), and a dose-dependent and rather similar response was found in the production of, for example, TNF-α for all 3 organisms, contrary to *Bacillus* species, which showed minimal response in this *in vitro* setting (Pylkkänen et al, unpublished data). None of these genera represents true gram-negative bacteria, because *Sphingomonas* species, although categorized as gram-negative, do not possess

LPS but have sphingolipids instead. Interestingly, we found that the dominant (>85%) bacterial genus in dust from urban-suburban homes was *Bacillus* species (Saris and Andersson, unpublished data), lending further support to the view that urban home dust might have minimal, if any, immunomodulatory capacity. Nonetheless, the ability of the common soil saprophytes *Streptomyces* species, *Sphingomonas* species, and *Macrococcus* species to elicit robust proinflammatory cytokine responses *in vitro* raises the question of tolerance, which must have evolved during the long history of coexistence of these saprophytes and man.[13]

TOLERANCE AND Treg CELLS

Repeated or persistent exposure appears to be one fundamental factor in the induction of tolerance. Repeated intranasal antigen exposure leads to decreased bronchial reactivity and tolerance in T_H2-sensitized mice.[105,106] In addition, allergen desensitization therapy (injection of a specific allergen extract at increasing doses) has for years been successfully used, particularly in patients with hay fever and insect venom allergy.[107] Tolerance is mediated by several mechanisms, including anergy and deletion of effector T-cell clones, and particularly by the induction and function of Treg cells.[108] These Treg cells are defined as cells that actively control the function of other cells, mostly in an inhibitory way.[109] Two major lineages of Treg cells have been identified: (1) naturally occurring, thymus-derived Treg cells expressing the transcription factor Foxp3, which are associated primarily with the control of autoantigens, and (2) induced (adaptive), antigen-specific Treg cells, which require IL-10, TGF-β, or both for their differentiation and function.[107,110] These cells ameliorate inflammation through the release of IL-10, TGF-β, or both in repeated or persistent exposure to prevent immune pathology (a form of tolerance) and maintain the persistence of low numbers of antigens in the body, which is necessary in certain cases to provide long-lasting immunity against reinfections.[111] Induction of antigen-specific Treg cells has been performed by administration of heat-killed *Mycobacterium vaccae* and allergen (ovalbumin) into mice. These specific Treg cells were found to release IL-10 and TGF-β and suppress eosinophilia and bronchial hyperresponsiveness.[112] Desensitization therapy has also been found to operate through IL-10.[107] In general, Treg cells are able to prevent the development of highly polarized T_H cells,[113] and one of the mechanisms involved in the development of asthma and atopy has been suggested to be a failure in Treg cell function.[107,109,113] Several excellent review articles of Treg cells are available for further reading.[107,109,110,113]

Treg cells can be preferentially induced at mucosal surfaces, particularly in the gut and respiratory tract.[111] In urbanized Western societies the natural environment might no longer have the ability to maintain the respiratory and gut mucosal system in a state that favors the development of Treg cells and mucosal tolerance to harmless bioparticles.[109] Persistent exposure to saprophytic bacteria in soil and vegetation, in addition to commensals in the gut and respiratory tract, might be needed to stimulate the production of IL-10 and TGF-β through the innate immune system, which in turn are required for the development of inducible Treg cells.

TLRs AND GENE-ENVIRONMENT INTERACTION

Innate immunity is now recognized as a central element also in the gene-environment interaction. The significance of particularly the TLR2 gene in this respect has been demonstrated in several studies. In European children TLR2 gene expression has been found to be higher in blood cells obtained from farmers' children compared with that seen in children not growing up on a farm. A similar difference was not found for the TLR4 gene.[114] Furthermore, a genetic variation in the TLR2 gene was shown to be a major determinant of reduced susceptibility to asthma and atopy in farmers' children but not in nonfarmers' children.[115] No clear association could be found between variations in the TLR4 gene and asthma or hay fever either in farmers' or nonfarmers children in most,[115,116] albeit not all,[117] studies.

These studies provide convincing evidence for gene-environment interactions: a certain polymorphism is expressed only in a certain environment. They also underscore the significance of TLR2 in environments associated with high exposure to soil microorganisms. Because TLR2 is the main receptor for gram-positive bacteria and their structural molecules, lipoteichoic acid and lipoproteins,[64,67] these studies might also point to the significance of particularly gram-positive bacteria in this context. Interaction of TLR2 with its ligands has been found to lead to rapid release of IL-10, which can block the induction of IL-12p35 and IFN-γ by TLR3 and TLR4.[118] It remains to be clarified whether TLR2 is the crucial Toll receptor in mediating IL-10 release for the development of inducible Treg cells.

CONCLUDING REMARKS

Several lines of evidence support the view that the environment in modern industrialized societies is unable to provide the stimulation for the developing immune system that might be beneficial or even necessary: disruption of the ancient connection of humankind and the soil might have had unexpected consequences.

The immunomodulatory role of saprophytic bacteria in soil and vegetation is now increasingly recognized. The innate immune system recognizes such saprophytes or their nonviable components encountered at respiratory and gut mucosal surfaces; however, robust inflammatory responses are not normally elicited but kept in tight control through mechanisms that involve the function of Treg cells that in turn might control the development of atopic

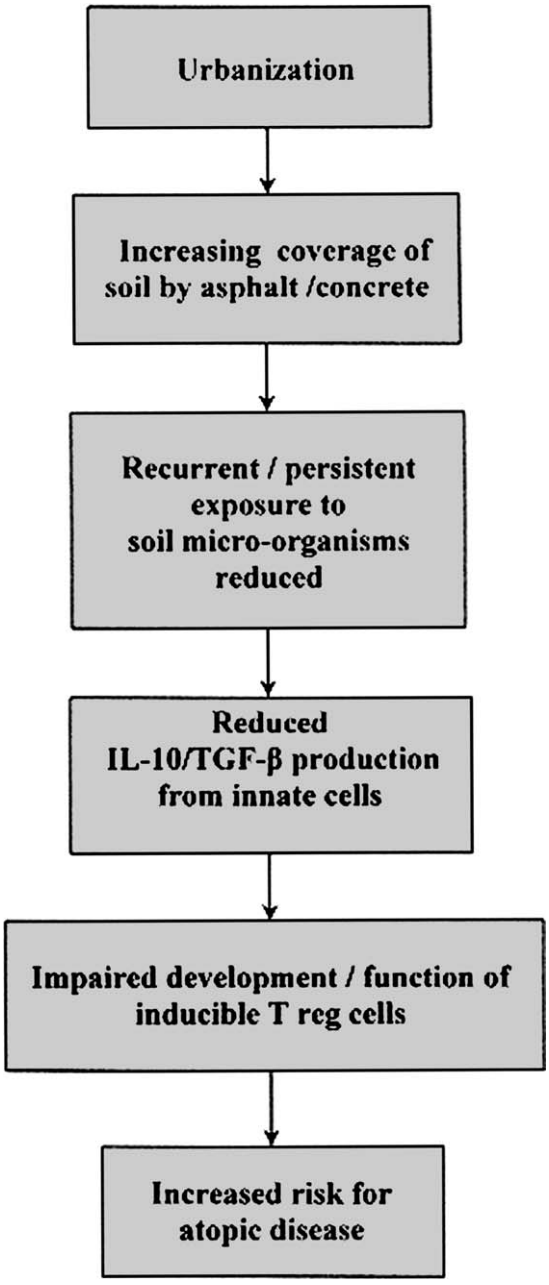

FIG 2. A model of the asphalt theory in the development of atopic diseases. Supplemental information is available in the Online Repository at www.jacionline.org. Because of the implementation of new technologies, including recycling of asphalt material, the figures for the use of asphalt from 1991 onward are not comparable with the earlier ones and have not been included in the analysis. Figures for the years 1960 through 1974 are based on unofficial data from the Road Administration, Ministry of Traffic and Communication, because no official statistics are available for that period. Data on the prevalence of diagnosed asthma and allergic rhinitis are based on Latvala et al.[6]

diseases. A number of microorganisms and their components have been found to induce the production of IL-10, the differentiation factor of inducible Treg cells, by innate cells.[111,119]

Here we proposed a hypothesis that the disconnection of man and the soil might be one major factor in the current asthma and atopy epidemic, as shown in Fig 2. The hypothesis could be tested in animal models and in further comparative and more clearly defined epidemiologic settings, and if correct, a strategy that involves enhancement of the development and activity of Treg cells[117] without concomitant induction of inflammation by bacterial products is evidently the goal to pursue. Central to the outcome of this strategy is probably timing (with respect to the primary sensitization), duration, dose, and route of exposure. The nature of the microbe might also play a role, although it is likely that there is no single agent or agent group behind the protective effect; rather, a mixture, including members of the phylum Actinobacter, might be involved. In addition, the product might not be based on bacterial cell-wall components only because bacterial DNA might have beneficial effects in this respect.

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