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## Toll-like receptors 2 and 4 and innate immunity in neutrophilic asthma and idiopathic bronchiectasis

We read with interest the article by Simpson *et al*<sup>1</sup> relating parameters of innate immunity, particularly expression of toll-like receptors (TLR)2, TLR4, CD14, SP-A and cytokines interleukin (IL)8 and IL1 $\beta$ , to disease in neutrophilic asthma and bronchiectasis. We agree that there is much to be gained from further analysis of innate mechanisms in both diseases. This is especially so in light of our findings implicating a role for natural killer (NK) cells in idiopathic bronchiectasis and the work from Umetsu's laboratory (Harvard Medical School, Boston, USA) on NKT cells in asthma.<sup>2,3</sup>

It may be useful to add one caveat and additional data to the case for innate mechanisms in neutrophilic asthma and idiopathic bronchiectasis.

Although it is self-evident that TLR2, TLR4 and CD14 activation may well indicate differences in endotoxin stimulation, considerable attention has recently focused on expression of TLR2 and TLR4 by T lymphocytes. This has implications both for the interface between innate and adaptive immunity, and for the regulation of the T cell response itself: a body of evidence now suggests that TLR2 is expressed by regulatory T cells (Treg), that TLR2 activation has a role in driving Treg expansion and that, in some cases, the ligands driving this may be endogenous rather than microbial.<sup>4,5</sup> In the light of such findings, it is important not to interpret TLR activation solely in terms of innate, microbial activation, particularly if it has not been possible to define the cell type responsible for the increase in mRNA. Put simply, changes in expression of TLR2 and TLR4 can, in some cases, bear on alterations in populations of regulatory and effector T cells, rather than differences in microbial exposure. In the sputum samples analysed by Simpson *et al* from patients with asthma, the relatively low lymphocyte counts might make this unlikely; in their bronchiectasis samples, where the lymphocyte counts are higher, it is impossible to clarify the situation without further analysis—for example, by multiparameter flow cytometry.

Simpson *et al* ask whether polymorphisms in TLRs may account for different phenotypes observed in disease studies. Single nucleotide polymorphisms of TLRs have been linked to susceptibility to infectious diseases.<sup>6</sup> With such a hypothesis in mind, we recently compared the frequency of the TLR2 Arg753Gln and the TLR4 Asp299Gly and Thr399Ile polymorphisms in patients with idiopathic bronchiectasis and controls. The Asp299Gly TLR4 polymorphism (and the co-segregating Thr399Ile polymorphism) is, as indicated by Simpson *et al*, proven to be bona fide, with respect to both effect on endotoxin binding and disease associations. Similarly, the TLR2 Arg753Gln polymorphism is functional and has been associated with altered susceptibility to several infectious diseases including herpes simplex virus, cytomegalovirus and rheumatic

**Table 1** Toll-like receptor (TLR)2 and TLR4 restriction fragment length polymorphism analysis\*

Gene polymorphism	Restriction enzyme	Restriction fragment length (bp)	Primer sequence
TLR2 Arg753Gln	Msp I	Wild type (G allele): 104 + 25 Arg753Gln (A allele): 129	F: cattccccagcgtcttgcagctcc R: ggaacctaggactttatcagctc
TLR4 Asp299Gly	Nco I	Wild type (A allele): 188 Asp299Gly (G allele): 168 + 20	F: agcaatactagactactactccatg R: gagagatttgatttcaatgctggg
TLR4 Thr399Ile	Hinf I	Wild type (C allele): 124 Thr399Ile (T allele): 98 + 26	F: gggtgctgttccaagtgatttgggagaa R: ggaatccagatgttctagttgtcaagcc

  

Gene polymorphism	Allele	Controls n = 86 (TLR2) n = 85 (TLR4)	Idiopathic bronchiectasis n = 94	Odds ratio (95% CI)	p Value
TLR2 Arg753Gln	G allele	169 (98.2)	180 (95.7)	—	—
	A allele	3 (1.8)	8 (4.3)	2.50 (0.65 to 9.59)	NS
TLR4 Asp299Gly	A allele	162 (95.3)	177 (94.1)	—	—
	G allele	8 (4.7)	11 (5.9)	1.26 (0.49 to 3.21)	NS
TLR4 Thr399Ile	C allele	163 (95.9)	177 (94.1)	—	—
	T allele	7 (4.1)	11 (5.9)	1.45 (0.56 to 3.82)	NS

F, forward; R, reverse; TLR, toll-like receptor.

The table shows the frequencies of TLR2 and TLR4 polymorphisms in patients with idiopathic bronchiectasis and in controls.

\*As described previously by Folwaczny *et al*.<sup>7</sup>

fever. The polymorphism was initially described in the context of a possible enhanced risk of staphylococcal sepsis.<sup>6</sup>

A total of 94 unrelated individuals with a diagnosis of idiopathic bronchiectasis recruited at the Royal Brompton Hospital, London, UK, and 86 heart/lung transplant donor controls from the Harefield Hospital, London, UK, were studied. The ethics committee of the Royal Brompton & Harefield & NHLI approved the study and all patients gave written informed consent for participating in the study. A diagnosis of idiopathic bronchiectasis was made where there was bilateral, predominately lower lobe bronchiectasis on CT, chronic rhinosinusitis and all known underlying causes had been excluded.<sup>2</sup> Genomic DNA was extracted from peripheral blood using a high-salt technique. Typing of the TLR2 Arg753Gln, TLR4 Asp299Gly and TLR4 Thr399Ile polymorphisms was carried out using polymerase chain reaction restriction fragment length polymorphism analysis as described previously. Allele frequency was determined and statistical analysis carried out using the Simple Interactive Statistical Analysis. Allele frequency comparisons were made using the Fisher's exact test. Only minor differences in gene frequency were observed, none of which were statistically significant (table 1).

In summary, we make the following points, building on the case made by Simpson *et al*. First, although it seems likely that the pathogenesis in both asthma and idiopathic bronchiectasis has a strong innate immunity component, it is important to note that the components contributing to TLR changes can be complex, including events at the innate-adaptive immune response interface, as well as interactions between effector T cells and Tregs. Specifically, it can be hard to interpret increases in the expression of TLR in the absence of data on the particular cell type(s) accounting for the change. Second, faced with clinical phenotypes of this level of complexity, it is perhaps not surprising that, as has been the case in analysis of sepsis and other complex disease end points, the effects of TLR polymorphisms are not striking.

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