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Using house dust extracts to understand the immunostimulatory activities of living environments ☆

Glenda Batzer[a], Diane P. Lam[a], Petra Paulus[a], Jared Boasen[a], Nicholas Ng[a], Anthony A. Horner[a,b,c,*]

[a] *Department of Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0663, USA*
[b] *Department of Pediatrics, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0663, USA*
[c] *The Sam and Rose Stein Institute for Aging, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0663, USA*

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Abstract

Laboratory and epidemiological studies have provided indirect but compelling evidence that toll-like receptor (TLR) signaling pathways play an important role in host responsiveness to ambient immunostimulatory factors. Nonetheless, direct evidence is limited. This paper will present our experience investigating the innate immunostimulatory activities of sterile house dust extracts (HDEs). In initial studies, bone marrow derived dendritic cells (BMDDCs) were cultured with HDEs, and cytokine production and co-stimulatory molecule expression were evaluated. In additional experiments, the TLR dependence of these responses was determined. HDEs induced concentration-dependent BMDDC activation. Moreover, the relative bioactivities of HDEs correlated with their endotoxin content. Finally, HDE-mediated responses were found to be partially dependent on TLR2, TLR4, and TLR9 and almost completely dependent on MyD88. These investigations provide the first direct evidence that TLR signaling pathways play a key role in innate responsiveness to non-infectious factors ubiquitous in living environments.

Keywords: Allergy; Dendritic cell; Endotoxin; House dust; Hygiene hypothesis; Toll-like receptor

Introduction

Over the last century, prevalence rates for asthma and other allergic diseases have increased dramatically in the industrialized world but not in underdeveloped countries (Horner and Raz, 2003; Latvala et al., 2005; Wills-Karp et al., 2001). While a topic of intense speculation and investigation, it remains to be determined why. Nonetheless, there is consensus agreement that allergic disease prevalence rates in affected countries have increased too rapidly to be a consequence of genetic drift (Horner and Raz, 2003; Liu and Murphy, 2003;

Abbreviations: BMDDC, Bone marrow derived dendritic cell; HDE, house dust extract; ISS, immunostimulatory sequence phosphorothioate oligodeoxynucleotide; MAMP, microbe-associated molecular pattern; P-3-C, lipopeptide Pam-3-Cys; TLR, toll-like receptor

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*Corresponding author. University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0663, USA. Tel.: +1 858 534 5435; fax: +1 858 534 0409.
E-mail address: ahorner@ucsd.edu (A.A. Horner).

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Martinez and Holt, 1999; von Mutius, 2002; Wills-Karp et al., 2001). From this perspective, there is a strong imperative to understand how external factors impact on host immunity in general and allergic risk in particular.

Allergen exposure is a clear prerequisite for the development and persistence of antigen-specific hypersensitivities (Huss et al., 2001; Platts-Mills et al., 1991, 2004). Nonetheless, while for some allergens (i.e. cockroach and mites), higher exposure levels have been associated with an increased risk of sensitization, for other allergens (dogs, cats, molds), this correlation has not been found (Frew, 2005; Huss et al., 2001). Furthermore, several studies have shown that for allergens associated with animals, increased levels of exposure are associated with decreased sensitization rates (Frew, 2005; Hesselmar et al., 2005; Platts-Mills et al., 2004). These and other observations have prompted speculation that aside from allergens, additional factors ubiquitous in living environments influence the balance between allergen tolerance and hypersensitivity. In line with this view, a number of epidemiological and laboratory studies provide indirect evidence that non-invasive contact with microbes influences immune development, homeostasis, and as a consequence, allergic risk. For example, intestinal flora have been shown to facilitate post-natal immune development (Mazmanian et al., 2005; Sudo et al., 1997) and prevent Th2 polarized responses to dietary allergens (Rask et al., 2005; Sudo et al., 2004) in mice. Moreover, human studies suggest that the intestines of atopic and non-atopic infants tend to be colonized with unique bacterial species (Bottcher et al., 2000). Finally, in several clinical trials, ingestion of probiotic bacteria has been found to be effective in treating eczema and in preventing development of additional allergic manifestations in high atopic risk infants (Bjorksten, 2005; Rautava et al., 2005). Nonetheless while such observations provide supportive evidence, our present understanding of the mechanisms that underlie this apparent symbiosis between physiologic microbial exposures and allergic risk remains incomplete.

Atopy and toll-like receptors

In order to ensure survival, the major task of mammalian immunity is the rapid detection and neutralization of infectious organisms (Gazzinelli et al., 2004; Picard et al., 2003; Takeda et al., 2003; Zhang et al., 2004). In part, surveillance is achieved by germline-encoded receptors that recognize a wide range of microbe-associated molecular patterns (MAMPs) not produced by higher eukaryotes (Medzhitov and Janeway, 2002; Philpott and Girardin, 2004; Takeda et al., 2003). Innate MAMP recognition provides for rapid, robust, and relatively microbe-specific immunity. With the possible exception of toll-like receptor (TLR) 11, all TLRs identified to date are expressed at varying levels by a wide range of mononuclear cells involved in innate and adaptive immunity and by the polymorphonuclear cells that participate in end organ inflammatory responses (Takeda et al., 2003; Zhang et al., 2004). Moreover, heterogeneity in extra-cellular domains allows for TLR recognition of a wide range of biochemically distinct microbial elements, while variability in their intra-cellular signaling pathways suggests the potential for ligands of different TLRs to induce distinct immunological responses (Horner and Raz, 2003; Takeda et al., 2003). Finally, purified ligands for several TLRs have been found to prevent or promote the development of Th2-biased hypersensitivities, in animal models of asthma and other atopic diseases (Chisholm et al., 2004; Eisenbarth et al., 2002; Horner and Raz, 2002, 2003; Racila and Kline, 2005; Tsalik, 2005). Such characteristics have prompted speculation that in addition to their role in innate defense against infection, TLRs might also mediate the modulatory influence of microbial exposures on allergic diseases and other diseases of immune dysregulation (Braun-Fahrlander et al., 2002; Gereda et al., 2000; Horner and Raz, 2003; Martinez and Holt, 1999; Wills-Karp et al., 2001).

In support of this view, endotoxin (TLR4) has been found to be ubiquitous in living environments, with higher concentrations reported in homes that have regular exposures to animals than in homes without (Braun-Fahrlander et al., 2002; Gereda et al., 2001). Moreover, infants raised in homes with high ambient endotoxin levels have been suggested to be at low relative risk for developing allergic hypersensitivities in many, although not all published reports (Braun-Fahrlander et al., 2002; Gereda et al., 2000). However, it is important to note that despite this apparent association between ambient endotoxin exposure levels and allergic risk, endotoxin-rich environments also generally contain elevated levels of other immunostimulatory microbial products. These include muramic acid, a breakdown product of peptidoglycan (TLR2), and bacterial DNA (TLR9) (Roy et al., 2003; van Strien et al., 2004). Furthermore, several man-made pollutants have been found to promote the development of allergic hypersensitivities (Saxon and Diaz-Sanchez, 2005). While much has been learned in recent years, such complexity in the content of daily exposures has hampered efforts to develop a comprehensive understanding of their impact on the development of allergic diseases.

Rationale for studying the immunological activities of house dust extracts

Given the difficulties inherent in determining which environmental exposures have the greatest impact on