

Toll-like receptor 2 ligands inhibit T_H2 responses to mite allergen

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Background: There is intense interest in the interaction between microbial compounds and allergy. Although Toll-like receptor (TLR)–2 ligands derived from Gram-positive bacteria alter allergic sensitization in animal models, it is not clear what effect TLR2 ligands have on allergen-specific T-cell memory in human beings.

Objective: To determine whether *in vitro* exposure to TLR2 ligands modifies the immune response to house dust mite allergen (HDM).

Methods: Blood mononuclear cells were obtained from individuals both allergic (n = 23) and not allergic (n = 22) to HDM, and stimulated with HDM in the presence or absence of TLR2 ligands.

Results: In subjects allergic to HDM, IL-5 and IL-13 responses to HDM were inhibited by heat-killed *Staphylococcus aureus*, staphylococcal lipoteichoic acid, and the synthetic lipoprotein Pam3CSK4 (*P* < .005; all stimuli). Although the whole staphylococcal bacteria increased IFN- γ responses, the purified TLR2 ligands lipoteichoic acid and Pam3CSK4 inhibited HDM-specific IFN- γ synthesis. In contrast, TLR2 ligands had minimal effects on responses to HDM in subjects without allergy. TLR2 ligands induced upregulation of HLA-DR expression but did not inhibit antigen uptake or processing by antigen-presenting cells.

Conclusion: Toll-like receptor 2 ligands inhibit allergen-specific T_H2 responses in sensitized individuals. This effect appears to be mediated by the actions of TLR2 ligands on antigen-presenting cells, and at least for the purified TLR2 ligands does not involve the induction of a strong T_H1 immune response.

Clinical implications: These findings provide an impetus for further preclinical studies examining the potential use of TLR2 ligands in allergic disease. (J Allergy Clin Immunol 2006;117:1148–54.)

Key words: Atopy, Toll-like receptors, innate immunity, T_H2 cytokines

Abbreviations used

DC: Dendritic cell
HDM: House dust mite allergen
LTA: Lipoteichoic acid
MFI: Mean fluorescence intensity
NK: Natural killer
SPT: Skin prick test
TLR: Toll-like receptor

There is intense interest in the interaction between exposure to microbial products and the clinical expression of human allergic diseases such as asthma, allergic rhinitis, and eczema. Microbial infections are frequently implicated in exacerbations of asthma and eczema, for example.^{1,2} In contrast, epidemiologic studies have suggested that children growing up in microbial-rich environments may be protected from the development of allergic sensitization.^{3,4} This protective effect was originally attributed to endotoxin (LPS) derived from Gram-negative bacteria, although more recent studies have suggested that exposure to components of Gram-positive bacteria may also protect against the development of allergic disease.⁵

Microbes are recognized by the innate immune system using pattern recognition receptors, with the Toll-like receptor (TLR) family the best characterized. Currently there are 10 identified TLRs in human beings that recognize different pathogen-associated molecular patterns, such as LPS (TLR4) and double-stranded RNA (TLR3), whereas TLR2 is the receptor involved in the recognition of lipoproteins and lipoteichoic acid (LTA) from Gram-positive bacteria. Although it was originally thought that signaling through TLRs is required for adaptive T_H1 responses,⁶ more recent studies suggest that the relationship between TLR stimulation and T_H1/T_H2 polarization is more complex, varying with both the concentration and type of TLR ligand studied.^{7–9}

Previous reports looking at the interaction between TLR2 stimulation and allergy have provided contradictory data. Initial studies using murine models of allergic asthma reported that TLR2 ligands administered during the sensitization period led to an enhancement of T_H2-mediated allergic inflammation.^{10,11} Conversely, other studies have found that synthetic lipopeptides administered immediately before airway allergen challenge inhibit T_H2-type responses and IgE production.¹² Recent studies also suggest that TLR2 stimulation inhibits established allergic airway inflammation, an effect mediated by an

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Supported by the National Health and Medical Research Council Australia. Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication October 3, 2005; revised January 16, 2006; accepted for publication February 6, 2006.

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0091-6749/\$32.00

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doi:10.1016/j.jaci.2006.02.014

augmented T_H1 response.¹³ Notably, these studies have all used murine models of asthma, and none has looked at the effects of TLR2 stimulation on established allergic responses in human beings.

This study investigated the effect of microbes on the immune response to allergens in human beings. In particular, we sought to examine the effects of various components of Gram-positive bacteria, acting through TLR2, on the established allergic immune response to house dust mite allergen (HDM). Our studies show that all 3 TLR2 ligands examined, including a heat-killed preparation of *Staphylococcus aureus* (Pansorbin; Calbiochem, LaJolla, Calif), staphylococcal LTA, and a synthetic lipopeptide (Pam3CSK4), were able to inhibit T_H2 cytokine secretion by HDM-stimulated PBMCs from individuals allergic to mite, but had no effect on immune responses from individuals without allergy. The effects of TLR2 ligands appeared to be mediated via antigen-presenting cells, but did not involve induction of an allergen-specific T_H1 (IFN- γ) response.

METHODS

Subjects

Forty-five laboratory volunteers aged between 19 and 63 years were recruited for the study. All subjects underwent blood sampling and skin prick test (SPT) to common environmental allergens including HDM, domestic animals, grass pollens, cockroach, and staple foods, and completed a questionnaire detailing symptoms of asthma, allergic rhinitis, and eczema. On the basis of SPT results, subjects were classified into those who were allergic to HDM ($n = 23$; mean wheal size 7 ± 2 mm) and those who were not sensitized to HDM ($n = 22$; negative SPT). Subjects with weakly positive SPT reactions to HDM (wheal size, 1–3 mm) were not included. No subjects were taking inhaled or systemic corticosteroids or antihistamines. The study was approved by Human Research Ethics Committee, Sir Charles Gairdner Hospital, Perth, and all subjects provided written informed consent to participate in the study. For mechanistic experiments requiring large numbers of cells, buffy coats were also obtained from the Red Cross blood transfusion center from healthy adult volunteers, whose atopic status was unknown.

Reagents

The following ligands for TLR2 were purchased: heat-killed *S aureus* strain Cowan I (Pansorbin), *S aureus* LTA (InvivoGen, San Diego, Calif), and the synthetic lipopeptide Pam3CSK4 (certified endotoxin-free; InvivoGen). An aqueous extract of lyophilized *Dermatophagoides pteronyssinus* (HDM) was provided courtesy of Professor P. G. Holt. The HDM extract was prepared in-house using mites purchased from CSL (Parkville, Australia) and has been extensively used in studies from our laboratory over the last decade.^{14–17} Previous dose-response experiments have shown that a concentration of 10 $\mu\text{g/mL}$ provides optimal discrimination between individuals with mite allergy and normal individuals. The extract contains the 2 major allergens Der p 1 and Der p 2, but does not contain some of the minor lipid soluble allergens found in *Dermatophagoides* species (B. Hales and W. Thomas, Unpublished data, December 2005). Although the HDM extract contains trace amounts of endotoxin, removal of endotoxin from the allergen preparation (Acrodisc Chromatography Unit; Pall Corp, Ann Arbor, Mich) has no effect on IL-5 and IL-13 synthesis by HDM-stimulated PBMCs (B. Hales, Personal communication, December 2005). Biotinylated

antihuman TLR2 mAb (clone TL2.1) was obtained from eBioscience (San Diego, Calif), whereas streptavidin allophycocyanin, anti-CD3 peridinin-chlorophyll-protein, anti-CD14 fluorescein isothiocyanate, anti-CD19 phycoerythrin, anti-CD56 phycoerythrin, and isotype-matched control mAbs were purchased from BD Biosciences (San Jose, Calif).

Cell preparation and culture

All laboratory experiments were performed and analyzed without knowledge of the atopic status of the subjects. Peripheral blood was collected into an equal volume of RPMI-1640 (Gibco BRL, Melbourne, Australia) containing preservative-free heparin (20 U/mL). PBMCs were isolated by density gradient centrifugation and cryobanked in liquid nitrogen as previously described.¹⁸ Previous studies from our laboratory and elsewhere have demonstrated that this procedure does not distort cellular immune responses.^{18–20}

Allergen-specific lymphocyte responses were determined as previously described.^{15,16} Briefly, PBMCs were thawed and resuspended in AIM-V medium (Gibco Life Technologies, New York, NY) supplemented with 1% autologous plasma and 2-mercaptoethanol (final concentration, 4×10^{-5} mol/L; Sigma, Sydney, Australia). Cell viability was >90%. Cytokine production and lymphoproliferation were assessed by culturing PBMCs in round bottom 96-well plates at 2.5×10^5 cells/well, in duplicate for cytokine production for 24 hours or 5 days, and in triplicate for proliferation, for 5 days. Cells were cultured in medium alone or with optimal stimulating concentrations of HDM (10 $\mu\text{g/mL}$) in the presence or absence of Pansorbin 0.01% and 0.0001%, LTA 20 $\mu\text{g/mL}$, or Pam3CSK4 300 ng/mL. In some experiments, PBMCs were also cultured with a control antigen, tetanus toxoid (0.5 Limes flocculation (LF)/mL; CSL Ltd, Melbourne, Australia) in the presence or absence of TLR2 ligands. Optimal concentrations of all 3 TLR2 ligands were determined in preliminary dose-response experiments. Proliferation was measured by incorporation of ^3H -thymidine for 16 to 18 hours before harvest. After subtraction of results obtained from unstimulated control cultures, data are presented as Δ disintegrations per minute per culture.

In some experiments, PBMCs were cultured for 24 hours in the presence or absence of TLR2 ligands, and HLA-DR expression was determined by flow cytometry, as a measure of the activation status of antigen-presenting cells (APC).

Cytokine protein detection

The levels of IL-5, IL-6, IL-10, IL-13, TNF- α , and IFN- γ were measured in culture supernatants by time-resolved fluorometry, as previously described.¹⁴ Capture and detection antibody pairs were from BD Pharmingen: rat IgG₁ antimouse/human IL-5 (clone TRFK5) and biotinylated rat IgG_{2a} antihuman IL-5 (clone JES1-5A10), rat IgG₁ antihuman IL-6 (clone MQ2-13A5) and biotinylated rat IgG_{2a} antihuman IL-6 (clone MQ2-39C3), rat IgG₁ antihuman IL-10 (clone JES3-9D7) and biotinylated rat IgG_{2a} antihuman IL-10 (clone JES3-1298), rat IgG₁ antihuman IL-13 (clone JES10-5A2) and biotinylated mouse IgG_{1,k} antihuman IL-13 (clone B69-2), mouse IgG₁ antihuman TNF- α (clone MAb1) and mouse IgG₁ antihuman TNF- α (clone MAb11), and mouse IgG_{1,k} antihuman IFN- γ (clone NIB42) and biotinylated mouse IgG_{1,k} antihuman IFN- γ (clone 45.33). IL-12 was measured by DuoSet ELISA Development System for human IL-12 p70 (R&D Systems, Minneapolis, Minn) as per the manufacturer's instructions. The lower limit of detection of the IL-12p70 assay was 50 pg/mL and for all other cytokines, 10 pg/mL. Levels of cytokine synthesis are presented as Δ above unstimulated control cultures. Preliminary experiments showed that IL-6, IL-10, and TNF- α synthesis was maximal at 24 hours, whereas IFN- γ , IL-5, and IL-13 were maximal at day 5 (data not shown).

Cellular expression of TLR2

Constitutive TLR2 expression on cell subsets of PBMCs was determined by flow cytometry. Cells were washed in PBS supplemented with 0.1% sodium azide and 1% normal human serum and incubated for 30 minutes on ice with anti-TLR2 in combination with anti-CD3 (T cells), anti-CD14 (monocytes), anti-CD19 (B cells), anti-CD56 (natural killer [NK] cells), or corresponding isotype-matched control mAbs, followed by streptavidin allophycocyanin. Dendritic cells (DCs) were identified by using a commercial kit (BD Biosciences) as HLA-DR⁺ cells lacking CD3, CD14, CD16, CD19, CD20, and CD56 expression, and further divided into myeloid DCs and plasmacytoid DCs on the basis of differential expression of CD11c (myeloid) or CD123 (plasmacytoid). Analysis was performed by using a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ) and FlowJo software (Tree Star, Ashland, Ore) to determine the percentage of various cell populations positive for TLR2.

Antigen uptake/processing

Antigen uptake was determined by using dextran fluorescein (Molecular Probes, Eugene, Ore), whereas antigen processing was evaluated by using DQ-Ova (Molecular Probes). The latter compound is a self-quenched conjugate of ovalbumin that only exhibits bright green fluorescence on proteolytic degradation within antigen-presenting cells.²¹ These studies used monocytes purified by adherence that had been cultured for 48 hours in RPMI supplemented with IL-4 (10 ng/mL), GM-CSF (100 ng/mL), and 5% FCS, hereafter referred to as Mono48 cells. This short period of culture induces monocytes to become nonadherent and highly endocytic, and therefore ideally suited to studies of antigen uptake and processing, although they have not fully differentiated into DCs.

Mono48 cells were pulsed with Pansorbin 0.01% for 45 minutes at 37°C and then washed extensively with RPMI + 5% FCS at 4°C. Cells were transferred to 37°C, and 0.25 µg/mL dextran fluorescein or 5 µg/mL DQ-Ova were added. Excess dextran fluorescein was removed by washing with PBS supplemented with 0.1% sodium azide and 1% normal human serum and quenched with 0.25 µg/mL trypan blue. The uptake of dextran fluorescein and the processing of ovalbumin into peptide were assayed by measuring the increase in mean fluorescence intensity (MFI) over time. Dextran fluorescein and DQ-Ova peptide were quantified by using the fluorescein isothiocyanate channel of the flow cytometer.

Statistical analysis

Group data were expressed as medians and interquartile ranges unless otherwise stated. Comparison between different groups was performed by using the Mann-Whitney *U* test and within groups using the Wilcoxon signed-ranks test. Values of $P \leq .05$ were considered significant. Antigen uptake and processing were expressed as the percentage MFI relative to the untreated media control at the final time point.

RESULTS

Subjects

Twenty-three subjects (6 men and 17 women; mean age, 42 years) were classified as allergic to HDM by having a positive HDM SPT (mean wheal size, 7 mm; range, 4–11) and 22 (6 men and 16 women; mean age, 37 years) as not allergic to HDM (wheal size, 0 mm). Positive SPT (wheal size, >3 mm) to other allergens (grass, cat, dog, peanut, mold, ovalbumin, milk) were noted but not used to distinguish between groups. In the group allergic to HDM, 87% ($n = 20$) had a positive SPT to 1 or more of the other allergens tested, whereas only 27% ($n = 6$)

of the group not allergic to HDM had a positive SPT to any allergen. There were no significant differences in age or sex between groups.

TLR2 ligands produce varying patterns of cytokine synthesis

Before determining the effects of TLR2 ligands on allergen-specific T-cell responses, it was important to examine the effects of TLR2 ligands in the absence of allergen. PBMCs were therefore stimulated with Pansorbin, LTA, or Pam3CSK4, and supernatants were collected at 24 hours and 5 days.

Although all 3 ligands stimulate through TLR2, they produced varying patterns of cytokine synthesis. Whereas all 3 stimuli induced IL-6 synthesis, only the higher concentration of Pansorbin (0.01%) produced substantial amounts of IFN-γ or TNF-α (Fig 1). Pansorbin and LTA, but not Pam3CSK4, induced IL-10 synthesis. Production of the T_H2 cytokines IL-5 and IL-13 was minimal with all ligands (Fig 1, B).

TLR2 ligands decrease T_H2 cytokine response to HDM

As expected, PBMCs from subjects allergic to HDM produced more IL-5 and IL-13 than PBMCs from subjects without allergy when stimulated with HDM *in vitro* ($P < .001$, $P = .014$, respectively). HDM-stimulated IFN-γ production was similar in both groups of subjects (Fig 2). When HDM and TLR2 ligand stimulation were combined, all 3 TLR2 ligands induced a significant inhibition of IL-5 (40% to 80%) and IL-13 (40% to 75%) synthesis in the subjects allergic to HDM. In contrast, the addition of TLR2 ligands had only a minor effect on HDM-stimulated IL-5 and IL-13 synthesis in the group without allergy, aside from a small reduction associated with LTA ($P = .016$, IL-5; $P = .006$, IL-13). In subjects both with and without allergy, TLR2 ligands had no significant effect on PBMC responses to the control antigen tetanus toxoid (see this article's Fig E1 in the Online Repository at www.jacionline.org).

We next asked whether the ability of TLR2 ligands to inhibit T_H2 cytokine production might be associated with deviation toward a T_H1 immune response. The combination of HDM and TLR2 ligands exhibited variable effects on IFN-γ production, and there was no difference between atopic and nonatopic subjects. High-dose Pansorbin (0.01%) combined with HDM was associated with high levels of IFN-γ production (Fig 2), although this appeared to be a result of the direct effects of Pansorbin 0.01% per se, as shown in Fig 1. However, low-dose Pansorbin (0.0001%) did not change IFN-γ production, whereas the more highly purified TLR2 ligands LTA and Pam3CSK4 led to a small reduction in HDM-stimulated IFN-γ production in the group with allergy (Fig 2). Similarly, there was no consistent effect of the various TLR2 ligands on IL-10 synthesis. Pam3CSK4, for example, did not induce detectable IL-10 production at 6, 24, or 48 hours (Fig 1 and data not shown), yet was able to inhibit allergen-induced IL-5 and IL-13 production at day 5

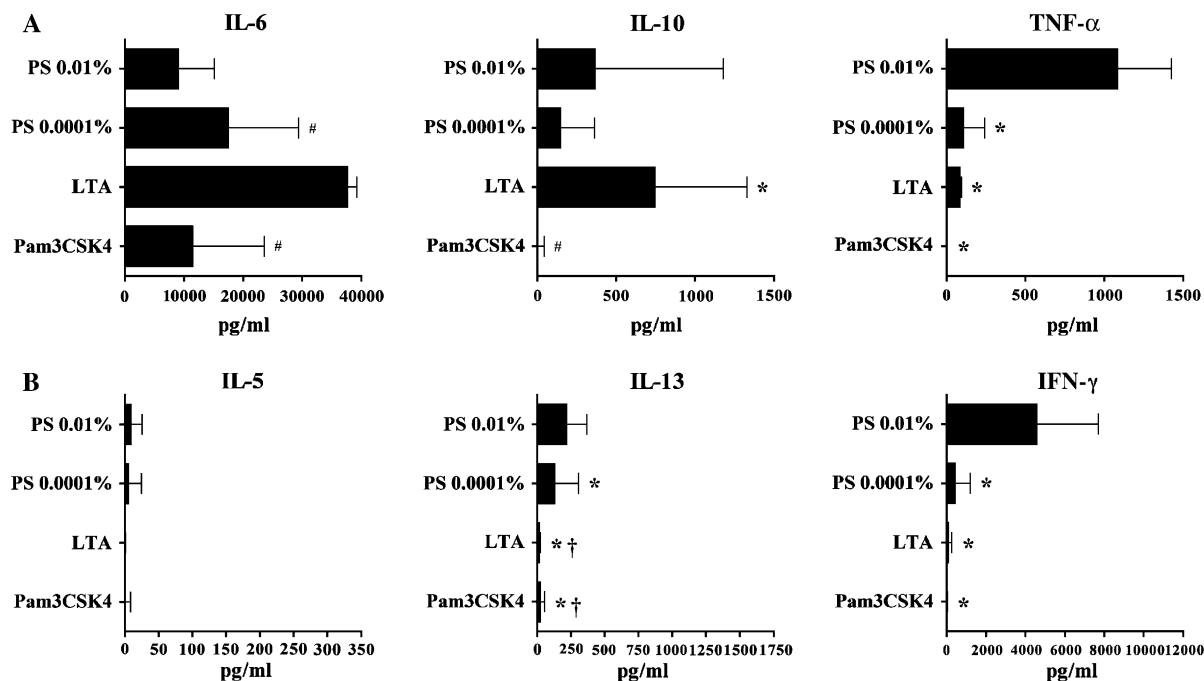


FIG 1. Effects of TLR2 ligands on PBMCs in the absence of allergen. **A**, Concentrations of IL-6, IL-10, and TNF- α were measured at 24 hours poststimulation; $n = 4$. **B**, Concentrations of IFN- γ , IL-5, and IL-13 were measured at day 5 poststimulation; $n = 45$. $P < .05$ compared with *Pansorbin 0.01%, †Pansorbin 0.0001%, and #LTA.

(Fig 2). TLR2 ligands also did not alter allergen-induced IL-10 production (data not shown). IL-12p70 was not detected under any condition (<50 pg/mL; data not shown). Thus, it appears that the mechanisms by which all 3 TLR2 ligands inhibit allergen-induced T_H2 cytokine production are unlikely to involve IFN- γ , IL-12p70, or IL-10.

The various TLR2 ligands had no consistent effect on HDM-induced lymphoproliferation. Although Pansorbin 0.0001% and Pam3CSK4 combined with HDM increased ^3H -thymidine incorporation above that seen with HDM alone, Pansorbin 0.01% and LTA did not significantly alter ^3H -thymidine incorporation. It thus appeared unlikely that the ability of all TLR2 ligands to inhibit T_H2 cytokine production could be attributed to changes in lymphoproliferation.

TLR2 expression on PBMCs

To examine further the cellular mechanisms by which TLR2 ligands exert their effects, the pattern of TLR2 expression within PBMCs was examined by flow cytometry. PBMCs were double-stained with TLR2 mAb in combination with cell-specific mAbs for B cells, T cells, NK cells, monocytes, and DC subsets. TLR2 expression was prominent on $CD14^+$ monocytes (88%) but few $CD19^+$ B cells (7%), and $CD3^+$ T cells (5%) were TLR2 $^+$ (Fig 3). Overall, 17% of PBMCs express TLR2 on their surface. TLR2 was also detected on myeloid DC ($Lin^-/HLA-DR^+/CD11c^+$), but not on plasmacytoid DC ($Lin^-/HLA-DR^+/CD123^+$) or on NK cells (data not shown). Subjects allergic and not allergic to HDM had similar expression of TLR2 according to both the

proportion of TLR2 $^+$ cells (Fig 3) and the MFI (data not shown).

Effects of TLR2 ligands on antigen-presenting cell function

Because TLR2 was predominantly expressed on monocytes and myeloid DC, we next examined the effects of TLR2 ligands on antigen-presenting cell (APC) function, including antigen uptake, antigen processing, and HLA-DR expression.

The ability of Mono48 cells to take up the model antigen dextran fluorescein was not inhibited by the TLR2 ligand Pansorbin, but rather was increased at all time points (Fig 4, A). Similar results were obtained with the synthetic lipopeptide Pam3CSK4 (data not shown). Mono48 cells exposed to DQ-ovalbumin in the absence of stimulation showed a time-dependent increase in fluorescence, corresponding to intracellular processing of this model antigen. Exposure of cells to the TLR2 ligand Pansorbin did not significantly alter the appearance of the fluorescent signal (Fig 4, B).

Activation of PBMCs with all 3 TLR2 ligands induced upregulation of HLA-DR expression at 24 hours (Fig 4, C), indicative of APC activation.

DISCUSSION

The key finding to emerge from this study was that TLR2 ligands have the capacity to inhibit T_H2 cytokine production by mononuclear cells stimulated with mite allergen.

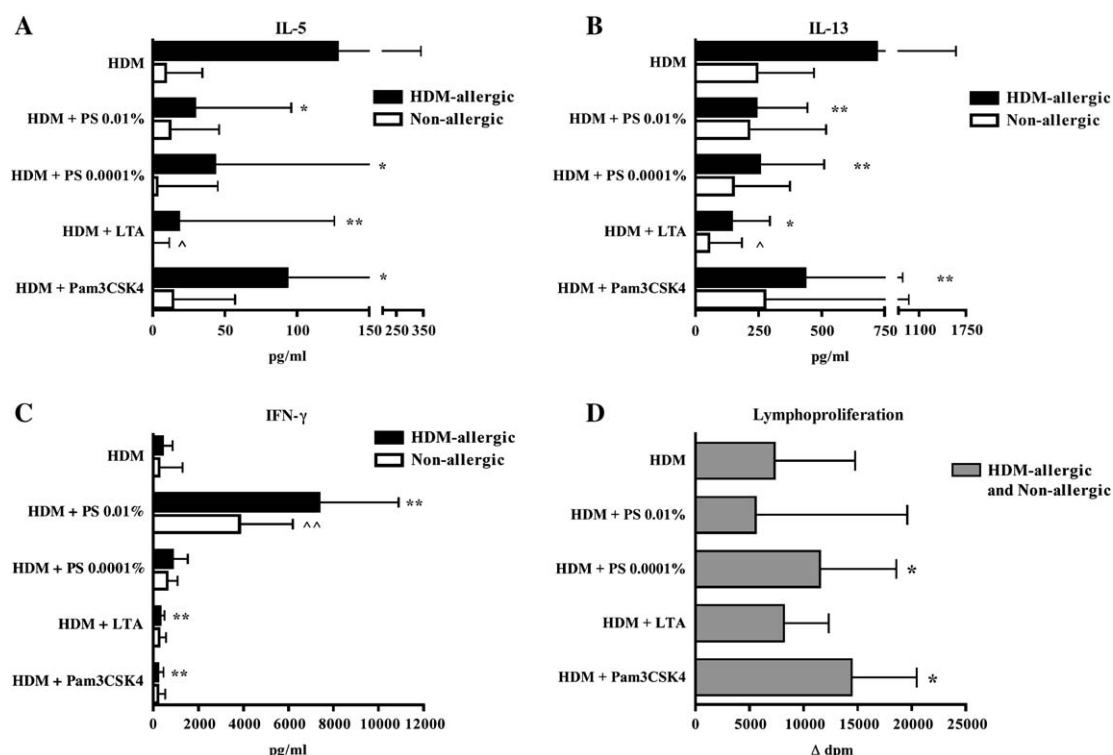


FIG 2. Effect of TLR2 ligands on cytokine synthesis and lymphoproliferation in the presence of HDM allergen. Graphs show the concentrations of (A) IL-5, (B) IL-13, and (C) IFN- γ measured at day 5. * $\Delta P < .03$, ** $\Delta P < .003$ compared with HDM alone (*allergic to HDM, Δ not allergic). (D) Effect of TLR2 ligands and HDM allergen on lymphoproliferation; $n = 8$. Data are expressed as the change in disintegrations per minute above media control (Δ dpm). PS, Pansorbin.

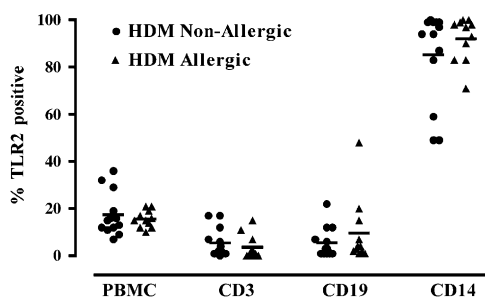


FIG 3. Constitutive TLR2 expression on PBMCs from subjects allergic and not allergic to HDM. Surface expression of TLR2 was examined on whole PBMCs, CD3 $^{+}$ T cells, CD19 $^{+}$ B cells, and CD14 $^{+}$ monocytes.

All TLR2 ligands studied induced significant and substantial reduction in IL-5 and IL-13 synthesis by mononuclear cells from adults with mite allergy. The lack of a similar effect in the subjects without allergy can probably be explained by the relatively small responses to mite allergen in this group, especially because TLR2 expression was similar in subjects allergic and not allergic to mite.

Increasing evidence suggests that exposure to microbial stimuli, acting via the innate immune system, can influence adaptive immune responses to allergens and the development of asthma and other allergic diseases.²⁻⁴

Although most experimental studies have examined the TLR4 ligand LPS in this context, recent studies in mouse models of asthma have suggested that TLR2 ligands can also enhance or inhibit T_H2 immune responses.¹¹⁻¹³ In the current study, we show in human beings that TLR2 ligands inhibit recall/memory responses to HDM. The inhibition of T_H2 cytokine production was observed with a variety of TLR2 ligands, including high and low concentrations of heat-killed *S aureus*, LTA, and Pam3CSK4. The fact that both heat-killed *S aureus* and the purified TLR2 ligands inhibit allergen-specific T_H2 cytokine production is consistent with the notion that the findings described in Fig 2 are mediated via a TLR2-dependent mechanism.

It does not appear that this involves deviation to an allergen-specific T_H1 response pattern, because induction of IFN- γ production was not consistently observed with the various TLR2 ligands. Although the higher dose of Pansorbin did induce substantial IFN- γ synthesis, this appeared to be a result of direct effects of Pansorbin on PBMCs, as shown in Fig 1, and may reflect a T_H1 response to staphylococcal proteins. Importantly, the lower dose of Pansorbin had no effect on IFN- γ production, whereas the purified TLR2 ligands LTA and Pam3CSK4 actually inhibited HDM-stimulated IFN- γ production in the subjects with allergy (Fig 2). Similarly, we were unable to detect the T_H1-polarizing cytokine IL-12p70 under any

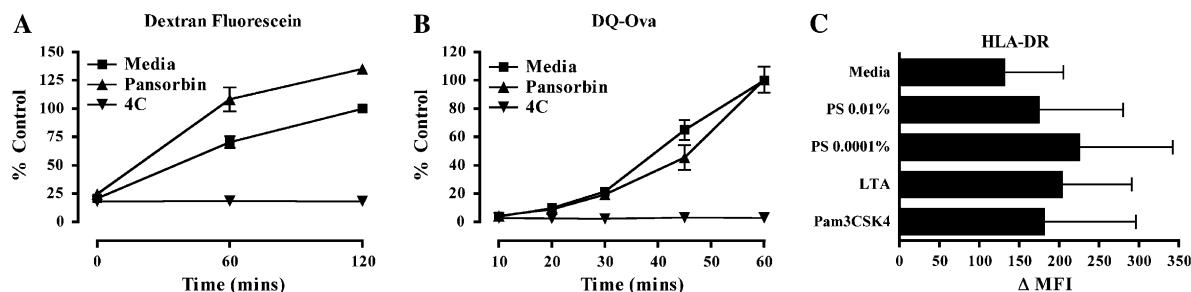


FIG 4. Effects of TLR2 ligands on antigen-presenting cell function. **A**, Antigen uptake by Mono48 cells was determined by using dextran fluorescein; $n = 5$. **B**, Antigen processing by Mono48 cells was determined by using DQ-ovalbumin; $n = 6$. **C**, HLA-DR expression was determined on whole PBMCs after 24-hour stimulation with TLR2 ligands; $n = 10$.

conditions studied (data not shown). The apparent lack of involvement of IFN- γ in mediating these effects is in contrast to the findings of Patel et al,¹³ who recently reported in a mouse model of asthma that the ability of Pam3CSK4 to inhibit allergic airway inflammation was IFN- γ -dependent but did not involve the induction of regulatory T cells. A previous study in human beings reported that the lipopeptide LP40 enhances IFN- γ and IL-12p70 production,¹² although in that study, LP40 also induced a residual cytokine response in TLR2 knockout mice, suggesting that the LP40 lipopeptide may activate receptors in addition to TLR2. Further studies are needed to examine a wide range of lipoproteins and lipopeptides to determine those that exhibit the most favorable effects on adaptive immune responses to allergens, without inducing the potentially deleterious proinflammatory cytokines seen with heat-killed *S aureus*.

Toll-like receptor 2 expression was most prominent on monocytes and myeloid DCs, implying that the effects of the TLR2 ligands on T_H2 cytokine synthesis are mediated via these APCs. However, TLR2 ligands did not inhibit the uptake or processing of ovalbumin by APC. Formal examination of the uptake and processing of HDM was not undertaken in the current study because of the lack of fluorochrome-conjugated purified allergens, although at this stage it seems unlikely that major changes in allergen uptake or processing explain the ability of TLR2 ligands to inhibit T_H2 cytokine synthesis.

Toll-like receptor 2 ligands did induce APC activation, as shown by increased HLA-DR expression. This in itself may not be sufficient to explain the observed inhibition of T_H2 cytokine synthesis, especially because APC activation or maturation usually induces a strong T_H1 immune response.²² Sun et al²³ recently showed that TLR engagement on mouse DCs could inhibit the development of T_H2 cells via an unidentified pathway, and further studies are needed to examine the mechanisms by which TLR2 ligands alter human APC function, including the induction of various costimulatory molecules and secreted cytokines, or the generation of regulatory T cells.

In conclusion, this study shows that TLR2 ligands have the ability to inhibit the T_H2 response to mite allergen in previously sensitized individuals by an unknown mechanism. The individuals studied had only mild clinical

disease, and it remains to be determined whether TLR2 ligands will have similar beneficial effects on allergen-specific responses from individuals with severe asthma or eczema, for example. However, these findings do provide an impetus for future studies designed to uncover novel mechanisms by which allergic responses can be ameliorated, and may open new treatment modalities.

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