

Coming back to a missing immune deviation as the main explanatory mechanism for the hygiene hypothesis

Sergio Romagnani, MD Florence, Italy

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The expression of the allergic phenotype is dependent on the interaction between 2 major factors: a genetic predisposition and gene-environment interactions. The pattern of allergic inheritance is that of a complex polygenic disorder. The gene-environment interactions, however, heavily influence the development of atopic allergy, and several changes in lifestyle that have occurred over the past few decades are certainly responsible for the increased prevalence of allergy in Western countries. Several epidemiologic studies have clearly shown that modifications of the pattern of microbial exposure of children associated with Westernization represent a critical factor underlying the rising severity and prevalence of atopic disorders (the so-called *hygiene hypothesis*).^{1,2} If the hygiene hypothesis is the most reasonable explanation for the allergy epidemics, its immunologic basis has been controversial. The initial interpretations, based on several *in vitro* and *in vivo* models, concluded that a lack of shifting of allergen-specific responses from the T_H2 to the T_H1 phenotype (ie, a missing immune deviation) because of the reduced microbial burden in the early childhood was accountable.¹⁻³ It was indeed widely shown that cytokines such as IL-12, IL-18, IFN- γ , and IFN- α polarize the allergen-specific T_H response toward the T_H1 profile both *in vitro* and *in vivo*. Moreover, many pathogen-associated molecular patterns, such as LPS, double-stranded RNA, and CpG-containing oligodeoxynucleotides, exhibit the same effect by interacting with Toll-like receptors (TLRs) expressed by dendritic cells (DCs) and by other cells of the innate immunity. This results in the production of high concentrations of different cytokines, the most important being the T_H1 -polarizing IL-12.⁴

Abbreviations used

ALEX: Allergy and Endotoxin
DC: Dendritic cell
TLR: Toll-like receptor

However, after the discovery of regulatory T cells,⁵ the importance of their decreased activity (reduced immune suppression) as the main, or even unique, explanatory mechanism for the hygiene hypothesis was emphasized.^{6,7} Three main arguments were given to support the validity of such a mechanism. First, prevalence of allergy is low in world areas characterized by diffuse and chronic helminthic infestations that induce strong T_H2 responses.⁶ Second, high pathogen burden results in the production of elevated amounts of suppressive cytokines (IL-10, TGF- β).⁶ Finally, prevalence of insulin-dependent diabetes mellitus, multiple sclerosis, and Crohn disease (thought to be T_H1 -dependent disorders) is also increasing in developed countries.⁷ On the basis of these arguments, a new paradigm was erected, and an impressive amount of previously reported *in vitro* and *in vivo* data showing the existence of a process of immune deviation was fully discarded, as in a sort of iconoclastic fury. Some reports came to interpret the allergic inflammation merely as the result of a defective regulatory T-cell activity,^{8,9} and even the possibility to cure allergy by tools inducing IL-10 production was postulated.¹⁰ More recently, however, new, more cogent epidemiologic findings have been provided that challenge the paradigm of reduced immune suppression and reinforce the validity of immune deviation. First, it was found that T_H1 -mediated nephropathies are increasing in developing countries, whereas T_H2 -mediated nephropathies are increasing in developed countries.¹¹ Second, it has been shown in a cohort of 500,000 enrolled people that pre-existing asthma prevents autoimmune disorders.¹² Finally, neonatal BCG vaccination, which polarizes to T_H1 , significantly reduced the asthma risk in a cohort of more than 5000 children.¹³

Among all epidemiologic findings supporting the validity of the hygiene hypothesis as explanation for the increased prevalence of allergy in developed countries, the most impressive are certainly those derived from the study on children of European farmers, known as Allergy and

From the Department of Internal Medicine, University of Florence.
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Reprint requests: Sergio Romagnani, MD, Department of Internal Medicine, viale Morgagni 85, Firenze 50134, Italy. E-mail: s.romagnani@dmf.unifi.it.
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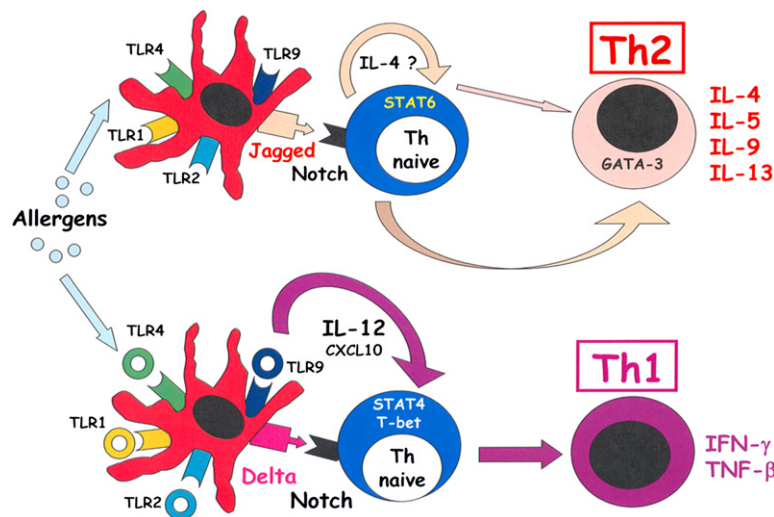


FIG 1. A missing immune deviation as the main explanatory mechanism for the hygiene hypothesis. Immature DCs, in absence of their TLR ligation, trigger a T_H2 -polarized program in allergen-specific naive T_H cells, because of the Jagged-1/Notch interaction, via an IL-4-dependent and/or IL-4-independent pathway (*top*). When allergen is presented by DCs undergoing chronic and multiple ligation of their TLRs by microbial components (*open circles*), strong upregulation of Delta-4, high IL-12 production, and subsequent T_H1 polarization occur (*bottom*). STAT, Signal transducer and activator of transcription.

Endotoxin (ALEX) study. The results of the ALEX study have clearly demonstrated that children who grow up in farming environment show lower levels of atopic sensitization, hay fever, and asthma than children of the same age living in the same villages, but not in such environments,¹⁴ and that this difference is a result of an early life contact of farm children with cowsheds and farm animals and/or consumption of products like raw milk.¹⁴ In this issue of the journal, Debarry et al¹⁵ provide evidence that 2 of the many bacterial species identified in cowsheds of farms, *Acinetobacter lwoffii* F78 and *Lactococcus lactis*, were able to reduce allergic reactions in mice by inducing a T_H1 -polarizing program in DCs. Murine DCs have been found to polarize naive T_H cells toward the T_H2 profile of cytokine production when Jagged is prevalently expressed as ligand for the Notch receptor of T_H cells. By contrast, the prevalent expression on DCs of Delta ligand polarizes naive T_H cells toward the T_H1 profile.¹⁶ Whether the mechanism by which Jagged/Notch interaction polarizes to T_H2 is IL-4-dependent or IL-4-independent is still unclear, but the mechanism by which the Delta/Notch interaction polarizes to T_H1 is clearly dependent on its stimulation of DCs to produce high concentrations of IL-12.¹⁶ In their study, Debarry et al¹⁵ demonstrate that both bacteria isolated from cowsheds not only induced high IL-12 production by DCs but also strongly upregulated on the same cells the expression of Delta-4.¹⁵ These findings are fully in agreement with recent data obtained in my laboratory, showing that immature myeloid DCs isolated from adult blood trigger in autologous $CD4^+$ T cells a T_H2 -polarized program, and this effect associates with their expression of Jagged-1, but not Delta-4. Maturation of the same DCs via ligation of their TLR4, TLR7, TLR8 or TLR9 induced a strong

upregulation of the Delta-4 ligand, inverted Jagged-1/Delta-4 ratio, production of high IL-12 concentrations, and T_H1 polarization (Liotta et al, unpublished data, 2007). These observations are consistent with those reported by Napolitani et al,¹⁷ showing that synergic stimulation of more TLRs increased production of both IL-12 and IL-23 and increased Delta-4/Jagged-1 ratio, leading to DCs with enhanced and sustained T_H1 -polarizing capacity.¹⁶ Taken together, these findings convincingly suggest that when immature DCs begin to present environmental allergens to T_H cells before birth and in early childhood, they tend to activate a program of T_H2 polarization because of their prevalent Jagged-1 expression. As full as the environment is of pathogen-associated molecular patterns, or even products of saprophytic microbes, favoring chronic and multiple TLR ligation on DCs, as high is the possibility that these cells trigger a less T_H2 -polarized or even a T_H1 -polarized program in the allergen-specific naive T_H cell as a consequence of strong Delta-4 upregulation and consequent stimulation of IL-12 production (Fig 1). Thus, a missing immune deviation, rather than a decreased immune suppression as a result of reduced microbial burden, again appears to be the most convincing explanatory mechanism for the hygiene hypothesis and therefore for the increased prevalence of allergy and asthma in Westernized countries.

This conclusion has also obvious implications for both prevention and therapy of allergic disorders. It is indeed clear that treatment of children or even newborns at high risk for allergy with food regimens or other modalities that promote chronic and multiple stimulation of TLRs on DCs by innocuous ligands may be effective in reducing the risk of developing T_H2 responses against common environmental allergens. Likewise, in people with already

established allergic disorders, specific immunotherapeutic strategies based on the use of synthetic adjuvants that induce TLR ligation on DCs^{18,19} may be effective and also safe. It is indeed of note that in the study of Debarry et al¹⁵ only negligible signs of T_H1-driven inflammation were observed after repeated intranasal treatment of mice with rather high doses of the bacterial preparations. The safety of allergen-specific T_H1 responses is in agreement with the observation that virtually all healthy individuals without allergy possess circulating Der p 1–specific T cells showing a clearly T_H1-polarized profile.⁴ Likewise, the great majority of children of the ALEX study, including those who did not become allergic, had in their serum allergen-specific IgG₁ antibodies,²⁰ a subclass that is produced by B cells in collaboration with T_H1 lymphocytes, further supporting the concept that atopic subjects have no defective, but differently polarized, T-cell effector responses to common environmental allergens.

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