Rostrum

Can bacterial endotoxin exposure reverse atopy and atopic disease?

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Studies have shown that endotoxin exposure in childhood is associated with a reduced risk of atopy and atopic asthma. It is commonly assumed that these effects only occur in early life. However, recent epidemiologic studies suggest that immune deviation might take place throughout life. Assuming that the immune system is not fixed after the first years of life, we hypothesize that endotoxin exposure might not only inhibit the development of atopic sensitization and disease at any time throughout life but might also reverse this process. This novel extension of the hygiene hypothesis is primarily based on the indirect evidence of several epidemiologic observations showing a reduction in atopy in adults highly exposed to endotoxin that is unlikely to be explained by protective effects alone. In addition, some animal studies demonstrated the potential of endotoxin to downregulate pre-existing airway eosinophilia and hyperreactivity. However, there is currently little direct evidence that endotoxin might reverse atopy and allergic diseases. Observational studies and randomized trials to test this hypothesis could ultimately lead to the development of novel treatments for atopic diseases, such as allergic asthma, hay fever, and eczema. (J Allergy Clin Immunol 2004;114:1051-4.)

Key words: *Asthma, atopy, allergies, endotoxin, protection, hygiene hypothesis*

The purpose of this rostrum is to propose a novel hypothesis for the treatment of atopic disease. The hygiene hypothesis postulates that microbial pressure early in life protects against atopy and asthma by stimulating T_H1 and inhibiting T_H2 (atopic) immune responses.[1,2] Some evidence for this has been reported both for infectious and noninfectious microbial agents.[3] In particular, several studies have shown that childhood exposure to endotoxin, a major component of the outer membrane of gram-negative bacteria that is present ubiquitously in the environment, is associated with a reduced risk for atopy and atopic asthma.[1,4] We propose that endotoxin can not only inhibit the development of atopy and atopic disease but can also reverse pre-existing atopic sensitization and related diseases.

Abbreviation used
AH: Airway hyperreactivity

ENDOTOXIN INHIBITS ATOPY

Bacterial endotoxins are strong immunomodulating substances that can inhibit the atopic T_H2 response by promoting and enhancing T_H1 responses through IL-12 and IFN-γ production in antigen-presenting cells.[2,5] A study in 61 infants showed that allergen-sensitized infants had significantly lower house dust endotoxin levels than nonsensitized infants, and levels correlated with IFN-γ–producing T cells (T_H1) but not with IL-4–, IL-5–, or IL-13–producing cell proportions (T_H2).[6] Furthermore, several studies have demonstrated that atopy is associated with a genetic polymorphism for CD14, the endotoxin receptor on monocytes and other inflammatory cells,[7-9] although this was not confirmed in other recent studies.[10,11] Children raised on farms with livestock had less atopy and asthma than nonfarming children, and endotoxin exposure has been suggested to play a major role.[12] Studies in both rural and nonrural environments reported a significant inverse association between indoor endotoxin levels and atopic sensitization,[13] hay fever, and atopic asthma.[4] In contrast, a birth cohort study conducted by the same researchers found that early endotoxin exposure was associated with an increased risk of atopy at the age of 2 years.[14] However, a similar birth cohort study found a protective effect on atopy in 2-year-old children.[15] Finally, several animal studies have reported findings consistent with the hypothesis that endotoxin might

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protect against atopy and asthma,[16,17] although some have shown mixed results.[18] Thus despite some inconsistencies in the available evidence that might be related to the hypothesized bimodal effect of endotoxin on the T_H1/T_H2 balance,[19] it appears that endotoxin exposures might protect against atopy. As recently suggested,[20,21] these effects might be modified by variations in the gene encoding Toll-like receptor 4, an important LPS receptor on antigen-presenting cells, macrophages, and monocytes.

It is commonly assumed that these protective exposures have their effects during the first years of life and that the immunologic reactivity expressed in adulthood is already established at the age of 5 years.[22] However, as recently argued by Kemp and Björkstén,[23] there is little evidence to support this. In fact, they concluded that the principal environmental influences on atopic disease are likely to occur throughout life, particularly because a number of the postulated protective infectious exposures are unlikely to exert their effect in early life because most of these infections predominantly take place later in childhood. In addition, one of the key studies linking endotoxin exposure to a reduced risk of atopy and asthma found this association with current endotoxin exposure in school-age children (rather than infants).[4] This was confirmed in 2 other studies in children beyond the age of infancy.[13,24] Also, a number of recent studies (discussed further below) have suggested that endotoxin exposure in adulthood might protect against atopy and atopic asthma.[20,25] Finally, most animal studies indicating that endotoxin exposure might protect against atopy and asthma were conducted in adult mice.[16,17] These studies thus strongly suggest that immune deviation might take place well beyond the age of infancy.

CAN ENDOTOXIN REVERSE ATOPY?

Assuming that the immune system is not fixed after the first years of life, we hypothesize that endotoxin exposure might not only inhibit the development of atopic sensitization at any time throughout life but might also reverse this process. This novel extension of the hygiene hypothesis is based on a number of epidemiologic observations that cannot be explained by primary protective effects alone. For example, several studies have shown a low prevalence among adult or adolescent farmers of allergic symptoms, atopic sensitization, or both.[26-29] This could be due to childhood exposures, but one study also showed a decreased prevalence among adolescent farmers who were not raised on a farm,[30] suggesting that current exposures might play a role as well. This suggestion was recently confirmed in a study among adult farmers, in which it was shown that current exposure to endotoxin was associated with a 3-fold reduction in atopic asthma.[25] Another study reported trends between high endotoxin levels in house dust and decreased prevalence of atopy and hay fever in nonfarming adults, which was statistically significant only for hay fever.[20] However, in the same study asthma and wheeze were more prevalent in the high-exposure group. Finally, among pig farmers in The Netherlands, it was shown that current endotoxin concentrations were associated with a lower prevalence of atopic sensitization (Professor Heederik, personal communication).

Given that atopy generally develops early in life and is well established in later life, a strongly reduced prevalence of atopic sensitization in adults or adolescents (as described above) can have several possible explanations. First, the effect might be explained by early life exposures. However, we do not consider this very plausible because current occupational exposures are unlikely to be strongly correlated with protective exposures in infancy. Second, those with atopy and symptomatic sensitivity to endotoxin might avoid further exposure in later life (including occupational exposure). Alternatively, events later in life might reverse (rather than just protect against) atopy, as we hypothesize. To our knowledge, only one population study has presented direct evidence that endotoxin (or other farm related exposures) might reverse atopic sensitization. In this 3-year follow-up study, it was shown that children living on a farm were more likely to lose atopic sensitization (50% in part-time and 60% in full-time farming families, $P < .05$) than their peers who did not live on a farm (15% in this group).[31] Parental farming was also inversely related to the prevalence and new occurrence of atopic sensitization. These results are, however, preliminary because the numbers of farming children in whom atopy was reversed was small (6 children from part-time farming families and 5 from full-time farming families). In addition, it is not clear whether these findings can be extrapolated to adults.

CAN ENDOTOXIN REVERSE ATOPIC DISEASE?

If endotoxin exposure could indeed reverse atopic sensitization, it could potentially also reverse atopic diseases, such as allergic asthma, hay fever, and eczema. Evidence that endotoxin might reverse atopic disease has been presented in some animal studies.[32-34] Studies in mice presensitized and prechallenged to cockroach allergens demonstrated that intranasal endotoxin exposure could suppress airway hyperreactivity (AH) and airway and lung eosinophilia.[32] A decrease in T_H2 cytokines and an increase in T_H1 cytokines in the airways were also observed. Similarly, an endotoxin-induced reduction of AH has been shown in rats presensitized and prechallenged with ovalbumin.[34] The mechanisms through which endotoxin might downregulate allergic airway inflammation and subsequently suppress AH are not clear. As mentioned earlier, endotoxin might induce immune deviation toward a T_H1 response, resulting in inhibited T_H2 immunity. However, in contradiction with this hypothesis, AH was suppressed independently of IL-12 and IFN-γ in the animal studies described above. Alternative mechanisms for a suppressive effect of endotoxin on the T_H2 immune response have been proposed, including the upregulation of immunoregulatory IL-10 production by

T cells[35] and endotoxin-induced changes in nitric oxide production.[33]

If these hypotheses are true and can be extrapolated to human subjects, then there is potential for the development of novel approaches to treat allergies and asthma. However, there is reason for caution because (1) not all asthma and rhinitis is caused by atopic mechanisms,[36,37] and (2) it is well known that high endotoxin exposures can cause nonallergic adverse respiratory effects.[38] Nonetheless, atopy still is an important factor in the development of allergic asthma, which we estimate to account for about one half of all cases of asthma.[36,37] Furthermore, although endotoxin might cause nonallergic respiratory symptoms,[38] these might not necessarily occur at the same levels required for a reversal of atopy and atopic diseases (but instead might occur only at higher levels).

Finally, it is not entirely clear whether endotoxin is solely responsible for the observed protective effects or whether other (microbial) agents highly correlated with endotoxin exposure might also play a role. Currently, most of the evidence points toward bacterial endotoxin, which is why we have focused on this particular exposure. However, if other exposures, such as peptidoglycans[39] or other agents, such as those discussed below, prove to be equally or more important, either on their own or synergistically in combination with endotoxin,[40] then this would not invalidate our more general hypothesis that certain environmental exposures (at sufficiently high levels) can modify the immune response from an atopic to a nonatopic state at any time in life. In fact, some evidence for this has been presented in a murine model of allergic asthma for another bacterial agent (ie, DNA motifs containing CpG oligodeoxynucleotides).[41] Similarly, mycobacterial antigens have been suggested to reverse atopic immune responses, but the evidence from clinical trials is weak.[42] Finally, it has been shown that influenza infection might suppress eosinophilia, T_H2 cytokine production, and airway hyperresponsiveness in already established allergic airway–sensitized mice.[43] The beneficial effects were shown to be mediated by IFN-γ–producing lung resident memory $CD8^+$ T cells. However, similar to the evidence for endotoxin, detrimental effects (exacerbation of allergic airway inflammation) have also been reported.[43,44]

Thus on the basis of the mainly indirect evidence presented above, we believe it is plausible that increased levels of endotoxin (or other environmental factors associated with endotoxin) might reverse pre-existing atopy and atopic disease in children and adults. Randomized clinical trials exposing atopic subjects for a limited time to endotoxin could be conducted to test this extension of the hygiene hypothesis, but these would have considerable practical and ethical implications. Alternatively, studies could make use of an existing situation, with real-life continuous exposures to high levels of endotoxin mimicking a long-term experimental exposure study. Some occupational environments with known high endotoxin exposures, such as wool processing, poultry slaughter, and animal feed processing,[38] are ideally suited for this. Prospective observational studies in atopic subjects newly exposed to high levels of endotoxin through their work environment would thus be of great value and could ultimately lead to the development of randomized trials and subsequently to novel treatments for atopic disease. However, a better understanding of the underlying immunologic mechanisms is crucial before any such interventions can be developed.

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