

Receptors of the innate immune system help identify the nature of invading pathogens. We now show that these receptors also participate in the recognition of aeroallergens

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Aims of the Project

- Aim 1: (a) To determine the contribution of alveolar epithelial cell TLR4 expression in the pathogenesis of emphysema and
(b) To ascertain whether LPS-induced emphysema is mediated through modulation of sphingolipids.
- Aim 2: To ascertain whether ceramide-mediated emphysema is dependent on TLR4 ligation.
- Aim 3: To temporally profile three phenotypically similar experimental models of emphysema to identify common and early signature genes that underpin lung destruction.

In recent times, the molecular characterisation of pattern recognition receptors and the identification of their cognate ligands, termed pathogen-associated molecular patterns, has been elucidated. This has provided a framework to better understand how the host recognises micro-organisms, and then decodes this information to elicit an appropriate innate inflammatory and adaptive immune response.

For example, the pattern recognition receptor toll-like receptor (TLR)2 recognises lipoteichoic acid, a lipid contained in the wall of most bacteria, to promote a monocyte and neutrophilic inflammation and induce the production of the CD4+ T helper type (Th)1-inducing cytokine, interleukin (IL)-12 from antigen presenting cells.

In turn, the effector Th1 cells produce IFN γ which enhances the microbicidal activity of the innate inflammatory cells at the site of infection. One of the immunopathological features of allergic inflammation is the infiltration of Th2 cells to the site of disease. As with the Th1 cell subset, Th2 cells release key effector cytokines such as IL-4, IL-5 and IL-13, which feedback and promote the survival and activation of innate inflammatory cells.

For instance, IL-5 is the terminal differentiation and survival factor of eosinophils and primes their activation state at sites of allergic inflammation. However, although Th2 cytokine expression can induce the cardinal features of eosinophilic asthma, the contribution of pattern recognition receptors to the generation of Th2 responses to clinically relevant aeroallergens remains poorly defined.

To determine the role of specific TLRs in the development of allergen-specific Th2 responses in vivo, we employed a mouse model of allergic asthma using the clinically relevant aeroallergen house dust mite (HDM). Critically, mice were sensitised and challenged to HDM via the airways, and in the absence of the exogenous adjuvant aluminium hydroxide, now known to activate the pattern recognition receptor, cryopyrin.

In initial experiments we employed mice deficient in the Toll-IL-1 receptor domain adaptor molecule MyD88 since all of the known TLRs with the exception of TLR3, signal through this adaptor protein. We observed that mice deficient in MyD88 were protected from the cardinal features of allergic asthma, including granulocytic inflammation, Th2 cytokine production and airway hyperreactivity, suggesting that the activation of TLRs and/or IL-1 family receptors (which also employ MyD88) was necessary for the development of antigen-specific Th2 responses.

To address whether HDM extract was able to activate any of the known TLRs, HEK cells that selectively over-expressed specific TLR were cultured with HDM extract and luciferase activity was measured as a marker of NF- κ B activation. Using these reporter cell lines we demonstrated that HDM extract activated TLR2 and TLR4, but not TLR3, TLR5 or TLR9. Armed with this information, we repeated our HDM model using mice deficient in TLR2 or TLR4. We

observed that the magnitude of allergic airway inflammation and hyperreactivity was attenuated only in mice deficient for TLR4.

Since the activation of distinct TLR may directly or indirectly licence local antigen presenting cells, we next addressed whether the absence of TLR2, TLR4 or MyD88 would alter the expression of co-stimulatory molecules associated with the polarisation of naïve T cells toward a Th2 phenotype.

Remarkably, whereas the pro-Th2 co-stimulatory molecule OX40 ligand was elevated on myeloid dendritic cells in the draining lymph nodes during allergic sensitization of WT and TLR2-deficient mice, this up-regulation failed to occur in the absence of TLR4 or MyD88. Thus, the diminished Th2 response present in MyD88- and TLR4-deficient mice was associated with the inability of local antigen-presenting cells to provide Th2-inducing factors at the time of T cell priming.

Finally, we observed that HDM exposure *in vivo* led to the development of antigen-specific IL-17-producing cells. Notably, IL-17 is produced by a newly characterised and distinct subset of CD4+ T helper cells, termed Th17 cells, that promote neutrophilic inflammation.

Intriguingly, the development of HDM-specific Th17 responses and associated airway neutrophilia was attenuated in the absence of MyD88 but not TLR4. Indeed, TLR4-deficient mice generated a heightened Th17 response and elevated numbers of airway neutrophils.

Taken together, these data suggest that Th2- and Th17-mediated inflammation generated upon inhalational HDM exposure is differentially regulated by the presence of microbial products and the activation of distinct MyD88-dependent pattern recognition receptors.

Significantly, our findings suggest that the altered expression or responsiveness of TLR4 may modulate the type of granulocytic inflammation within the airways and hence the development of distinct endophenotypes of asthma.

The award of the fellowship partly supported Dr. Phipps' salary for 2 years. In addition to performing the experiments described in the project, he has also published work on the role of TLRs in the development of allergic asthma.