

Images in Allergy and Immunology

Toll-like receptors and atopy

Pierre Olivier Fiset, BSc, Meri Katarina Tulic, PhD, and Qutayba Hamid, MD, PhD, Editors

Editor's note: This feature, Images in allergy and immunology, is designed to highlight current concepts of the immunopathology of allergic diseases and other common immunologically mediated diseases. The presentation will appear as sets of images that involve cross-pathology, histopathology, and molecular pathology and will cover a range of topics of interest to allergists and immunologists.

The Toll-like receptors (TLRs) are a recently discovered family of receptors involved in the innate recognition of pathogens. TLRs have much homology to the IL-1 receptor family and the *Drosophila* Toll protein, and at least 10 distinct TLRs have now been identified in human subjects (Fig 1). TLR ligands are highly conserved structures and molecules present on many pathogens, the so-called pathogen-associated

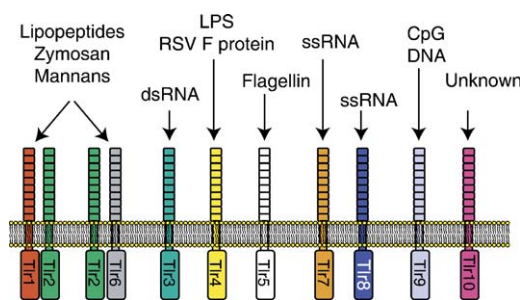


FIG 1. Ten distinct TLRs exist in human subjects, recognizing many PAMPs. TLRs can associate as heterodimers changing their ligand specificity.

From Meakins-Christie Laboratories, Department of Pathology and Medicine, McGill University, Montreal, Canada.

Received for publication April 21, 2005; accepted for publication April 22, 2005.

Available online June 17, 2005.

Reprint requests: Qutayba Hamid, MD, PhD, McGill University, Meakins-Christie Laboratory, 3626 St Urbain St, Montreal, Canada H2X 2P2. E-mail: qutayba.hamid@mcgill.ca.

J Allergy Clin Immunol 2005;116:467-70.

0091-6749/\$30.00

© 2005 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2005.04.034

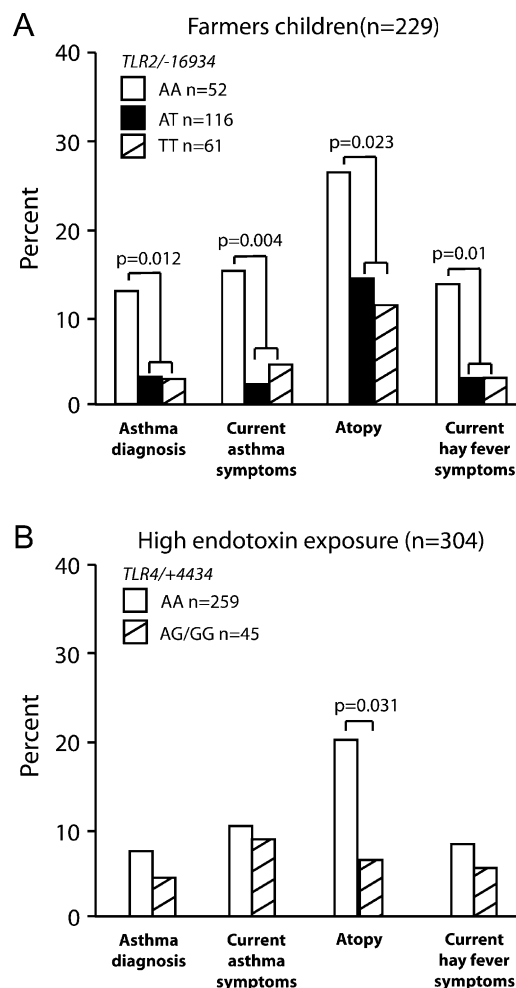


FIG 2. A, Prevalence of asthma, atopy, and current hay fever symptoms in *TLR2*-16934 in farmers' children. B, Prevalence of asthma, atopy, and current hay fever symptoms in *TLR4*+4434 in children exposed to high endotoxin concentrations.¹

molecular patterns (PAMPs). Some PAMPs are bacterial molecules, such as lipopeptides, mannans, LPSs, flagellin, and CpG DNA. Other PAMPs recognized by TLRs include virus- and fungus-associated molecules. Triggering of TLRs leads to expression of many genes involved in inflammatory responses to pathogens, leading to cell activation, differentiation, proliferation, and cell recruitment. Because of their strong immunostimulatory capacities, many PAMPs are currently studied as potential treatment agents for allergic diseases.

Because the TLRs are part of the innate immune system, they are not modified during an immune response and are passed on to the progeny with little genetic change. This has prompted genetic studies to determine whether specific single nucleotide polymorphisms in the TLR genes are associated with atopy. A recent study has suggested a polymorphism in *TLR2* and *TLR4* in Europeans to be associated with decreased atopy, dependent on PAMP exposure

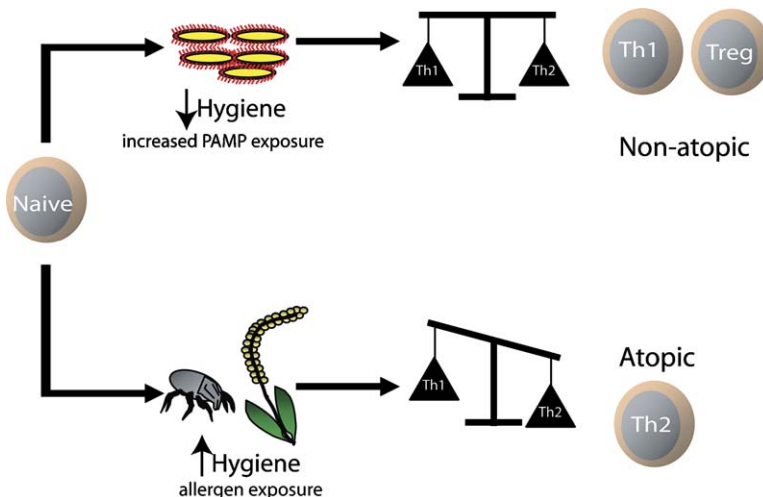


FIG 3. The hygiene hypothesis states that exposure to microorganisms (decreased hygiene) during early age is important for the development of a balanced immune system. Increased hygiene leads to an uncontrolled T_H2 immune response to allergens, resulting in atopic diseases.

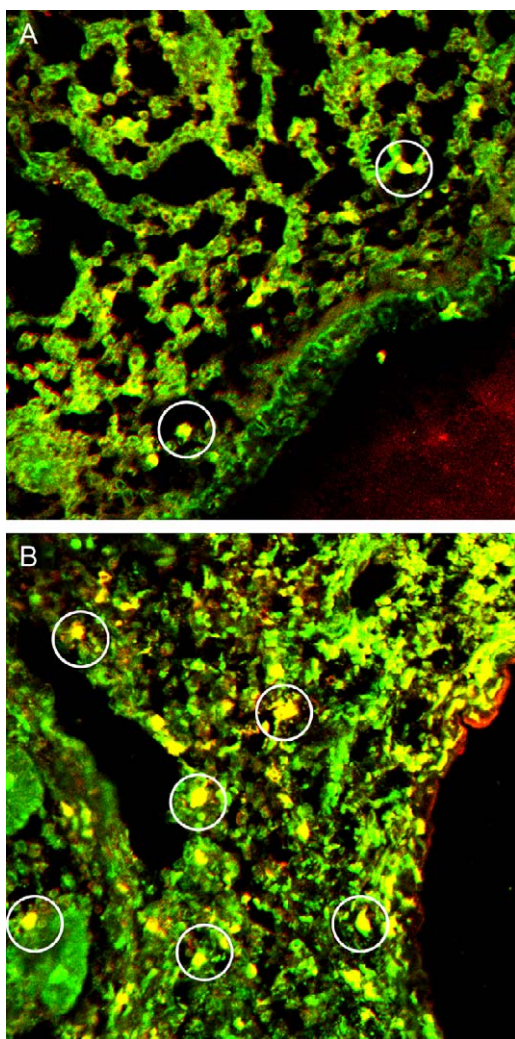


FIG 4. Detection of bromodeoxyuridine-positive proliferating cells colocalized with CD3-positive cells in explants of nasal mucosa of children. The explants were stimulated with LPS (0.1 $\mu\text{g}/\text{mL}$) for 2 hours (**A**) and 24 hours (**B**). Ten percent colocalization was seen at 2 hours, and 70% colocalization was seen at 24 hours.³

(Fig 2).¹ On the other hand, another study showed no association of atopy and polymorphisms in *TLR2*, *TLR3*, *TLR4*, and *TLR9* in Japanese populations.²

Evidence from epidemiologic studies has shown an association between high exposure to PAMPs during early life with decreased levels of atopic diseases and asthma. This has led to the proposal of the hygiene hypothesis, which states that lack of a “pathogenic pressure” (increased hygiene) in early childhood results in an imbalanced immune system hypersensitive to allergens (Fig 3). Thus atopy is associated with increases in T_H2 cytokines compared with T_H1 or immunoregulatory cytokines. Tulic et al³ have shown that LPS can cause a proliferation of CD3-positive cells in the nasal mucosa of children (Fig 4). This was associated with increases in IL-2-positive, IL-12-positive, and IFN- γ -positive cells without increases in T_H2 cytokines and MBP-positive cells (Fig 5). It has been also shown that LPS can inhibit allergen-induced increases in IL-4-positive, IL-5-positive, and IL-13-positive cells, as well as in MBP-positive and tryptase-positive cells.⁴ These effects were determined to be due to the increase of IL-10, IL-12, and IFN- γ induced by LPS. Additionally, in this same study TLR4-positive cells were higher and more responsive to LPS in children compared with adults.

PAMPs are also studied to treat patients with atopic diseases. In this context PAMPs are used as adjuvants for current immunotherapy regimens to reduce the antigen dose needed for therapy and to promote the development of immunoregulatory mechanisms. For example, combining CpG DNA with ragweed immunotherapy has been shown to provide potential clinical benefits. In a mouse model of allergy, physical linking of CpG DNA to ragweed protein inhibited IgE expression, inhibited IL-5 expression, and promoted IFN- γ expression.⁵ Physical linking of the CpG DNA to the ragweed protein enhanced the effects, suggesting that cells reacting to the allergen also have TLR9

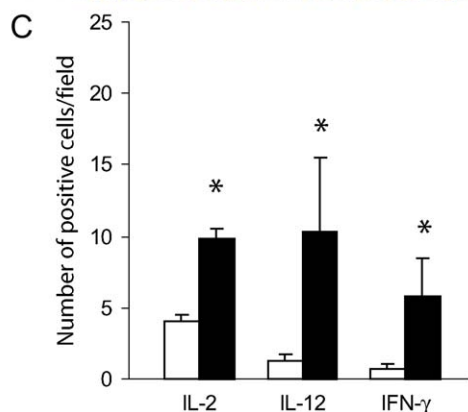
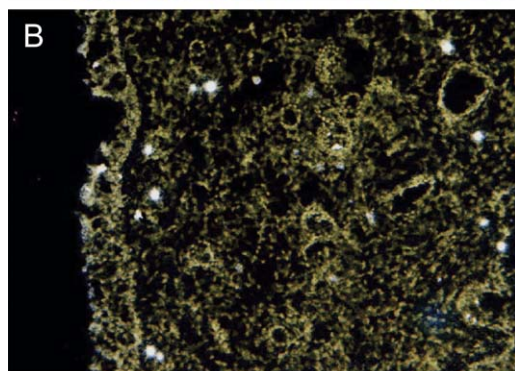
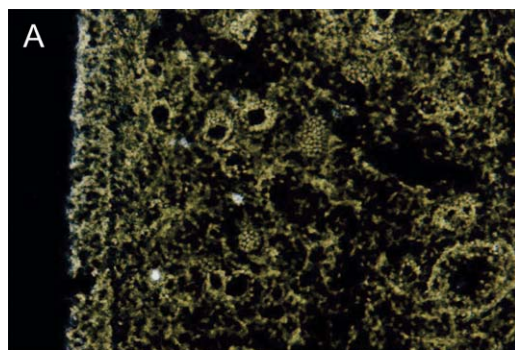


FIG 5. IL-12 *in situ* hybridization of sections taken from nasal explants without stimulation (A) and stimulated with 0.1 µg/mL LPS (B). IL-2, IL-12, and IFN-γ profile after stimulation with 0.1 µg/mL LPS (C). Open bars indicate no stimulation, and filled bars indicate LPS stimulation.³

activated by the CpG DNA (Fig 6). Ragweed CpG DNA injections in human clinical trials has been shown to inhibit allergen-induced IL-4 mRNA expression, IL-5 mRNA expression, and MBP-positive cell numbers in the nasal mucosa of allergic patients (Fig 7) and to reduce chest and nasal symptom scores.⁶ The compound can also increase the number of TLR9-positive cells in the nasal mucosa (Fig 8). As the role of PAMPs and TLRs is clarified in atopy and TLRs are better characterized, development of new therapies to both prevent atopic diseases and treat existing disease will be possible.

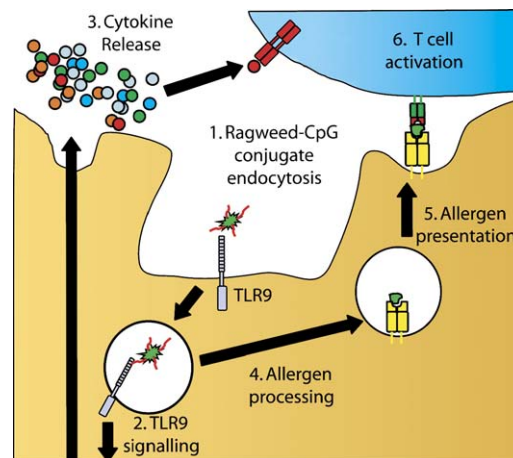


FIG 6. Linking of the allergen to an immunostimulatory CpG DNA sequence increases the potency of the ragweed CpG DNA vaccine for immunotherapy. The same antigen-presenting cell is activated by the complex, through TLR9, to synthesize cytokines and increase antigen presentation of the allergen. Allergen presentation and cytokines activate allergen-specific T cells to change their cytokine profile.

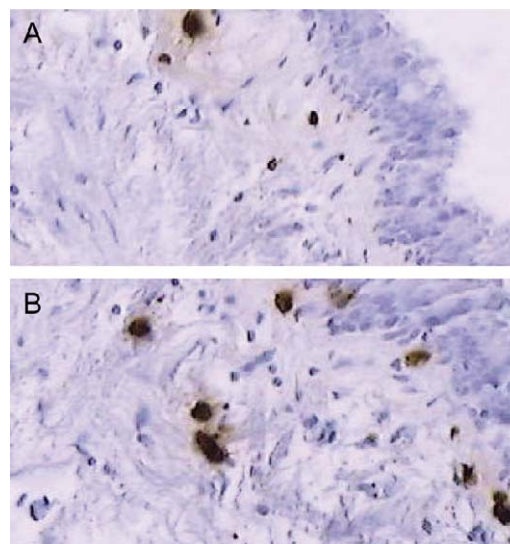


FIG 7. Horseradish peroxidase immunocytochemistry for MBP-positive cells in sections of patients receiving the ragweed CpG DNA vaccine for immunotherapy (A) or placebo (B).

REFERENCES

1. Eder W, Klimecki W, Yu L, von Mutius E, Riedler J, Braun-Fahrlander C, et al. Toll-like receptor 2 as a major gene for asthma in children of European farmers. *J Allergy Clin Immunol* 2004;113:482-8.
2. Noguchi E, Nishimura F, Fukai H, Kim J, Ichikawa K, Shibasaki M, et al. An association study of asthma and total serum immunoglobulin E levels for Toll-like receptor polymorphisms in a Japanese population. *Clin Exp Allergy* 2004;34:177-83.
3. Tulic MK, Manoukian JJ, Eidelman DH, Hamid Q. T-cell proliferation induced by local application of LPS in the nasal mucosa of nonatopic children. *J Allergy Clin Immunol* 2002;110:771-6.
4. Tulic MK, Fiset PO, Manoukian JJ, Frenkiel S, Lavigne F, Eidelman DH, et al. Role of toll-like receptor 4 in protection by bacterial lipopolysaccharide in the nasal mucosa of atopic children but not adults. *Lancet* 2004;363:1689-97.

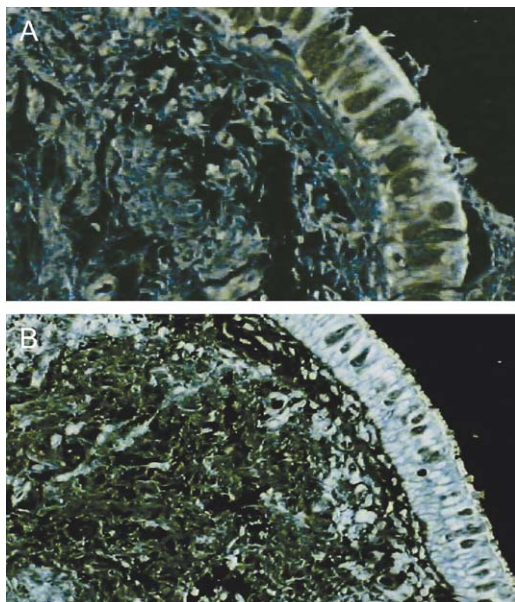


FIG 8. *In situ* hybridization for TLR9 mRNA—positive cells in sections of patients receiving placebo (A) or the ragweed CpG DNA vaccine for immunotherapy (B).

5. Tighe H, Takabayashi K, Schwartz D, Van Nest G, Tuck S, Eiden JJ, et al. Conjugation of immunostimulatory DNA to the short ragweed allergen Amb a 1 enhances its immunogenicity and reduces its allergenicity. *J Allergy Clin Immunol* 2000;106:124-34.
6. Tulic MK, Fiset PO, Christodoulou P, Vaillancourt P, Desrosiers M, Lavigne F, et al. Amb a 1-immunostimulatory oligodeoxynucleotide conjugate immunotherapy decreases the nasal inflammatory response. *J Allergy Clin Immunol* 2004;113:235-41.

Chronic active Epstein-Barr virus infection of natural killer cells presenting as severe skin reaction to mosquito bites

Susan E. Pacheco, MD, Stephen M. Gottschalk, MD, Mary V. Gresik, MD, Megan K. Dishop, MD, Takayuki Okamura, MD, and Theron G. McCormick, MD, Guest Editors

Discovered more than 40 years ago, EBV is known to exhibit tropism for lymphocytes, especially B-cells. This γ herpes virus is capable of immune evasion,

From Baylor College of Medicine, Pediatric Allergy and Immunology Service, Texas Children's Hospital, Houston, Tex.

Received for publication February 25, 2005; revised April 11, 2005; accepted for publication April 20, 2005.

Available online July 15, 2005.

Reprint requests: Theron G. McCormick, MD, Baylor College of Medicine, Pediatric Allergy and Immunology Service, Texas Children's Hospital, 6621 Fannin St. FC330.01, Houston, TX 77030-2399. E-mail: Tgmccorm@texaschildrenshospital.org.

J Allergy Clin Immunol 2005;116:470-2.

0091-6749/\$30.00

© 2005 American Academy of Allergy, Asthma and Immunology
doi:10.1016/j.jaci.2005.04.044

producing latent infections that might lead to B-cell and other lymphoproliferative diseases. A relatively unusual target of EBV infection involves natural killer (NK) cells. Despite varying classifications, a form of chronic active EBV infection (CAEBV) involving NK cells presents with severe inflammatory and necrotic skin reactions considered pathognomonic of EBV⁺ NK cell lymphoproliferative disease.¹⁻³ Most patients presenting with this condition are of Asian descent, and there is no sex predominance.



FIG 1.

Fig 1 shows a 7-year-old Latin American boy with NK cell CAEBV. Typical of this condition is the presence of bullous and ulcerative skin lesions after exposure to mosquito bites. In addition, patients develop high fever, lymphadenopathy in draining nodes, and marked hepatosplenomegaly. Bullous



FIG 2.