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Heat-Killed Listeria monocytogenes as an Adjuvant Converts Established Murine Th2-Dominated Immune Responses into Th1-Dominated Responses

V. Peter Yeung, Randall S. Gieni, Dale T. Umetsu, and Rosemarie H. DeKruyff

We investigated the capacity of heat-killed Listeria monocytogenes (HKL) as a vaccine adjuvant to modify both primary and secondary Ag-specific immune responses. Mice immunized with the Ag keyhole limpet hemocyanin (KLH) mixed with HKL generated a KLH-specific primary response characterized by production of Th1 cytokines and large quantities of KLH-specific IgG2a Ab. Moreover, administration of KLH with HKL as an adjuvant reversed established immune responses dominated by the production of Th2 cytokines and high levels of KLH-specific IgE and induced a Th1-type response with high levels of IFN-γ and IgG2a and low levels of IgE and IL-4. Neutralization of IL-12 activity at the time of HKL administration blocked the enhancement of IFN-γ and reduction of IL-4 production, indicating that IL-12, induced by HKL, was responsible for the adjuvant effects on cytokine production. These results suggest that HKL as an adjuvant during immunization can successfully bias the development of Ag-specific cytokine synthesis toward Th1 cytokine production even in the setting of an ongoing Th2-dominated response. Thus, HKL may be clinically effective in vaccine therapies for diseases such as allergy and asthma, which require the conversion of Th2-dominated immune responses into Th1-dominated responses. The Journal of Immunology, 1998, 161: 4146–4152.

The profile of cytokines produced by CD4+ T cells during an immune response determines the nature of effector functions that develop and regulates the outcome of an infection (1, 2). Production of IL-2 and IFN-γ during Th1-dominated responses is associated with vigorous cell-mediated immunity, with an induction of IgG2a and inhibition of IgE synthesis (3, 4), and with resistance to intracellular pathogens (1, 2). In contrast, the production of IL-4, IL-5, and IL-10 during Th2-dominated responses is associated with humoral immunity and protection from autoimmune pathology (5–8). Overproduction of Th2 cytokines by allergen-specific CD4+ T cells also results in the development of allergic disease and asthma (9–16).

An important mechanism that influences the specific profile of the cytokines produced by responding CD4+ T cells is the cytokine environment present at the time of initiation of the immune response. IL-12, a heterodimeric cytokine produced by macrophages and dendritic cells, is potent in driving the development of Th1 cytokine synthesis in naive and memory CD4+ T cells (17–21). However, several in vivo studies have demonstrated that rIL-12 as an adjuvant, while enhancing IFN-γ synthesis, paradoxically also increases IL-4 and IL-10 synthesis in Ag-primed CD4+ T cells in some cases (22–25).

In this report, we studied the innate adjuvant activity of heat-killed Listeria monocytogenes (HKL) to provoke Th1-dominated immune responses. Infection with L. monocytogenes, a potent stimulator of the innate immune system, induces production of high levels of IL-12 and results in cell-mediated immunity against this intracellular bacterium. We used L. monocytogenes as an adjuvant with the Ag keyhole limpet hemocyanin (KLH) to generate a KLH-specific immune response characterized by high Ag-specific IFN-γ production and large quantities of KLH-specific IgG2a Ab. Moreover, vaccination with HKL and KLH reversed an established Th2-dominated, KLH-specific immune response and resulted in significant reductions in IL-4 and IL-10 synthesis, increases in IFN-γ production, and reduction in KLH-specific IgE. Reduction in IL-4 and IL-10 synthesis did not reduce the intensity of the Ag-specific immune response, since T cell proliferation to KLH was not reduced. Neutralization of IL-12 activity with anti-IL-12 mAb at the time of boosting with Ag blocked reduction of IL-4 and enhancement of IFN-γ production, indicating that HKL-induced IL-12 was responsible for the adjuvant effects on cytokine production.

Taken together, these results suggest that employing a strong nonspecific activator of IL-12 and IFN-γ production such as HKL as an adjuvant during Ag-specific immunization can successfully bias the development of Ag-specific cytokine synthesis toward Th1 cytokine production in both primary and secondary immune responses. Reversal of an established Th2-dominated response is particularly difficult and has not been effectively achieved by previous protocols using recombinant cytokines (22, 23, 25) or by agents that neutralize IL-4 (26). A modified version of this technique may be useful in clinical situations to induce appropriate cytokine synthesis during vaccination and in the treatment of ongoing diseases such as allergy and asthma, which are caused by...
highlighted Th2 cytokine responses to exogenous allergens. Such therapies, which alter the underlying immunologic processes, could potentially provide a cure for such diseases, which currently are treated only symptomatically.

**Materials and Methods**

**Animals**

BALB/cByJ mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Animal protocols used in this study were approved by the Stanford University Committee on Animal Welfare.

**Antigens**

KLH was obtained from Calbiochem (San Diego, CA).

**Immunizations**

BALB/c mice were immunized in the footpads with KLH (100 μg/mouse) emulsified in CFA or in IFA with or without HKL (10^8 bacteria/mouse). CFA contains the same oil base as does IFA, but CFA also contains killed mycobacteria. In some experiments, BALB/c mice were first primed in the footpads with KLH (10 μg) adsorbed to 2 mg of alum (Al(OH)_3), a priming method that invokes a strong Th2 response. After 4 wk, mice were treated with KLH (100 μg) in either CFA or IFA with or without HKL (10^8 bacteria). After an additional 10 days, all mice received another dose of KLH (100 μg) in PBS.

**Monoclonal Abs**

Anti-IFN-γ mAb R46A2 (HB170, American Type Culture Collection, Manassas, VA) and anti-IL-4 mAb 11B11 (generously provided by Drs. J. Ohara and B. Paul, National Institutes of Health, Bethesda, MD), were prepared from serum-free culture supernatants by ammonium sulfate precipitation and depleted of resting B cells by adherence to goat anti-mouse IgG depletion, XMG1.2 (for IFN-γ), W3/25 (for IL-10, and 4G10 (rat IgG2a control) in PBS. The recovered supernatants were harvested, centrifuged, and washed three times in PBS. The recovered supernatants were used as an isotype control.

**Treatment of mice with anti-cytokine Abs**

BALB/c mice were injected i.p. with 1 mg of mAbs C17.8 (for IL-12 depletion), XMG1.2 (for IFN-γ depletion), or 4G10 (rat IgG2a control) in 0.5 ml PBS on the day before, the day of, and 3 days following immunization with KLH in IFA or KLH in IFA with HKL.

**Medium**

Cells were cultured in DMEM (Life Technologies, Grand Island, NY), which was supplemented as described previously (28) and contained 5 × 10^-5 M 2-ME and 10% FBS (HyClone Laboratories, Logan, UT).

**Restimulation of lymph node (LN) cells in vitro**

Draining LN were removed at 7 days after priming or booster immunization and depleted of resting B cells by adherence to goat anti-mouse Ig-coated plates; cells (4 × 10^5) were restimulated in vitro with KLH at 1 or 10 μg/ml as indicated in the figure legends. B cells are depleted from the LN before culture because they consume cytokines, particularly IL-4, produced by the T cells. Cultures were set up in 96-well microtiter plates in 150 μl of medium. Supernatants were harvested after 4 and 5 days for the determination of IL-4, IL-10, and IFN-γ levels. Cytokine levels for each sample were measured in triplicate by ELISA. Proliferation was assessed by pulsing cultures overnight with ^3H]thymidine after 36 h of culture.

**Cytokine ELISA**

Plates with 96 wells were coated overnight with primary anti-cytokine capture Ab, washed, and blocked, and dilutions of supernatants or standards were added. Dilutions of culture supernatant were incubated over-night at 4°C. After washing, the wells were incubated with biotin-conjugated anti-cytokine-detecting mAb. After a 2-h incubation, the plates were washed, and a horseradish peroxidase (HRP)-streptavidin conjugate (Southern Biotechnology Associates, Birmingham, AL) was added. The plates were incubated for an additional hour, and o-phenylenediamine (OPD) substrate was added after washing. After developing, the OD was determined at 492 nm. The absorbance of cytokine in each supernatant was extrapolated from the standard curve. The Ab pairs used were as follows (listed by capture/biotinylated detection): IFN-γ, R4-6A2/XMG1.2; IL-12, C17.8/C15.6; IL-10, 2A5/SXC1; and IL-4, 1B11/BVD6-24G2. The standards were recombinant cytokine curves generated in 1/2 dilutions from 20 to 0.156 ng/ml for IFN-γ, 4000 to 30 pg/ml for IL-10, 20 to 0.1 ng/ml for IL-10, and 0 to 0.15 U/ml for IL-4. One unit of IL-4 is equivalent to 30 pg.

**Measurement of anti-KLH Ab isotypes**

Mice were bled at the time of sacrifice, and KLH-specific Ab was measured using a modified Ag-specific ELISA. For measurement of KLH-specific IgG1 and IgG2a, plates were coated overnight with 2 μg/ml of KLH. After washing and blocking, serial diluted sera were added to the plates. Following overnight incubation, the plates were developed using HRP-conjugated goat anti-IgG subclass-specific Abs (Southern Biotechnology Associates). After additional washing, OPD substrate was added, the plates were developed, and the OD was determined at 492 nm. The concentration of anti-KLH Ab was estimated using standard curves constructed by coating wells with 1 μg/ml of goat anti-mouse IgG1 or anti-IgG2a (Southern Biotechnology Associates) and adding polyclonal mouse Ig standards of the pertinent subclass. Determination of KLH-specific IgE was performed by ELISA using rat anti-mouse IgE mAb EM95 (0.5 μg/ml) to coat plates. After the samples were applied and incubated overnight, plates were washed, and biotinylated KLH (5 μg/ml) was added. After 3 h, plates were washed, and HRP-conjugated streptavidin (Southern Biotechnology Associates) was added. Plates were developed with OPD substrate, and the OD was determined at 492 nm.

**Preparation of HKL**

A clinical isolate of _L. monocytogenes_ was kindly provided by Dr. L. Tompkins and B. Allen (Stanford University). A heat-killed preparation of bacteria (HKL) was prepared by growing nutrient broth cultures (Difco, Detroit, MI) overnight at 37°C on a rotator. Cultures in log-phase growth were harvested, centrifuged, and washed three times in PBS. The recovered bacteria were resuspended in PBS and incubated at 80°C for 1 h. After two additional washes in PBS, the absence of viable colonies was confirmed by lack of growth on nutrient agar plates. Bacteria concentration was enumerated by comparing the absorbance of a serial dilution of HKL at 570 nm with a standard dilution of a known concentration of _Listeria_ that had been enumerated previously by counting the outgrowth of colonies from serial dilutions of bacteria plated on nutrient agar. The HKL was kept at −80°C.

**Results**

**HKL as an adjuvant at the time of Ag priming enhances IFN-γ but inhibits IL-4 and IL-10 production**

We investigated the ability of HKL to act as a Th1-inducing adjuvant in vivo. The HKL activates a potent innate immune response characterized by the induction of IL-12, which induces rapid production of IFN-γ, but inhibits the synthesis of IL-4 in NK cells and CD4+ T cells (17, 20, 29, 30). For these experiments, we immunized BALB/c mice in the footpads with KLH (100 μg) plus HKL (10^8 bacteria) in IFA. Control mice received KLH in IFA or in CFA. At 7 days postimmunization, draining LN were removed, and LN cells were restimulated with KLH in vitro. Figure 1 shows that HKL induced the development of LN cells with a strongly biased Th1-like cytokine pattern, characterized by production of large quantities of IFN-γ and very low levels of IL-4. The level of IFN-γ was significantly greater and the levels of IL-4 were significantly lower than the quantities produced by LN cells from mice that had received KLH in IFA or KLH in CFA. Furthermore, the cells from mice immunized with KLH plus HKL produced much lower quantities of IL-10, demonstrating the shift from a Th2-like to a Th1-like cytokine profile. These data indicate that HKL is a potent Th1 cytokine-inducing adjuvant in vivo.
Although the cytokine profiles of LN cells from the three groups of mice differed significantly, the magnitude of the proliferative response was similar regardless of the priming condition used (Fig. 2). LN cells from mice primed with KLH in IFA, KLH in CFA, or KLH plus HKL in IFA all proliferated to the same extent when stimulated with a wide range of Ag concentrations. Thus, addition of HKL as an adjuvant to KLH did not alter the intensity of the KLH-specific immune response. The similar dose-dependent proliferation between groups primed under different conditions and the lack of significant cytokine production in the absence of Ag indicated that priming with HKL has a minimal effect on the specificity of the response to KLH.

Administration of HKL during secondary Ag challenge

We subsequently examined the capacity of HKL to enhance Th1 cytokine synthesis in established Th2 cytokine-dominated immune responses. In these experiments, BALB/c mice were first primed with KLH (10 μg/mouse) adsorbed to alum (2 mg/mouse), which is known to provoke Ag-specific Th2-dominated immune responses (31, 32). After 4 wk, mice were immunized in the footpads with KLH (100 μg/mouse) plus 10⁶ HKL in IFA, KLH in CFA, or KLH in IFA. After an additional 10 days, all mice received a s.c. booster immunization of KLH (100 μg) in no adjuvant to mimic persistent although nonbiasing in vivo antigenic stimulation. The draining LN were removed 7 days later, and the cells were cultured in vitro with KLH. Figure 3 shows that HKL as an adjuvant was extremely effective in enhancing IFN-γ and in reducing IL-4 and IL-10 synthesis in the draining LN cells compared with controls. There was a slight reduction in IL-4 production in mice receiving CFA as an adjuvant compared with mice receiving IFA as an adjuvant, but the reduction in IL-4 production was much greater in mice receiving HKL as an adjuvant. In addition, HKL was much more effective than CFA in reducing IL-10 synthesis. Finally, the proliferative responses in all groups were similar, with negligible proliferation in the absence of in vitro Ag (data not shown). Taken together, these data demonstrate that HKL can act as a potent Th1 cytokine-inducing adjuvant and also reduce preexisting Th2 cytokine production in an Ag-specific manner.

The effect of HKL on IL-4 and IFN-γ production is mediated by IL-12

Since HKL is a potent inducer of IL-12 production, and since IL-12 is known to inhibit production of IL-4 and enhance production of IFN-γ (18–20, 33), we asked whether the effects of HKL as an adjuvant on cytokine production were mediated primarily by IL-12. Mice were first primed with KLH in alum to induce a Th2-dominated immune response. As in the experiments shown in Figure 3, the mice were vaccinated with KLH plus HKL in IFA or with KLH in IFA. Some mice received three i.p. injections of the anti-IL-12 mAb C17.8: one dose just before boosting with KLH plus HKL (4 wk after priming), another dose on the day of boosting, and the final dose at 3 days after boosting. Figure 4A shows that the treatment of the KLH-primed mice with KLH plus HKL, as expected, greatly enhanced IFN-γ production and greatly reduced IL-4 production in LN cells taken from these mice. Neutralization of IL-12 in such mice with anti-IL-12 mAb reversed the enhanced IFN-γ production and the reduction in IL-4 production, indicating that IL-12 mediated much of the in vivo effects of HKL on cytokine production. Although IL-12 was critical for enhanced IFN-γ and reduced IL-4 production, the presence of IFN-γ was not important in regulating cytokine synthesis by HKL, since neutralization in vivo of IFN-γ with an anti-IFN-γ mAb, XMG1.2, had a minimal effect on ex vivo IFN-γ and IL-4 synthesis (Fig. 4A). The effects of HKL on Th2 cytokine production involved other factors.
in addition to IL-12, since the reduction in IL-10 production by HKL was not reversed by treatment with anti-IL-12 mAb. These findings indicate that the effects of HKL as an adjuvant on IL-4 and IFN-γ production are mediated by IL-12 and not by the enhanced levels of IFN-γ; however, IL-12-independent mechanisms are also implicated, particularly in reducing IL-10 synthesis.

**Immunization with HKL as an adjuvant promotes the production of KLH-specific IgG2a and inhibits KLH-specific IgE synthesis**

Next, we asked whether the isotype and subclass distribution of anti-KLH Ab were altered by treatment with HKL. Mice were first primed with KLH in alum to generate a Th2-dominated immune response and then treated with KLH plus HKL. After an additional boost with Ag at 5.5 wk (same protocol as in Figs. 3 and 4), serum was collected, and KLH-specific Ab responses were determined by isotype- and IgG subclass-specific ELISA. Mice treated with KLH plus HKL showed enhanced anti-KLH IgG2a Ab responses (2.5- to 2.8-fold enhancement in three experiments) and reduced anti-KLH IgE responses (59.6–79.6% reduction in four experiments) as compared with control mice treated with KLH (Fig. 5). Levels of anti-KLH IgG1 Ab in KLH plus HKL-treated mice were reduced from those of KLH-treated mice (38%, 47%, and 72% reduction in three experiments) (Fig. 5). The increase in KLH-specific IgG2a by HKL treatment was dependent upon the presence of IL-12 and IFN-γ, since neutralization of these cytokines blocked the effects. In contrast, the reduction in both IgE synthesis and KLH-specific IgG1 was only partially dependent upon the presence of IL-12 and IFN-γ, since neutralization of these cytokines blocked the effects. In contrast, the reduction in both IgE synthesis and KLH-specific IgG1 was only partially dependent upon the presence of IL-12 and IFN-γ, since neutralization of these cytokines blocked the effects. These results demonstrating the effects of HKL on Ab isotype and IgG subclass production are consistent with the idea that HKL as an adjuvant can convert a Th2-dominated immune response into Th1-dominated one, and that the mechanisms by which HKL performs this conversion involve in part IL-12 and IFN-γ.

**FIGURE 3.** Conversion of established Th2- to Th1-like cytokine responses using HKL as an adjuvant. BALB/c mice were primed in the footpads with KLH (10 μg) adsorbed to 2 mg alum. After 4 wk, mice were injected in the footpads with KLH (100 μg/mouse) in IFA or CFA or with KLH plus 10⁸ HKL in IFA. After an additional 10 days, all mice received a footpad booster immunization of KLH (100 μg) in no adjuvant. The draining LN were removed 7 days later, and the cells were cultured in vitro with KLH (0 or 10 μg/ml) at 5 × 10⁵ cells/well. IL-4, IL-10, and IFN-γ levels in supernatants were determined after 4 days by ELISA. Cytokine production in the absence of Ag was negligible (IL-4 < 0.5 U/ml, IL-10 < 200 pg/ml, IL-12 < 60 pg/ml, and IFN-γ < 1.0 ng/ml). Data are the mean of triplicate cytokine determinations ± SEM. Representative results from one of five experiments are shown.

**FIGURE 4.** Administration of anti-IL-12 mAb blocks the effect of HKL adjuvant on cytokine production. Mice were treated as noted in Figure 3. At 4 wk after the initial priming with KLH in alum, mice were injected in the footpads with KLH (100 μg/mouse) in IFA or with KLH plus 10⁸ HKL in IFA. As indicated, BALB/c mice were injected i.p. with mAbs (1 mg/dose) C17.8 (for IL-12 depletion), XMG1.2 (for IFN-γ depletion), or 4G10 (rat IgG2a control) in 0.5 ml of PBS on the day before, the day of, and 3 days following the immunization with KLH plus HKL. After an additional 10 days, all mice received a 100-μg booster immunization of KLH in PBS. After 7 days, LN cells were removed and cultured (5 × 10⁵ cells/well) with KLH (1 μg/ml). The values represent the mean ± SD of triplicate determinations. A and B show results from two of four experiments.
recently that the amount of IFN-γ-regulating cytokine synthesis in primed CD4 T cells. We reported that macrophages from BALB/c mice. IL-12, which is induced by HKL, plays a critical role in responses and limit a Th2-dominated immune response in BALB/c APCs as a means to alter the cytokine profile of ongoing immune levels of IL-12. This results in high IFN-γ production in primed BALB/c than MHC identical (H-2d) DBA/2 mice. Listeria monocytogenes, a Gram-positive, intracellular, facultative bacterium, elicits a strong, classical, cell-mediated immune response that is characterized by the presence of potent Ag-specific CD8 killer cells (34–36). In the infected cell, Listeria escapes from the phagosome/endosome and replicates in the cytoplasm, preferentially shunting listerial Ags into the MHC class I-restricted pathway of Ag processing (37). The cytoplasmic localization of the bacteria permits Listeria to be an effective vector for tumor and viral Ags and function as a tumor/viral vaccine (38–41). However, Listeria also rapidly activates innate immunity and induces high levels of IL-12. This results in high IFN-γ production in NK cells and stimulates the induction of strongly polarized Th1 CD4 T cells (17, 18).

Our study exploits the capacity of Listeria to induce IL-12 in APCs as a means to alter the cytokine profile of ongoing immune responses and limit a Th2-dominated immune response in BALB/c mice. IL-12, which is induced by HKL, plays a critical role in regulating cytokine synthesis in primed CD4 T cells. We reported recently that the amount of IFN-γ and IL-4 produced by primed CD4 T cells upon restimulation in vitro depended upon the quantity of IL-12 present in culture, primarily produced by APCs (42). We showed that macrophages from BALB/c mice, which are pre-disposed to high IL-4 and high IgE synthesis, produced less IL-12 than did DBA/2 macrophages during Ag-driven interactions with CD4 T cells, resulting in higher IL-4 and less IFN-γ production by T cells from BALB/c than MHC identical (H-2d) DBA/2 mice. This observation suggested that enhanced IFN-γ and reduced IL-4 production in primed BALB/c T cells could be induced if greater IL-12 production by APCs could be mobilized by adjuvants such as HKL.

We demonstrated that HKL as an adjuvant is very effective in reversing cytokine synthesis in primed CD4 T cells and in reducing ongoing IgE synthesis. Although rIL-12 can reduce in vitro IgE synthesis (43) and in vitro IL-4 synthesis (20, 44), administration of rIL-12 in vivo results in more limited effects, particularly during ongoing Th2-dominated immune responses. In such secondary responses, IL-4 synthesis is often resistant to the effects of rIL-12 (22, 25); in fact, rIL-12 may increase IL-4 synthesis (22, 25), perhaps by inducing a rebound increase in IL-10 synthesis (23). In parasite models, treatment with rIL-12 can resolve ongoing Leishmania infection in susceptible BALB/c mice, but only when used in combination with the anti-parasite drug antimony (45) or if rIL-12 treatment is started within 14 days of challenge (46). In our system, administration of KLH plus rIL-12 (20 μg) to mice primed with KLH in alum failed to decrease IL-4 or IgE synthesis and resulted in increased production of IL-10 and IFN-γ (data not shown). In contrast, HKL as an adjuvant in our in vivo model system was effective in reducing ongoing IgE and IL-4 synthesis. This effect was due to the induction of endogenous IL-12 production by HKL, since neutralization of IL-12 activity with a mAb abolished the effect of the HKL. Administration of an anti-IFN-γ mAb did not affect the activity of HKL, suggesting that HKL functioned independently of IFN-γ.

There are several possible reasons why HKL is much more effective than rIL-12 in reducing ongoing Th2-dominated immune responses. HKL, in contrast to IL-12, reduces IL-10 synthesis (Fig. 3), which may result in enhanced IL-12 production. Furthermore, it is possible that the adjuvant effects of HKL are localized to sites of antigenic stimulation, whereas the effects of rIL-12, which diffuses rapidly into the systemic circulation, are more widespread in the host, less Ag-specific, and appear to evoke sustained NK cell activation. The restriction of the effects of HKL plus Ag to Ag-specific cells may be similar to that observed with fusion proteins consisting of Ag genetically linked to IL-12. Such fusion proteins are similarly effective in reducing IL-4 and IgE synthesis as HKL plus Ag and appear to limit the effects of IL-12 to Ag-specific cells (47).

Another reason for the effectiveness of HKL as an adjuvant is that HKL may induce the production of several cytokines in addition to IL-12, such as IL-18, which is 10-fold more potent than IL-12 in inducing IFN-γ (48) and also extremely effective in reducing IgE synthesis in B cells. HKL as an adjuvant may function similar to naked DNA containing unmethylated CpG motifs, which...
appear to induce production of IL-12 and IL-18. Plasmids containing such motifs and cDNA for allergen, when injected intramuscularly, are effective in reversing Th2-dominated immune responses (49, 50). However, HKL plus Ag may provide more consistent and potent immune responses. The magnitude of such responses with HKL is comparable with that seen with CFA, which is much greater than that seen with plasmid DNA vaccination (51). Since the difference between CFA and IFA is the presence of killed mycobacterium, administration of KLH with HKL was more effective in reversing Th2 responses than administration of KLH with mycobacterium.

The effectiveness of HKL as an adjuvant in reducing Th2-dominated immune responses and reducing Ag-specific IgE synthesis suggests that it may be clinically useful in the treatment of diseases caused by heightened allergen-specific Th2 responses, such as allergy and asthma. Allergen immunotherapy, which is currently performed by vaccination with aqueous extracts of allergen, is used as an effective therapy for these two diseases (52, 53), although treatment failures are frequent. Since disease improvement with allergen immunotherapy is associated with the reduction of allergen-specific IL-4 synthesis (54), and since HKL is potent in reducing Ag-specific Th2-dominated immune responses and Ag-specific IgE synthesis, modification of conventional allergen immunotherapy to include adjuvants such as HKL may render allergen immunotherapy much more efficacious. Preliminary data in our laboratory also suggest that immunotherapy with HKL as an adjuvant can reduce allergen-induced airway hyperreactivity (G. Hansen et al., unpublished observations) and may be more effective than intratracheal rIL-12 (55) in such a model. Safety issues with Listeria may not be of major concern, since killed rather than live Listeria is effective; in addition, even live Listeria is not a particularly invasive organism, and is a pathogen primarily in immunosuppressed patients or in the setting of pregnancy. Therefore, HKL may be an effective adjuvant for allergen immunotherapy, eliciting rapid innate immune system activation and production of Th1-inducing and Th2-reducing cytokines on vaccination.

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