

Correspondence

Confounding effect of atopy on functional effects of the CD14/-159 promoter polymorphism

To the Editor:

In the September 2004 issue of the Journal, Böttcher et al.¹ presented an interesting study reporting an association between a polymorphism in the Toll-like receptor 4 (*TLR4*) gene and asthma. They explained this association by altered cytokine production, presumably caused by this variation. In their study of Swedish children, *TLR4* Asp299Gly (AG) heterozygosity was associated with atopic asthma and with lower LPS-induced IL-12 and IL-10 levels compared with *TLR4* Asp299Gly (AA) homozygosity. In addition, CD14/-159 TT homozygote individuals showed higher LPS-induced IL-12 levels than individuals carrying CD14/-159 CC and CT. Finally, LPS-induced IL-10 and IL-12 levels were lower in individuals with atopic asthma. Thus, the *TLR4* Asp299Gly (AG) polymorphism is associated with atopic asthma and asthma by itself with lower cytokine production. Consequently, the polymorphism is also associated with lower cytokine production.

This raises the question whether the observation on cytokine production in the Swedish study is confounded by disease status.

We can illustrate this with an example. We have investigated 40 adult individuals of a Dutch family study in whom the association of the CD14/-159 polymorphism and atopy severity previously was shown.² We assessed the association of the CD14/-159 genotype and both membrane-bound CD14 expression and LPS-induced (1 ng/mL) IL-10 and IL-12(p70) cytokine production *in vitro* of PBMCs in the presence of IFN- γ . In an attempt to disentangle atopic status and CD14/-159 genotype, we investigated these associations in 4 groups: 10 CD14/-159 CC atopic individuals, 9 TT atopic individuals, 10 CC nonatopic individuals, and 11 TT nonatopic individuals. We found that CC atopic individuals had a significantly lower percentage CD14⁺ monocytes compared with TT nonatopic individuals (median, 62%; range, 49-82%; vs median, 73%; range, 58-80%). However, the difference was even larger comparing all atopic

individuals with all nonatopic individuals (median, 61%; range, 45-82%; vs median, 73%; range, 57-86%; Fig 1). Furthermore, we also found higher levels of LPS-induced IL-10 production in atopic individuals (median, 1976 pg/mL; range, 1307-2966 pg/mL) compared with nonatopic individuals (median, 1548 pg/mL; range, 629-2937 pg/mL; $P = .04$) irrespective of the genotype. We could not confirm an association of the CD14/-159 genotype and LPS-induced IL-12 production.

In conclusion, atopy is an important confounder of the effects of the CD14/-159 genotype on membrane-bound CD14 in our study. This could also apply to the study of Böttcher et al.¹ It would be of interest if the authors present their data on the effects of the *TLR4* Asp299Gly (AG) and CD14/-159 genotype on cytokines stratified by atopic asthma. Of importance, our data suggest taking the disease status into account when analyzing functional effects of single nucleotide polymorphisms in a human population.

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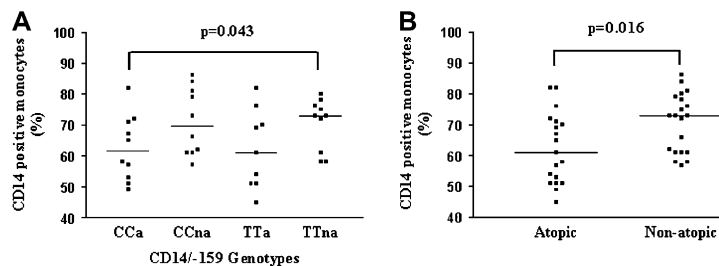


FIG 1. A, Significantly lower percentage of CD14⁺ monocytes in atopic individuals with CD14/-159 CC compared with nonatopic individuals with CD14/-159 TT. *a*, Atopic; *na*, nonatopic. Median is shown. **B**, Significantly lower percentage of CD14⁺ monocytes in atopic individuals compared with nonatopic individuals. Median is shown.