

Hygiene and the cytokine jungle in Brazil

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Cytokines are important mediators of immune responses and play an important role in the development of allergy and asthma. Diverging concepts of immune development have been proposed. One perception proposes antagonistic regulation of opposing signals emanating from T_H1 and T_H2 cells, respectively. According to this view, T_H2-derived cytokines mediate the onset of atopy by promoting specific IgE responses to allergens, whereas T_H1 cytokines antagonize this move. However, this paradigm does not go along with epidemiologic observations of increasing temporal trends in allergic and autoimmune diseases, the latter being related to increased T_H1 responses. An overarching paradigm to reconcile these apparently paradoxical approaches states that regulatory factors, in particular IL-10 and TGF- β , contribute to containing the immunologic balance.^{1,2} Although pro and con arguments can be derived from manipulating sophisticated animal models, not much is known from human populations.^{3,4}

This lack of knowledge is in part attributable to the enormous time and effort needed to assess immune responses in field studies. Because of large numbers of enrolled subjects, sorting out and characterizing single-cell populations is usually not feasible. Methods based on whole blood samples are much more practical but will appear crude to an immunologist attempting to understand the cellular origin of cytokines. However, whole-blood assays can capture more comprehensively immune responses taken as a whole. Moreover, the increased statistical power of large sample sizes allows the identification of environmental determinants against potentially confounding factors in a real-world scenario.

The question then arises of how to best describe and analyze cytokine responses. In the aftermath of genomic studies, hypothesis-driven approaches have been increasingly complemented and substituted by hypothesis-free approaches to uncover hidden associations in datasets rather than imposing theories on observations. In the epidemiologic world latent class analysis has become a powerful tool for the latter approach. This statistical

method attempts to detect underlying classes, creating patterns of associations in observations of interest.^{5,6} Figueiredo et al.,⁷ in the April 2013 issue of the *Journal*, have, for the first time, applied such an approach to the analysis of cytokine patterns in 1227 children aged 4 to 11 years recruited from a poor neighborhood of the Brazilian city of Salvador. Levels of the cytokines IFN- γ , IL-13, IL-5, and IL-10 were measured in supernatants of whole-blood assays after stimulation with pokeweed, a mitogen that activates lymphocytes. Three latent classes were identified according to the prevailing pattern of detectable cytokine responses. These classes were labeled as “responsive,” “underresponsive,” and “intermediate.” In the underresponsive class few subjects produced cytokines (Table I in Figueiredo et al.⁷), and if they produced cytokines, they did so at very low levels (Fig 1 in Figueiredo et al.⁷). In the intermediate grouping almost all children produced IFN- γ and, at the highest level, IL-10, whereas IL-5 and IL-13 were detected in only about a third of the children. In almost all subjects assigned to the responsive class, all 4 cytokines were detectable (Table I in Figueiredo et al.⁷). However, in contrast to the intermediate group, in which those producing IL-13 had low levels of this cytokine, the mean IL-13 level was more than 1000 times higher in the responsive group, suggesting that this group might also be labeled as T_H2 prone.

The identified classes of subjects with different patterns of cytokine responses were associated with the results of skin prick tests and measurements of specific IgE antibodies in these children. Interestingly, both ends of the spectrum were associated with atopy (ie, both the underresponsive class and the responsive T_H2-prone class). In this and a previous publication, the underresponsive class was associated with a cleaner environment (paved rather than unpaved roads and poor rather than appropriate garbage collection).⁸ These findings might suggest that a lack of environmental stimuli can result in an insufficient maturation of T-cell responses and thereby an increased risk of atopy.

In turn, the responsive T_H2-prone class was associated with fewer infections and more hygienic environmental living conditions, as described above. A light burden of infections was associated with more atopy in this population, as in other populations.² Interestingly, this effect was in part mediated by the responsive T_H2-prone class. This article is thus one of the first reports to show statistically that the hygiene hypothesis is at least partly attributable to infections affecting immune responses, mostly IL-13 responses, and thereby increasing the risk of atopy in human populations.

However, there was little difference in mean IL-10 and IFN- γ levels between the reference (intermediate) group and the responsive T_H2-prone class, suggesting that the increase in IL-13 levels in the responsive T_H2-prone and atopy-prone class might be attributable to factors other than the T_H1 antagonism and IL-10 regulatory balance reported from experimental studies. Alternatively, the relation between cytokine levels might play an important role. It can be hypothesized that in the responsive group both low and high IL-10 producers might significantly differ in

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their IL-13 levels. In consequence, children with a higher IL-13/IL-10 ratio might display stronger associations with environmental risk factors and might be more prone to atopy. In fact, 71% of all subjects were classified as responsive, whereas the prevalence of atopy was only 37.7%, suggesting that other characteristics of individual immune responses might matter.

The latent class approach used by the authors divided groups into those with and without detectable cytokines. Although such an approach seems reasonable for disease outcomes, such as wheeze (either present or absent), it might not be appropriate for genuinely quantitative outcomes, such as cytokine levels. Latent class analysis can also be performed on ordinal variables, such as tertiles of cytokine levels, and nondetectable levels can be substituted by a minimal value or excluded (at most 30% of children in this study), bearing in mind that the quantitative aspects might result in a more appropriate approach to cytokine analyses. Furthermore, relations between cytokine levels (eg, the IL-10/IL-13 ratio) can be tested in such an unbiased approach and might lead to a more functional view on the cytokine network.

To investigate the hygiene hypothesis with respect to the T-cell cytokine repertoire, the authors selected pokeweed as the mitogenic stimulus on lymphocytes.⁹ As with other lectins, the pokeweed stimulus induces a strong production of T-cell cytokines and is thereby suitable to exclusively assess the T-cell cytokine repertoire in whole-blood assays. However, innate immune responses instructing T-cell differentiation might be of particular interest in the context of the hygiene hypothesis and will have been missed with this approach. Stimulation of monocytes with LPS would have provided additional insights into the pattern of the frontline immune defense under poor living conditions, as demonstrated previously.¹⁰

The relations discussed above were only seen for atopy but not for wheeze and asthma. These observations go along with previous epidemiologic findings supporting the hygiene hypothesis for the development of allergic sensitization but much less for asthma and wheeze.¹¹ However, the contribution of single infections rather than the composite score of burden of infections to protection against either skin prick test positivity or measurable specific IgE antibodies (≥ 0.7 kU/L) remains unclear.^{8,12}

Therefore residual confounding of such a composite score cannot be excluded.

In sum, the latent class analysis using dichotomized variables (detectable vs nondetectable cytokines) might not be ideal to unravel patterns of cytokine networks in which the cytokine amount produced might matter for allergy development. However, better analytic approaches (eg, using ordinal variables) are urgently needed to address complex immune responses and to further explore the cytokine jungle off the beaten track.

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