

EDITORIAL

Learning from discrepancies: CD14 polymorphisms, atopy and the endotoxin switch

In 1999, Baldini et al. published the intriguing finding that a single nucleotide polymorphism (SNP) in the CD14 promoter, CD14-159/C→T, was associated with increased levels of soluble CD14 and decreased total serum IgE in the Children Respiratory Study (CRS), an unselected population enrolled at birth in Tucson, AZ [1]. This work received wide attention, coming as it did at a time when the role of innate immunity in interfacing with the environment and shaping adaptive responses was becoming the focus of intense interest. In this issue of *Clinical & Experimental Allergy*, a group led by Claudia Sengler and including researchers involved in the original work on CD14-159/C→T reports that no such association was found in the German Multicenter Allergy Study (MAS) cohort [2]. Genotype and phenotype assessments, ethnicity and age of the two populations were comparable if not identical, suggesting that this striking difference in results is not merely technical. Furthermore, inconsistencies between epidemiological findings in different populations are not isolated episodes. As an example, a SNP in the coding region of IL-13 which was found to be strongly associated with increased IgE levels, asthma and atopic dermatitis in populations from the US, Japan, Germany and the Netherlands [3–7] showed no association with allergic phenotypes in members of the Hutterite community of South Dakota [8]. An important question then arises. Are these discrepancies threatening cracks in an ambitious endeavour? Are they symptoms of an underlying weakness in the data or their analysis? Here we would like to argue that, far from undermining the value of genetic and epidemiologic studies, these discrepancies may reflect deep biological realities, and as such may highlight critical mechanisms that fine-tune gene expression and phenotype determination. In short, these are discrepancies we may learn from.

Developing our argument requires that we briefly discuss the potential role that genetic variation in CD14 may play in regulating IgE levels and the allergic phenotype. CD14 is unique among innate immunity genes in at least two respects. Although initially characterized as the endotoxin/LPS receptor [9], CD14 was later found to have a much broader spectrum of biological activity. While the signalling partners of CD14, the Toll-like receptors (TLRs), appear to be somewhat restricted in their ability to sense their ligands (TLR4 for gram-negative bacteria, TLR2 for gram-positive bacteria, TLR9 for bacterial DNA and so on; reviewed in [10]), CD14 mediates sensitive responses to different pathogens (bacteria, both gram-negative and gram-positive, mycobacteria, possibly viruses; reviewed in [11]). Genetically determined variation in CD14 expression may therefore affect a spectrum of responses wider than the one dependent on TLR-mediated recognition.

CD14 has another peculiar property. It exists not only on the membrane of monocytes and neutrophils, but also as a soluble protein found in serum at microgram concentrations. Soluble CD14 has the ability to confer pathogen responsiveness to cells,

such as epithelial and endothelial cells, that do not express CD14 on their membrane. As a consequence, variation in serum levels of soluble CD14 would be expected to have complex functional repercussions.

What are the mechanistic links between CD14 and IgE regulation? A genetically determined increase in CD14 expression could result in enhanced responsiveness to pathogen products (typically, endotoxin) in early life, when the relative balance between Th1- and Th2-mediated immunity is finely adjusted. Robust CD14-mediated reactions to pathogens would elicit strong IL-12/IL-18 expression by innate immune pathways. Thus Th1, rather than Th2, differentiation would be favoured, decreasing the likelihood of vigorous IgE-dependent responses following allergen exposure [12]. Consistent with this view, farmers' children who were protected from atopic asthma and atopic sensitization expressed higher levels of CD14 in their blood cells [13]. On the other hand, CD14-mediated stimulation of monocyte/macrophages results in the secretion of cytokines (such as IL-6) which potentially amplify IgE synthesis induced by IL-4/IL-13 and CD40 engagement [14]. Activation of the CD14 pathway may therefore have a dual effect on IgE expression, inhibitory or enhancing, depending on the biological context and timing of stimulation.

The role of innate immunity in shaping allergic responses has been explored in a variety of animal models. However, the data about the role of endotoxin in the regulation of Th2 responses and IgE production are apparently conflicting [15]. Protection as well as exacerbation has been reported. Part of this discrepancy depends on the time at which pathogen exposure occurs. In a rat model, administration of LPS before allergen challenge was strongly protective against sensitization, whereas exposure after sensitization resulted in exacerbation of inflammation [16]. On the other hand, rather than more prone to develop high IgE levels, mice with a mutation in TLR4 that causes LPS unresponsiveness were incapable of mounting strong antigen (ovalbumin)-specific Th2 responses because of deficient dendritic cell maturation before pathogen exposure [17]. Even more intriguingly, when mice were immunized by inhalation of an ovalbumin preparation which had been rigorously purified and was LPS-free, no response was elicited. By contrast, when LPS at low or high doses was added to ovalbumin at the time of immunization, the mice developed Th2 responses with a low LPS dose, and strong Th1 responses with a high LPS dose. The cells recruited to the lung were eosinophils in one case, and neutrophils in the other [18].

These data uncover a dose-dependent relationship between environmental exposure to bacterial products and the outcome of immune responses which is not linear but bimodal, if not multimodal. The key to this relationship is what we propose to call the 'endotoxin switch' (Fig. 1). While the mechanisms underlying the switch remain to be determined, its functional implication is that exposure to a certain antigen concentration may

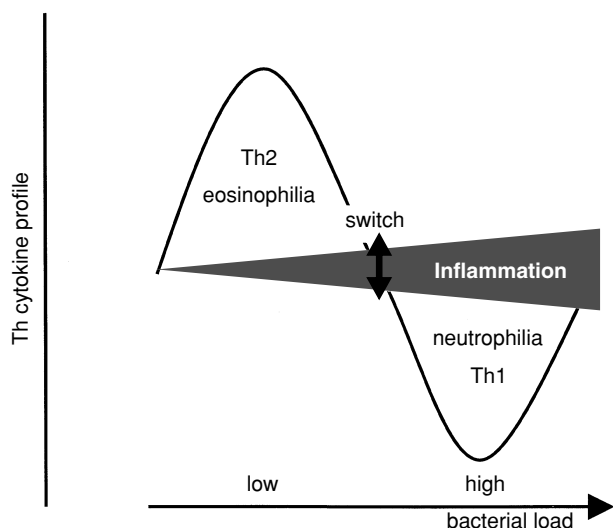


Fig. 1. The endotoxin switch. The curve represents the bimodal relationship between bacterial load in the environment and outcome of the immune response. The double pointed arrow corresponds to the occurrence of the switch from Th2 to Th1 responses.

result in opposite responses depending on the environmental context [19]. The immune system of our species may have been shaped by evolution to recognize antigens in a context relatively rich in bacterial pathogens [20]. A significant reduction in the endotoxin load, such as the one typical of western countries and lifestyles, may shift the response to the leftmost part of the curve, where Th2 responses predominate (Fig. 1). In this scenario, the endotoxin switch merges with the hygiene hypothesis in considering environmental pathogens sensed through pattern recognition receptors as major determinants of the type of response evoked by antigens (reviewed in [21]).

In the context of this model, polymorphisms in genes at the host/environment interface – CD14 first and foremost – may modulate immune responses by repositioning the endotoxin switch along the bacterial load axis (Fig. 2). Each polymorphic allele (i.e. different levels of gene expression or function), or more likely each haplotype, would have its own response curve and a distinct endotoxin concentration associated with the switch. For very low or very high endotoxin loads, Th responses would be similar across the population. The effect of subtle differences in levels of gene expression would become detectable only for bacterial loads within a narrow window (hatched area in Fig. 2). Carriers of CD14/-159 T with higher CD14 expression would require less environmental endotoxin to switch to Th1 responses and would therefore be more protected from allergic inflammation than the CD14/-159 C individuals.

In such a scenario, the discrepancy in the effects of CD14/-159 in the Tucson CRS and German MAS populations would reflect differences in the endotoxin loads to which these populations are exposed. The Tucson population, unlike the MAS cohort, would be within the intermediate range sensitive to small variations in CD14 expression (Fig. 2). This would explain the lack of association between allergic phenotypes and CD14/-159 in the German group. Of note, the model also accounts for the intriguing finding that the association between CD14/-159 and atopy recently reported in the Hutterites (whose main occupation is farming) was with the T rather than the C allele [8]. Extremely high endotoxin loads may bring about

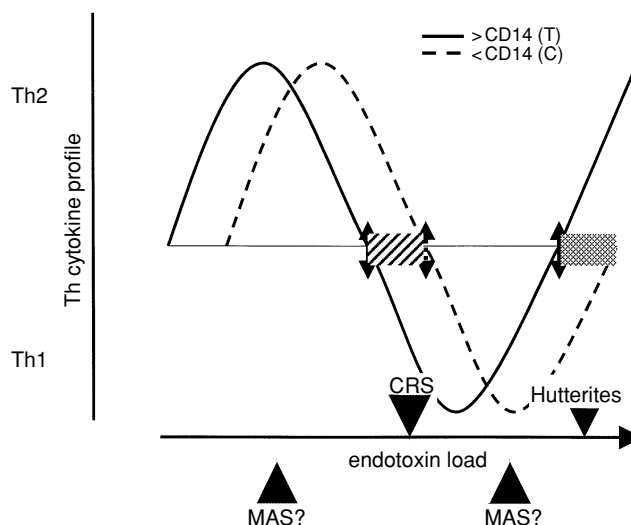


Fig. 2. CD14 SNPs and the endotoxin switch. The two curves represent CD14/-159 allele-specific relationships between endotoxin load and immune responses. The double pointed arrow corresponds to the occurrence of the switch from Th2 to Th1 responses. The hatched and dotted areas identify the window of opportunity within which genetic variation may impact on Th responses. Hypothetical levels of exposure for different populations ([1, 2, 8]) are noted on the endotoxin load axis.

a second switch, away from Th1 and again towards Th2 responses, which would now preferentially affect individuals with higher CD14 expression (dotted area in Fig. 2).

In conclusion, the complexity of gene–environment interactions and their pathogenetic effects appears to be humbling. What we see now is probably only the tip of the iceberg. In the near future we will need to reason more in terms of haplotypes, and we will need to generate animal models that better recapitulate the regulation of Th responses in outbred populations. Indeed, the Th1/Th2 dichotomy seen in inbred mice may not have an immediate equivalent in humans, where we find complex patterns of Th2 responses which can be both pathogenic and protective (unpublished data). The fundamental principles, however, may remain the same. Genetic variation in innate immunity genes at the interface with the environment may skew the immune response in critical ways by modulating the impact of pathogen exposure. Far from being met with disbelief or uneasiness, discrepancies in the association between specific SNPs and specific phenotypes may (and perhaps should) be seen as cogent reflections of the complexity at the interface between immunity and the environment. By comparing and contrasting the features of populations between which these discrepancies exist, we may find clues about critical genetic and environmental factors. The most promising approach to understanding the pathogenesis of complex diseases is thus likely to lie in a lively synergism between epidemiology, molecular genetics and mechanistic biology.

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