

Review article

CD14: an example of gene by environment interaction in allergic disease

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The prevalence of allergic diseases (1, 2) has increased significantly over the last few decades. Drastic environmental modifications have been targeted as the main culprit of this rise. However, both asthma (3) and total serum immunoglobulin (Ig)E levels (4) are known to be genetically determined. Therefore, the dissection of the relative contribution of environmental and genetic factors will provide a deeper understanding of the pathophysiology underlying the trends in allergic diseases.

In this paper, we are reviewing a model of interaction between environmental and genetic factors in the expression of IgE-mediated allergy (5). We are discussing the role of bacterial exposure in the development of the mature immune response and its modulation by a polymorphism in *CD14*, the gene encoding an essential component of the receptor for bacterial lipopolysaccharide (LPS, endotoxin), a key factor in the initiation of the innate immune response to bacterial infections.

An attempt at explaining epidemiological trends in allergic diseases: the 'hygiene hypothesis'

During the last decades, a significant shift has

occurred in the main causes of morbidity amongst infants and young children worldwide. Until the 1950s and 60s, acute infectious diseases of the digestive tract were the most prevalent pathology in children. This pattern of morbidity persists in the most indigent regions of the world, and is still taking a large death toll. In developed countries, on the contrary, chronic, recurrent illnesses of the respiratory tract have replaced acute diarrhoea as the major cause of morbidity. Of note, this trend does not differ in developing areas where successful programs of vaccination and prevention of acute infection have been instituted. A very high proportion of children is affected by allergies, and the prevalence of asthma is on the rise (1). Surprisingly, the increase seems to be particularly noticeable in areas where improvements in outdoor air quality (6) and sanitary and nutritional conditions have been achieved.

The so-called 'hygiene hypothesis', an immunological extension of a concept introduced by Strachan (7), was proposed (8, 9) in an attempt to explain these findings. Its foundation is that enforcement of public health policies and markedly improved hygiene conditions have changed the type and level of stimulation from the microbial environment which

may have indirectly influenced the postnatal development of the immune system, leading to an increased predisposition to allergic diseases during childhood.

Development of mature immune responses in early life: the role of microbial exposure

The pattern of cytokine responses by mononuclear blood cells obtained from newborns and foetuses is significantly different from that observed in older children and adults. Immature responses are characterized by the dominance of cytokines that have been usually identified as belonging to the T-helper (Th) 2-type.

After birth, the environment to which the immune system is exposed changes dramatically and contributes to the transition from the foetal/low-Th1 to the adult/high-Th1 cytokine profile. Although it is not known which environmental exposures are involved in promoting this transition, animal experimental data (9, 10) suggest that microbial exposure in early life is a major trigger of such a switch and that lack of bacterial exposure tends to lock the immune system into a predominant Th2 state (11).

LPS is a major component of the bacterial cell wall and is known to stimulate interleukin (IL)-12 production from antigen presenting cells (APC) (12, 13), including dendritic cells (DCs) (14). It has been suggested that DCs are professional APCs that play a major role in primary responses to antigens and in determining the differentiation of naïve T-helper cells into either Th1-like or Th2-like cells (14). In the absence of IL-12-inducing factors, DCs have been shown to induce both IL-4 and interferon (IFN)- γ production in maturing naïve Th cells (15), supporting the hypothesis that IL-12 production by DCs may be an obligatory signal for the differentiation of naïve T cells into Th1-like cells (16). In conclusion, a reduced exposure to bacterial antigens such as LPS may be responsible for the delayed development of the mature, Th1-dominated immune response through deficient maturation of APCs and subsequent insufficient IL-12-mediated stimulation of uncommitted naïve Th cells.

This could be just one of the many effects that LPS exposure in early life may have on the development of the neuroendocrine-immune system. Indeed, recent data show that early life exposure to LPS alters the development of the hypothalamic-pituitary-adrenal axis and influences the predisposition to inflammatory diseases (17).

Development of mature immune responses in early life: the role of genetic background

The pattern of immune responses in mature T cells is the result of interactions between environmental exposure

during the development and the genetic background of the subject. The transition from the foetal low Th1 to the adult high Th1 pattern is significantly slower in subjects with familiarity for allergic diseases, as indicated by reduced IFN- γ production and failure to downregulate foetally primed allergen-specific Th2 responses during infancy (18).

The genetic basis for the delayed postnatal maturation of Th1 functions in allergy remains to be established. Among the many genes likely to be involved in this process, it is not surprising that a lot of interest has focused on *CD14*. In fact, *CD14* is located on chromosome 5q31.33, an area where linkage to Th2 prevalent phenotypes such as high total serum IgE, eosinophilia and allergic asthma has been repeatedly reported (4, 19–21). Moreover, *CD14* encodes the receptor for LPS. *CD14* is expressed on the surface of macrophages and monocytes and is also present as a soluble form (*sCD14*). Engagement of *CD14* by LPS and other bacterial cell wall-derived molecules (16, 22–25) greatly enhances IL-12 secretion from APCs. *CD14* may thus serve as a crucial link between nonadaptive and adaptive immune responses to environmental antigens (26). Interestingly, DCs do not express membrane *CD14* (*mCD14* 27, 28), but can mount IL-12 responses to LPS stimulation by recognizing complexes formed as a result of interactions between *sCD14* and LPS (26). Recent evidence suggesting that LPS-induced cytokine responses in DCs show dose-response relations with *sCD14* concentrations supports the potential involvement of these complexes in DC activation (26). Consequently, any variability in *CD14* function could affect this form of signalling through the adaptive immune system, contributing to the transient developmental defect in Th1 function now recognized as a characteristic of the atopic phenotype. *CD14*-independent pathways leading to LPS-mediated activation of monocytes and secretion of Th1-inducing cytokines function effectively in adults, but are inoperative in infants (29). Thus, the *CD14*-dependent effects of LPS exposure (30) may be more relevant in early life than later on.

A genetic variant (*CD14* C-159T) in the promoter of the *CD14* gene has been described and found to be directly associated with different levels of *sCD14* expression (31) and inversely associated with levels of total serum IgE (31–33). In addition, *sCD14* levels were directly correlated to IFN- γ responses and inversely correlated to IL-4 responses by mitogen-stimulated peripheral blood mononuclear cells (31). The preponderance of Th2-like cytokines such as IL-4 may thus tilt the immune response in favour of IgE production, hence explaining the higher levels of IgE observed in children homozygotes for the C allele, who had lower levels of circulating *sCD14* (31). Ongoing functional studies indicate that transcription factors of the Sp family bind to a consensus sequence that includes *CD14* C-159T. The T and C allele have different binding affinity for Sp

proteins and this results in differences in the transcriptional activity of the two variants (34). The inverse association between levels of *sCD14* and IgE raises the possibility that exposure to bacterial products, such as LPS, may modulate the IgE regulation, possibly by activating innate immune pathways that promote Th1 differentiation and suppress Th2-dependent IgE responses. In support of this hypothesis, it has recently been found that children raised in rural areas with heavy exposure to animals and LPS have remarkably lower prevalence of allergic diseases compared to children living in the same areas, but who are not exposed to animals (35, 36). In addition, decreased allergic sensitization has been reported among subjects raised in homes with indoor pets such as cats and dogs (37, 38). Genetic factors that modify LPS responsiveness may thus influence susceptibility to the development of allergic diseases (39). Interestingly, several studies have suggested that, in individuals with established asthma, exposure to LPS may increase the severity of the disease (40). It is therefore possible that the interaction between LPS exposure and genetic variants in the LPS response pathway may have different consequences depending on the timing of exposure. In a rat model, early exposure to LPS inhibits allergic sensitization, whereas the LPS administration in already sensitized animals exacerbated allergic inflammation (41). Interestingly, an association with total serum IgE levels and the *CD14*-159 T allele has been reported among subjects with documented allergy to animals who worked at Jackson Laboratories (Bar Harbor, Maine) the largest repository of mouse strains in the USA (42). It can therefore be speculated that the heavy LPS exposure associated with such an environment, acting on the established atopic background of these individuals, may stimulate a higher IgE production in those carrying the T allele, who are more likely to have higher levels of *sCD14* and, consequently, higher LPS-binding activity. This putative mechanism may explain the relationship between *CD14*-159 T and atopy observed in a highly inbred population (43).

A further element of complexity in the genetic variability of *CD14* has recently been introduced. Four additional SNPs in the promoter region have been identified (44, 45): A-1619G, G-1359T, A-1145C and A-809C. A large sample of the same population previously assessed for *CD14* C-159T (31) was also genotyped for G-1359T and A-1145C. Given the tight linkage disequilibrium existing between these SNPs, only 3 haplotypes (–1359T/–1145 A/–159C, –1359G/–1145G/–159T and –1359G/–1145 A/–159C, respectively) were virtually present out of all possible 1359/–1145/–159 haplotypes. Carriers of the –1359T/–1145 A/–159C haplotype on both chromosomes had significantly higher levels of IgE than any other group. The addition of preliminary data on A-1619G and A-809C did not modify such pattern.

Although the group with the highest level of IgE had the lowest concentration of *sCD14*, suggesting an inverse correlation between these two parameters, *sCD14* was not significantly associated to any specific haplotype.

Not surprisingly, these data suggest that the association between *CD14* C-159T and *sCD14* levels may be part of just one of the many mechanisms involved in IgE regulation. Modulation of IgE levels by the newly described SNPs in the upstream part of the *CD14* promoter is likely to be related to other pathways. For example, further research is needed to explore the relationship between SNPs in the *CD14* promoter and expression of *mCD14*.

Linkage disequilibrium with closely linked variants in chromosome 5q may also explain these findings. As previously mentioned, the –159 T allele was associated to atopy among the highly inbred population of the Hutterites; however, that was true only when –159 T was part of a haplotype containing a specific D5S642 marker allele (D5S642, 185-bp) and not other D5S642 alleles, suggesting linkage disequilibrium with another close, unknown variant (43). Furthermore, linkage but not association between *CD14* C-159T and atopy has been shown recently (21).

Conclusions

The data we reviewed suggest that LPS, *CD14* and Th polarization may be part of a complex network that is triggered by different environmental stimuli and can either activate or suppress IgE responses. Allergens stimulate adaptive responses that result in Th2 differentiation and enhanced IgE production. Downregulation of *CD14*, with reduced IL-12 and IL-18 expression, may be a critical factor in allowing the predominance of Th2 polarization. On the other hand, bacterial products trigger innate immune pathways, and, by enhancing *CD14* expression and LPS responsiveness, are able to increase IL-12 and IL-18 expression and decrease Th2 differentiation, which in turn results in inhibition of IgE responses.

CD14 is just one of the many genes that appears to contribute to the expression of the IgE-mediated allergic phenotype and further research is needed to investigate the mechanisms by which SNPs in the *CD14* promoter regulate the expression of this gene and its role in IgE regulation. Nonetheless, it provides a model of gene by environment interaction that highlights the importance of research efforts aimed at identifying candidate genes for allergy and their relation to pathways involved in the development and regulation of the immune response. Advances in this area of research may provide the basis for new preventive and therapeutic strategies.

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