Antigen coupled with Lewis-x trisaccharides elicits potent immune responses in mice

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Background: Glycoproteins containing Lewis-x (Le^x) trisaccharides are often associated with the host's adaptive T_H2-type immunity, but the mechanisms underlying the T_H2-biased response are at present unclear.
Objective: The modulatory effect of Le^x or its glycoconjugates on IgE/T_H2 responses was investigated.
Methods: The levels of serum antibodies and cytokines were analyzed by means of ELISA, RT-PCR, or both.
Results: In C3H mice Le^x coupled with BSA (Le^x-BSA) elicited higher levels of specific IgE and IgG1, but not IgG2a, which were associated with increased levels of splenic T_H2 cytokines when compared with those seen in BSA-sensitized mice. In BALB/c mice sensitized with Le^x-BSA or Le^x mixed with ovalbumin, significantly increased levels of specific IgE and IgG2a antibodies were found concomitant with reduced levels of serum IL-12p70. These effects were attenuated in IL-12–deficient BALB/c mice. Le^x and an isomer, Le^y, but not other isomers, inhibited the production of LPS-induced IL-12p70, associated with a significant reduction of nuclear NF-κB, in bone marrow–derived dendritic cells from BALB/c mice, suggesting that Le^x-induced suppression of IL-12p70 results in an enhanced T_H2 response. The addition of mannan, a known ligand for dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin, abrogated the suppressive effect of Le^x trisaccharides.
Conclusion: These results provide evidence for a potential role of Le^x trisaccharides in shaping the immune responses through, at least in part, its suppressive effect on IL-12p70 production. Considering the relative ubiquity of glycoproteins with Le^x or similar oligosaccharides, including plant-derived (or food-derived) allergens, these findings might have a broad implication.
Clinical implications: The adjuvant activity of Le^x trisaccharides might aid in vaccine design and might be important in determining the allergenicity of proteins containing this or other similar structures. (J Allergy Clin Immunol 2007;119:1522-8.)

Key words: *Dendritic cells, C-type lectin receptor, Lewis-x trisaccharides*

Heteroglycans are involved in homeostasis and are critical in cellular adhesion, innate and adaptive immunity, and tumor metastasis.[1-3] Among various heteroglycan structures, Lewis-x (Le^x) trisaccharide (β-D-Gal-(1,4)-[α-L-Fuc-(1,3)]-β-DGlcNAc-OH) is one of the carbohydrate moieties commonly found in mammalian and nonmammalian complex glycans. Several human pathogens, including *Helicobacter pylori* and *Schistosoma mansoni*, are known to express Le^x-containing glycans, which are often associated with pathogen-induced T_H2-biased adaptive immunity,[3,4] featuring increased levels of the T_H2 cytokines IL-4 and IL-13 and IgE antibodies. For example, *Schistosoma mansoni* egg antigen has been shown to induce strong T_H2-associated cytokine and antibody responses,[5] and the Le^x-containing glycan lacto-*N*-fucopentaose III acts as an adjuvant for the T_H2 response.[5] Although the biased T_H2 responses mediated through the Le^x–containing complex glycans in pathogens have been repeatedly observed, the molecular mechanisms underlying the T_H2 polarization remain to be defined. It is also unclear whether Le^x trisaccharide in the complex glycan structure is directly involved in these biased responses. It is of interest to note that complex glycans bearing an α(1-3)-fucosylated core are also commonly found in plant, insect, and food allergens that cause T_H2- and IgE-mediated allergy and have been suggested to be important in IgE cross-reactivity.[6,7]

It has been established that dendritic cells (DCs), a potent antigen-presenting cell type, are critical in the development of adaptive T-cell responses and in the generation of host innate immunity.[8,9] The expression of C-type lectin receptors (CLRs) on DCs is shown to be crucial in the recognition of complex glycan structures on various pathogens and has evolved to facilitate the endocytosis and presentation of antigens.[3,10,11] Several CLRs have also been implicated in modifying the cellular response through cross-regulation of the Toll-like receptor (TLR)–mediated effect.[12] For example, dectin-1,[13] a receptor for the yeast zymosan, cooperates with TLR2 to enhance the production of TNF-α and IL-12 (a potent T_H2 inhibitor[14]) by human DCs. In contrast, signaling through DC-specific intercellular adhesion molecule 3–grabbing nonintegrin (DC-SIGN; CD209) antagonizes the TLR4-mediated

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Abbreviations used

BM-DC:	Bone marrow–derived CD11c$^+$ dendritic cell
CLR:	C-type lectin receptor
DC:	Dendritic cell
DC-SIGN:	Dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin
Le^x:	Lewis-x
Le^x-BSA:	Le^x coupled with BSA
NF-κB:	Nuclear factor κB
OVA:	Ovalbumin
TLR:	Toll-like receptor

effect.[15] Human DC-SIGN contains C-type carbohydrate recognition domains and is known to bind to high-mannose oligosaccharides and several Lewis glycoforms, including Le^a, Le^y, and Le^x, with different binding patterns.[2,4,16,17] In contrast to human DC-SIGN, the recently identified murine homologues mDC-SIGN and mSIGNR1-8 reveal structural and functional differences when compared with human DC-SIGN or closely related DC-SIGNR (L-SIGN), and at present, their ligand specificity and expression patterns are much less well defined.[18,19] At this time, the nature of the ligand specificity for mDC-SIGN is unknown; mSIGNR1 has been shown to have binding specificity for mannose-, fucose-, and N-acetylglucosamine–terminating oligosaccharides when the soluble mSIGNR1-Fc fusion proteins are used.[20] Fucose recognition is demonstrated to be similar to that of human DC-SIGN, namely Le^x, Le^y, Le^a, and Le^b.

Although this has been an active area of investigation, we have used mouse bone marrow–derived CD11c$^+$ DCs (BM-DCs) as a model and demonstrated, for the first time, that Le^x antagonizes LPS-induced IL-12 expression, a potent T_H2 regulator, in BM-DCs. In addition, unconjugated Le^x is a potent adjuvant for eliciting IgE response to BSA, an immunogenic and otherwise T_H1 antigen,[21] which was particularly evident in C3H mice. This effect is possibly mediated through the suppression of nuclear factor κB (NF-κB) activation and subsequent release of IL-12, as seen in activated BM-DCs *in vitro*. Therefore these results suggest a direct involvement of Le^x trisaccharides in shaping the immune response.

METHODS

Mice and immunization

Mice (BALB/c, IL-12 deficient on a BALB/c background, TLR4-defective C3H/hej, or TLR4-wild type C3H/HeOuj mice; 6-8 weeks old; male or female; 6-12 per group) were sensitized with saline, BSA (50 μg; Sigma-Aldrich, St Louis, Mo), or Le^x-coupled BSA (Le^x-BSA, 50 μg; V-LABS, Covington, La) by means of intraperitoneal injection once a week for 2 weeks. All experiments were approved by the Animal Use and Care Committee of Johns Hopkins University School of Medicine and conformed to the institutional and National Institute of Health guidelines. In some cases BALB/c mice were sensitized with saline, ovalbumin (OVA; 50 μg, Sigma-Aldrich), OVA in combination with the free form of Le^x (5 μg, V-LABS), or a common adjuvant, alum (1 mg; Pierce, Rockford, Ill).

Analysis and adoptive transfer of DCs

Mouse BM-DCs were prepared according to the method of Lutz et al,[22] and the surface expression of CD11c, MHC II, CD80, and CD86 was analyzed to confirm the purity (>98%) of the DCs. Mouse (BALB/c) BM-DCs (2×10^5 cells) were incubated with varying concentrations (0.02-2 μmol/L) of Le^x, Le^y, Le^a, sialyl-Le^x, or sulfated Le^x for 30 minutes, followed by activation of the cells with LPS (10 ng/mL; *Escherichia coli*, 0127:BB; Sigma), at which dose there was a significant induction of IL-12p40 and p70 expression; hence it was subsequently used for all assays. After 48 hours, the supernatants were collected for analysis of the cytokines IL-10 and IL-12p70 by means of ELISA (eBioscience, San Diego, Calif, and R&D Systems, Minneapolis, Minn). For blocking experiments, mouse BM-DCs were cultured in the presence of mannan (25 μg/mL; V-LABS, catalog no. PS129) for 30 minutes, and then Le^x-BSA (25 μg/mL) was added for an additional 30 minutes before LPS stimulation. The relative level of IL-12p40 expression normalized by G3PDH was assessed by means of RT-PCR 4 hours after LPS stimulation. For nuclear NF-κB detection, mouse BM-DCs (BALB/c; 2×10^5 cells) were incubated with or without Le^x (2 μmol/L) for 30 minutes and then treated with LPS (10 ng/mL) for 2 hours, and the concentrations of nuclear NF-κB were measured by using an NF-κB ELISA kit (Biosource, Camarillo, Calif). For adoptive transfer, BM-DCs (1×10^6 cells per mouse) from naive C3H/hej mice were pulsed with or without BSA (25 μg/mL) or Le^x-BSA (25 μg/mL) for 24 hours, washed, and then injected intraperitoneally into naive C3H/hej mice. In some cases the levels of serum IL-12p70 in sensitized mice were measured by means of ELISA.

T-cell stimulation and cytokine and serum antibody measurement

Twenty-one days after the initial sensitization, the relative levels of antigen-specific IgE, IgG1, and IgG2a in sera were determined by means of ELISA and expressed as OD, as previously described.[23] In analyses of serum antibody levels, titration experiments of pooled sera were performed initially to examine the levels of antigen-specific IgE and IgG1 and to determine the upper–lower limits of detection, and based on the results, optimal dilutions (1:1 dilution for IgE and 1:1000 for IgG2a measurement) of serum samples were selected for analysis of the relative levels of antibodies in each mouse sample. The splenocytes (2×10^6 cells) were stimulated either with medium control, antigen (BSA or OVA; 25 μg/mL), Le^x-BSA (25 μg/mL), or Le^x (5 μg/mL) plus OVA (25 μg/mL), the supernatants were collected 48 hours after stimulation, and cytokines (IL-4, IL-13, and INF-γ) were analyzed by means of ELISA (eBioscience and R&D Systems).

Statistical analysis

Data are expressed as means ± SEMs for each subject group. Statistical analysis was performed with the Student unpaired *t* test and the Mann-Whitney *U* test. A *P* value of less than .05 was considered significant.

RESULTS

Le^x is a potent adjuvant

C3H/hej mice with defective LPS-TLR4 signaling and TLR wild-type C3H mice were investigated to demonstrate the activity of uncoupled Le^x or Le^x-conjugated BSA in mice and also to rule out a potential influence of endotoxin on the induction of IgE.[24] The results from a series of titration experiments of pooled sera from the same group of mice showed that the titers of both IgE and IgG1

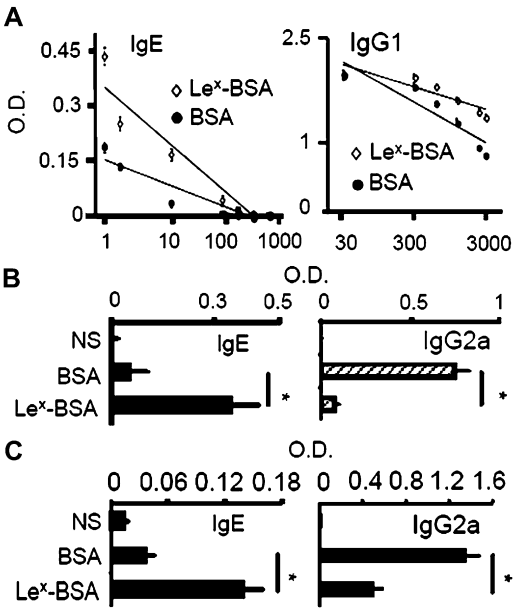

FIG 1. The relative levels of serum-specific antibodies in sensitized, C3H/hej, and TLR4 wild-type C3H/HeOuj mice. **A**, Serum titration analysis of specific IgE and IgG1 levels. **B** and **C**, The relative levels of serum IgE and IgG2a antibodies in sensitized C3H/hej (Fig 1, *B*) and TLR4 wild-type (Fig 1, *C*) mice. N = 9 to 12 mice per group. $*P < .05$. *NS*, Normal saline.

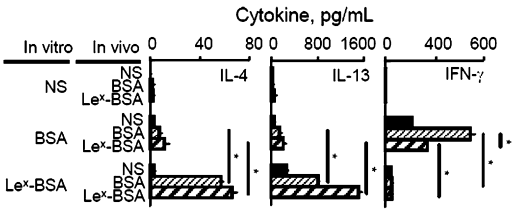

FIG 2. Levels (mean ± SE) of the cytokines IL-4, IL-13, and IFN-γ in spleen cells from sensitized C3H/hej mice with saline *(NS)* or antigen (BSA or Le^x-BSA) stimulated with NS, BSA, or Le^x-BSA. N = 9 mice per group. $*P < .05$.

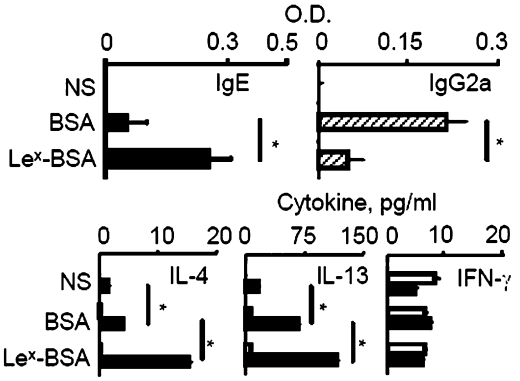

FIG 3. Analysis of antibody and cytokine levels in C3H/hej mice receiving DC transfers. **A**, Relative levels (mean ± SE) of serum-specific antibodies in mice receiving BM-DC transfers. **B**, Levels (mean ± SE) of the cytokines IL-4, IL-13, and IFN-γ in spleen cells of adoptively transferred mice. N = 9 to 12 mice per group. $*P < .05$. *NS*, Normal saline.

were significantly higher in C3H/hej mice sensitized with Le^x-BSA when compared with those seen in mice immunized with BSA (Fig 1, *A*). Furthermore, when individual serum samples were analyzed, significantly enhanced levels of specific IgE were found, whereas in contrast Le^x-BSA failed to significantly upregulate the levels of IgG2a (Fig 1, *B*). In TLR4 wild-type C3H mice, similarly enhanced levels of IgE, but not IgG2a, were also found (Fig 1, *C*), suggesting that Le^x selectively increased the IgE and IgG1 responses in C3H mice, and this was independent of the LPS-TLR4 signaling.

To examine the activity of Le^x on the T_H2 responses *in vitro*, splenocytes from mice immunized with saline control, BSA alone, or Le^x-BSA were stimulated either with medium alone or each of the sensitizing antigen mixture, and 48 hours later, the cytokine levels were analyzed. As shown in Fig 2, in the absence of antigen stimulation, the levels of cytokines were low, but significantly increased levels of both IL-4 and IL-13 production were observed in Le^x-BSA–stimulated cells from BSA- or Le^x-BSA–sensitized mice when compared with those in BSA-stimulated cells. In contrast, enhanced levels of IFN-γ were noted in BSA-stimulated splenocytes from BSA-sensitized mice, which were significantly higher than those seen in Le^x-BSA–stimulated cells (Fig 2).

To investigate whether DCs are responsible for the Le^x-mediated adjuvant effect, adoptive transfers of BSA- or Le^x-BSA–treated BM-DCs into naive C3H/hej mice were performed, and 1 week after cell transfer, the levels of serum BSA-specific IgE and IgG2a were determined. Consistent with results from active immunization experiments, significantly increased levels of IgE and reduced levels of IgG2a were found in mice receiving Le^x-BSA–pulsed DCs when compared with those seen in mice receiving BSA-pulsed BM-DCs (Fig 3, *A*). Also, significantly increased levels of both IL-4 and IL-13 production were observed in BSA-stimulated spleen cells from mice receiving Le^x-BSA pulsed DCs, whereas very low levels of T_H2 cytokines were detected in BSA-stimulated cells from mice receiving transfers of BSA-pulsed DCs. No significant difference in the levels of IFN-γ was seen in the splenocytes in all 3 groups (Fig 3, *B*).

Adjuvant activity of Le^x in BALB/c mice

To demonstrate the activity of uncoupled Le^x or Le^x-conjugated BSA in a different mouse genetic background, BALB/c mice were sensitized with BSA alone or Le^x-coupled BSA (Le^x-BSA). As shown in Fig 4, *A*, significantly higher levels of BSA-specific IgE and IgG2a antibodies were seen in mice sensitized with Le^x-BSA compared with that seen with BSA alone. When the free form of Le^x was used together with BSA, increased levels of IgE and IgG2a were also found, although its potency is less than that for Le^x-coupled BSA. Splenocytes from Le^x-BSA–sensitized mice produced significantly higher levels of T_H2 cytokines (IL-4 and IL-13) than those of

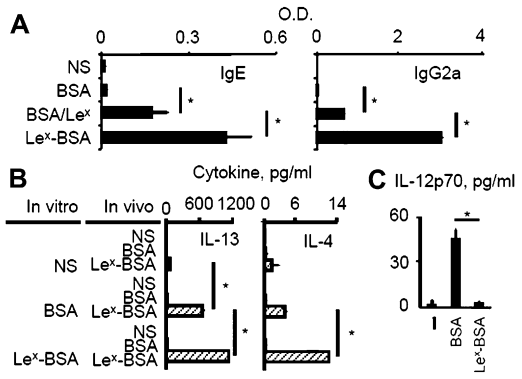

FIG 4. Analysis of antigen (BSA)–induced antibody and cytokine levels in BALB/c mice. **A**, Relative levels (mean ± SE) of serum BSA-specific antibodies in sensitized BALB/c mice. **B**, Levels (mean ± SE) of the T_H2 cytokines IL-4 and IL-13 in spleen cells. N = 9 to 12 mice per group. **C**, Analysis of serum IL-12p70 levels in mice sensitized with saline *(–)*, BSA, or Le^x-BSA. N = 4 mice per group. *$P < .05$. *NS*, Normal saline.

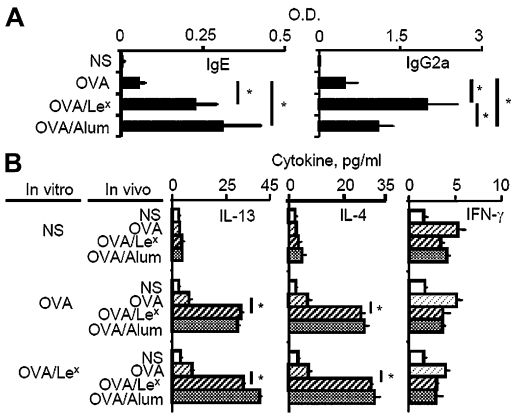

FIG 5. Measurement of antigen (OVA)–induced antibody and cytokine levels in BALB/c mice. **A**, Relative levels (mean ± SE) of serum OVA-specific antibodies in sensitized BALB/c mice. **B**, Levels (mean ± SE) of IL-4, IL-13, and IFN-γ in spleen cells. N = 6 to 9 mice per group. *$P < .05$. *NS*, Normal saline.

BSA-sensitized mice (Fig 4, *B*). The enhanced T_H2 cytokine expression was seen in cells stimulated with either BSA or Le^x-BSA, whereas the highest levels of T_H2 cytokines were seen in Le^x-BSA–stimulated cells from Le^x-BSA–sensitized mice. Furthermore, significantly reduced levels of serum IL-12p70 were seen in Le^x-BSA–sensitized mice when compared with those found in BSA-sensitized mice (Fig 4, *C*). The levels of IFN-γ production were very low and could not be reliably determined in all antigen-stimulated cultures (data not shown).

Next, we sought to examine whether the adjuvant effect of Le^x is dependent on the antigen used. We repeated the above experiments, replacing BSA with OVA. As seen in Fig 5, *A*, although immunization of BALB/c mice with OVA alone showed production of detectable levels of specific IgE and IgG2a, significantly enhanced IgE and IgG2a levels were found in mice sensitized with Le^x and OVA. In fact, the relative level of specific IgE was equivalent to that seen in mice sensitized with OVA and a common T_H2 adjuvant, alum; this is especially significant when considering that the dose of Le^x used was 3 orders of magnitude lower than that of alum. Mice immunized with OVA and Le^x demonstrated also increased levels of OVA-specific IgG2a. Moreover, significantly increased levels of both IL-4 and IL-13 production were observed in OVA-stimulated spleen cells from mice immunized either with OVA/Le^x or OVA/alum (Fig 5, *B*). Again, in this strain of mice, the levels of IFN-γ were low and showed no significant difference in all antigen-stimulated cultures (Fig 5, *B*). Therefore these results suggest that uncoupled Le^x is also a potent adjuvant for OVA-induced immune responses *in vivo*.

Le^x suppressed LPS-induced IL-12 production in BM-DCs

To investigate the modulatory effect of Le^x on DCs, the BM-DCs from BALB/c mice were treated with each of the Lewis glycoforms or activated by LPS in the presence or absence of varying concentrations of Le^x or other Lewis glycoforms, Le^y, Le^a, or sialyl or sulfated Le^x. As seen in Fig 6, *A*, a significant dose-dependent reduction of LPS-induced IL-12p70 expression was seen in the presence of Le^x and Le^y, but not other Lewis oligosaccharides, whereas the levels of LPS-induced IL-10 were similar in DCs treated with either Le^x or Le^y (Fig 6, *B*). Similar inhibitory effects were also found when Le^x was coupled with BSA (Fig 6, *C*), and none of the Lewis glycoforms, including Le^x, could directly modulate cytokine release in resting DCs (data not shown). Although blocking antibodies for mDC-SIGN or mSIGNR1 are not available at present, the effect of mannan, a known ligand for mDC-SIGN and mSIGNR1,[20] on Le^x-mediated suppression of IL-12 was examined. As shown in Fig 6, *D*, the addition of mannan (25 μg/mL) abrogated the Le^x-mediated suppression of LPS-induced IL-12p40 gene expression. Therefore these results suggest the potential involvement of mDC-SIGN or mSIGNR1 on Le^x-mediated effect.

Nuclear translocation of activated NF-κB[25] in LPS-activated BM-DCs (BALB/c) was examined to investigate the potential underlying mechanisms of the IL-12 suppression by Le^x. As shown in Fig 6, *E*, although as expected very low levels of nuclear NF-κB were seen in resting DCs, DCs pretreated with Le^x revealed a significant reduction in the level of nuclear NF-κB compared with that of untreated DCs after the stimulation of the cells with LPS, suggesting that Le^x-mediated suppression of LPS-induced IL-12 is mediated, in part, by its inhibitory activity on NF-κB activation in DCs. Of note, no significant difference was seen in the levels of class II MHC, CD80, and CD86 in DCs treated with or without unconjugated Le^x or Le^x-BSA, and the viability also remained similar during the culture time period.

IL-12–deficient mice on a BALB/c background were tested to investigate the involvement of IL-12 in Le^x-mediated induction of T_H2 response *in vivo*. As seen in Fig 7, *A*, immunization of IL-12–deficient mice with

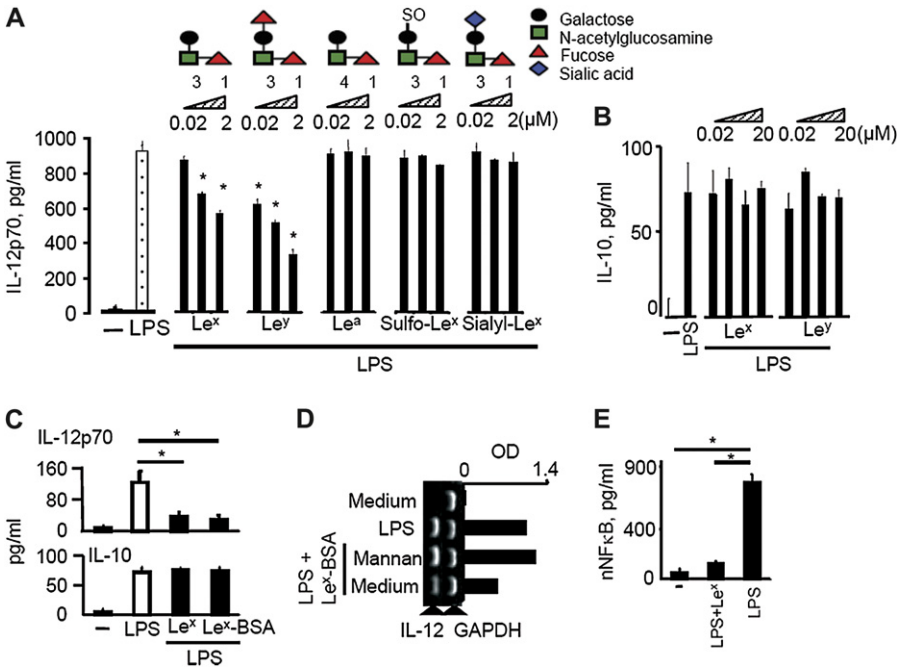

FIG 6. Analysis of cytokines in BM-DCs. **A** and **B**, Levels (mean ± SE) of IL-12p70 (Fig 1, *A*) and IL-10 (Fig 1, *B*) in BALB/c BM-DCs are shown. DCs were incubated with medium alone or LPS in the presence or absence of varying concentrations of glycoforms as indicated, with their respective structures and linked positions for fucose shown. **P* < .05 versus LPS alone. **C**, Levels (mean ± SE) of LPS-induced IL-12p70 and IL-10 production in BM-DCs. **P* < .05 versus LPS alone. **D**, Mannan blocks the inhibitory effect of Le^x-BSA on LPS-induced gene expression for IL-12p40 in BM-DCs. **E**, Relative level (mean ± SE) of nuclear NF-κB *(nNF-κB)* in BM-DCs. **P* < .05. N = 3 experiments.

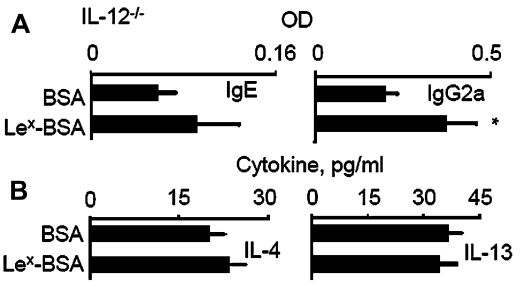

FIG 7. Levels of antibodies and cytokines in IL-12$^{-/-}$ mice. **A**, Relative levels (mean ± SE) of serum BSA-specific antibodies. **B**, Levels (mean ± SE) of the T_H2 cytokines IL-4 and IL-13 in spleen cells from BSA- or Le^x-BSA–sensitized mice stimulated with BSA. N = 9 to 12 mice per group. **P* < .05.

Le^x-BSA failed to demonstrate the enhancement of specific IgE levels when compared with those observed in wild-type mice. Although the difference in the levels of specific IgG2a remained significant, the difference was significantly reduced in IL-12$^{-/-}$ mice when compared with that found in wild-type BALB/c mice, and no significant increase in the levels of the T_H2 cytokines IL-4 and IL-13 was found in Le^x-sensitized IL-12$^{-/-}$ mice when compared with those seen in BSA-sensitized mice (Fig 7, *B*), suggesting the suppression of IL-12, a critical immune modulator for T_H2 responses,[14] as a contributing factor in the Le^x-mediated adjuvant effect.

DISCUSSION

In this report we provide evidence that Le^x and an isomer, Le^y, antagonize the production of LPS-induced IL-12p70, but not IL-10, in BM-DCs and that unconjugated Le^x serves as a potent adjuvant for eliciting immune responses in 2 inbred strains of mice. Furthermore, these results also suggest that although the levels of IgG2a antibodies, a surrogate marker for T_H1 response, vary in different strains of mice, the adjuvant activity of Le^x in IgE and T_H2 responses is independent of the strain used and is likely mediated through the suppression of IL-12.

In addition to the suppression of IL-12 production, it is likely that coupling of Le^x to antigen might also facilitate enhanced antigen uptake and processing by the DCs. This is particularly relevant because enhanced antigen uptake and presentation by the DCs through DC-SIGN has been shown.[26] The increased levels of T_H2 responses seen in mice sensitized with Le^x-BSA conjugates versus the free form of Le^x could be explained by enhanced antigen recognition and endocytosis in DCs. An alternative but not mutually exclusive possibility is that Le^x or Le^x coupled with antigen might directly activate T cells or other cell types, such as B cells and macrophages. Although the likelihood of this cannot not be determined at this time, Le^x-containing glycans have previously been shown to be able to activate B cells directly.[27] It is noted that similar to those found in BALB/c mice sensitized with antigen

and alum, the levels of IgG2a antibodies were also significantly increased in mice when Le^x was used, even in the absence of IL-12 *in vivo* and increased levels of splenic IFN-γ. IFN- γ–independent IgG2a production has been observed in mice after viral and parasitic infections,[28] suggesting a multifactorial influence on the expression of IgG2a, depending, perhaps in part, on the nature of the inciting antigens, the genetic background, or both.

Furthermore, the modulatory effect of Le^x was observed only when the DCs were activated through, for example, the LPS-TLR4 signaling pathway, suggesting a potential involvement of TLR in the Le^x-mediated adjuvant effect. Other mechanisms of activation, such as those through CD40-CD40L,[29] are likely to be involved in the adjuvant activity of Le^x and might be operative in the polarized T_H2 response seen in TLR4-defective C3H/hej mice. Also, the reduction of LPS-induced IL-12p70 expression in DCs was seen only in the presence of Le^x and Le^y, but not other Lewis oligosaccharides, including Le^a, suggesting that the conformation and the binding affinity of the oligosaccharides determine the functional consequences of CLR-ligand binding.

In our study the relative level of IgE response seen in Le^x-BSA–sensitized, IL-12–deficient mice was lower than that found in wild-type mice. This might reflect the fact that IL-12 is needed for proper maturation and migration of DCs and hence optimal immune response.[30] Also, it is noted that no significant increase in T_H2 response to pathogens[31,32] or OVA antigen is found in IL-12–deficient mice compared with that seen in the wild-type mice. This is perhaps due, in part, to the likely difference in the nature of the inciting antigens and the ensuing dynamic regulatory processes in the host.

Although the nature of the CLRs in mouse DCs responsible for the Le^x-mediated effect is, at present, unclear, in our study mannan, a known ligand for mDC-SIGN/mSIGNR1,[20] abrogated the suppressive effect of Le^x on LPS-induced IL-12 expression, suggesting a potential involvement of mDC-SIGN, mSIGNR1, or both in the Le^x-mediated effect *in vitro*. mDC-SIGN is known to be expressed exclusively in $CD8\alpha^-$ DCs[19] but not $CD8\alpha^+$ DCs. In contrast, mSIGNR1 is expressed in macrophages within the splenic marginal zone, lymph node medulla, and liver sinus endothelial cells, with a very low level of expression in $CD11c^+$ DCs.[18,19] Consistent with this previous finding, BM-DCs in our study expressed only mDC-SIGN but none of the other DC-SIGN homologues (data not shown). It is likely therefore that the $CD8\alpha^-$ DC subset is the target cell type for the Le^x-mediated effect *in vitro*. However, the target DC population *in vivo* remains to be determined. Based on its expression pattern, mSIGNR1 appears to show more homology to human L-SIGN rather than DC-SIGN. Also, although DC-based (or macrophage-based) studies of its binding specificity are needed, mSIGNR1 has been shown to have binding specificity for Le^x, Le^y, Le^a, and Le^b, a feature similar to that of human DC-SIGN.[20] It is thus likely that the Le^x-mediated effect *in vivo* might involve multiple DC subsets through a combination of mDC-SIGN and mSIGNR1. Additional studies are needed to define the regulatory pathways leading to the Le^x-mediated polarizing effect when necessary reagents become available.

It is of interest to note that α(1-3)-fucosylated and xylosylated glycans are commonly present in pollen and food allergens, and the conserved core α(1-3)-fucose epitope has been suggested to be a potential panallergen in IgE recognition.[7] It is thus likely that complex N-glycans bearing an α(1-3)-fucose residue to the innermost GlcNAc, such as those found in Le^x-containing glycans, might play a role in the polarization and persistence of the T_H2 responses. Also of interest is the fact that in human subjects the Le^x trisaccharide epitope, often found in glycoproteins (and glycolipids) and assigned to the CD15 cluster, exists in various cell types and tissues and is expressed at different stages of development, but the functional significance of its existence remain obscure. The known interactive and potentially modulatory activity of Le^x-containing glycans on DC functions might suggest a new dimension in cellular adhesion, homeostasis, and disease mechanisms.

In summary, our present data suggest that Le^x, in both free and conjugated forms, is a potent adjuvant for eliciting immune responses, particularly favoring the T_H2-type response through its suppressive effect on IL-12. The evidence provided herein might have strong implications with regard to its potential use as an adjuvant for vaccine design and might suggest its potential role in the development and persistence of pathogen- and perhaps allergen-induced T_H2 responses. Our current study provides an experimental basis for testing these hypotheses.

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