Prevention of IL-1 signaling attenuates airway hyperresponsiveness and inflammation in a murine model of toluene diisocyanate–induced asthma

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Background: IL-1 is a pleotropic cytokine that has been shown to play a prominent role in asthma induced by large-molecular-weight proteins. Increased IL-1 immunostaining in the submucosa of patients with toluene diisocyanate (TDI)–induced asthma has also been observed, suggesting that this cytokine might also be important in asthma associated with low-molecular-weight chemicals.

Objective: We sought to determine the role of IL-1 signaling in airway reactivity and inflammation by using a murine model of TDI-induced asthma.

Methods: C57BL/6 mice were exposed to TDI by means of vapor inhalation (20 ppb; 4 hours per day, 5 days per week, for 6 weeks) and then challenged 2 weeks later by inhalation with 20 ppb TDI vapor for 1 hour.

Results: Sensitized-challenged mice showed increased airway hyperresponsiveness (AHR), increased levels of TDI-specific IgG_1 antibodies, airway epithelial thickening, inflammation consisting of infiltrating lymphocytes and eosinophils, and increased mRNA expression of IL-4, intercellular adhesion molecule 1, and vascular cell adhesion molecule 1 in the lung. Prevention of IL-1 signaling through deletion of the IL-1 receptor type I or administration of neutralizing antibodies to both IL-1β and IL-1α abrogated the development of TDI-induced asthma. A partial reduction in AHR and TDI-specific IgG_1 levels was observed in mice administered anti-IL-1β, whereas anti-IL-1α had no effect on either parameter. Antibodies to IL-1β or IL-1α alone blocked airway inflammation and the expression of IL-4 and adhesion molecules in the lung.

Conclusions: These results suggest that IL-1 signaling is critical for AHR and airway inflammation, with IL-1β and IL-1α having unique and overlapping roles in TDI-induced occupational asthma. (J Allergy Clin Immunol 2005;116:851-8.)

Key words: *Toluene diisocyanate, occupational asthma, IL-1, IL-1 receptor type I, IL-1β, IL-1α, TDI*

Occupational asthma accounts for nearly 10% of all adult-onset asthma, with diisocyanates the most commonly reported cause.[1] Isocyanates are used for polyurethane foam manufacturing, autobody painting and repair, and plastics manufacturing. Nearly 250,000 workers are exposed to diisocyanates in the workplace annually, with clinical airways disease developing in 5% to 15%. Evidence from human and animal model studies suggests that T_H2 cells and their secreted cytokines play a prominent role in the pathogenesis of toluene diisocyanate (TDI)–induced asthma,[2,3] although T_H1 cytokines and CD8 T cells also participate.[2,4-6] Recent studies have indicated that proinflammatory cytokines might also be essential to the development of TDI-induced asthma. Lee et al[7] demonstrated increased production of IL-1β and TNF-α in the lungs of mice with TDI-induced asthma, and we recently identified an essential role for TNF-α and its cognate receptors using a mouse model.[8] In addition, bronchial biopsy specimens from workers with TDI-induced asthma have shown increased IL-1β and TNF-α expression in the submucosa.[9]

IL-1, a pleotropic cytokine that is produced in response to pathogenic infection, is a well-established mediator of chronic inflammatory diseases. Recent studies indicate that IL-1 also plays an important role in respiratory allergy and asthma.[10-13] IL-1 receptor type I–deficient mice (IL-1Rko) demonstrate reduced eosinophil recruitment to the bronchoalveolar lavage fluid (BALF) and lung tissue,[10] reduced ovalbumin-specific antibody production in mild asthma,[11] and reduced airway hyperresponsiveness (AHR) and T_H2 cytokine production after challenge in ovalbumin-sensitized mice.[12] The involvement of IL-1 in eosinophilic lung inflammation and AHR are supported by observations that IL-1 stimulates the release of IL-5 from airway smooth muscle cells,[13] a T_H2 cytokine important for eosinophil recruitment and activation, and induces endothelial cell production of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1).[14,15] In this respect the expression of IL-1β, ICAM-1, and VCAM-1 are increased after TDI challenge in sensitized mice.[7]

The purpose of the current study was to investigate the role of IL-1 in the pathogenesis of TDI-induced asthma

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Abbreviations used

AHR: Airway hyperresponsiveness
BALF: Bronchoalveolar lavage fluid
ICAM-1: Intercellular adhesion molecule 1
IL-1Rko: IL-1 receptor type 1–deficient mice
TDI: Toluene diisocyanate
VCAM-1: Vascular cell adhesion molecule 1
xIL-1α: Mice administered neutralizing anti-IL-1α antibodies
xIL-1β: Mice administered neutralizing anti-IL-1β antibodies
xIL-1β/xIL-1α: Mice administered neutralizing anti-IL-1β and anti-IL-1α antibodies

by using a previously characterized inhalation exposure murine model.[5,6] We hypothesized that disruption of IL-1 signaling would reduce or prevent TDI challenge–induced inflammation and AHR in sensitized mice, in part through preventing adhesion molecule expression. The IL-1 receptor type I is the functional signaling receptor,[15] and thus we used mice with a targeted deletion of this receptor. Ligands for the type I receptor include both IL-1β and IL-1α.[15] To investigate their individual contributions to TDI-induced asthma, we used an antibody-mediated neutralization strategy using *in vivo* treatment with antibodies to IL-1β and IL-1α separately and in combination. Our findings demonstrate critical roles for IL-1 in TDI-induced asthma.

METHODS

Materials

TDI (Mondur TD80 Grade A; 80% and 20% mixture of 2,4- and 2,6-isomers, respectively) was provided by Bayer Corporation, Polyurethanes Division (Pittsburgh, Pa). Rabbit polyclonal antisera against mouse IL-1β was produced by Biosource International (Camarillo, Calif). Neutralizing monoclonal hamster anti-mouse IL-1α was purchased from BD PharMingen (San Diego, Calif).

Experimental animals

Female wild-type C57BL/6 and IL-1Rko (B6.129S7-*Il1r1*^{tm1Imx}/J) mice were purchased from Jackson Laboratories (Bar Harbor, Me) at 5 to 6 weeks of age. IL-1Rko mice fail to respond to IL-1β or IL-1α by using established stimulators.[16] On arrival, the mice were quarantined for 2 weeks and acclimated to a 12-hour light-dark cycle. Animals were housed in microisolator cages in pathogen-free and environmentally controlled conditions (21°C ± 2°C and 50% ± 5% relative humidity) at National Institute for Occupational Safety and Health facilities in compliance with Association for the Assessment and Accreditation of Laboratory Animal Care International-approved guidelines and an approved Institutional Animal Care and Use Committee protocol (03-JM-M-005). Food and water were provided *ad libitum*. Groups of wild-type mice were administered neutralizing antibodies to IL-1β (200 μL of polyclonal antisera), IL-1α (100 μg in 200 μL of PBS), or both and are referred to as xIL-1β, xIL-1α, or xIL-1β/xIL-1α mice. Antibodies were administered through intraperitoneal injection on days 0 and 21 of the 6-week exposure period and again 24 hours before challenge. We demonstrated the blocking efficacy of the IL-1β antisera by showing that administration of the antisera 24 hours before treatment with 10 mg/kg LPS for 6 hours completely blocked the ability to detect IL-1β in the sera by means of ELISA (data not shown). The neutralizing capacity of the IL-1α antibody used was demonstrated elsewhere.[17]

Exposure

Generation of the TDI vapor atmosphere that was free of TDI aerosol was performed as described in detail by Matheson et al.[5,6] TDI vapors were generated by passing dried air through an impinger containing 3 mL of TDI. Chamber TDI concentration was regulated by mixing air from the TDI impinger with dilution air by using computer-interfaced mass flow controllers (Aalborg Instruments, Orangeburg, NY). Air flow through the system resulted in 5 to 8 complete air changes per hour. Temperature and relative humidity were maintained under controlled conditions of approximately 21°C to 24°C and 50% relative humidity, respectively. TDI concentration was monitored in real time with an Autostep continuous toxic gas analyzer (Bacharach, Inc, Pittsburgh, Pa), with TDI concentrations never varying more than 10% in the study. For sensitization, mice were exposed to TDI by means of inhalation of 20 ppb TDI for 6 weeks, 5 days per week, 4 hours per day, in a 10-L inhalation chamber, with only the heads of the animals extending into the chamber.[5,6] Challenge (1 hour, 20 ppb TDI) was performed on all groups 14 days after the last day of exposure. The control group represents mice exposed to air only during the 6-week period, followed by TDI challenge 14 days later.

Tissue collection

Mice were killed by mans of CO_2 inhalation 48 hours after airway challenge, and blood was collected. Serum was frozen at −80°C until analyzed for TDI-specific IgG_1 antibodies. After exsanguination, the chest cavity was opened, the right bronchus was sutured at the tracheal bifurcation, and the right lung was removed and stored at −20°C in RNAlater. The left lung was then inflated through a tracheal cannula with 350 μL of 10% neutral-buffered formalin, sutured at the trachea, and immersed in 10% neutral-buffered formalin for 18 hours. The tissues were embedded in paraffin, serially sectioned, and stained with hematoxylin and eosin for histopathologic assessment. Periodic acid–Schiff (PAS) staining was performed to identify goblet metaplasia, and Chromatrope 2R/Mayer's Hematoxylin staining was used for eosinophil identification. Additional groups of mice were killed 48 hours after challenge and used for BALF and blood collection. Mice were killed by means of CO_2 inhalation, exsanguinated from the abdominal artery, and intubated with a 20-gauge cannula positioned at the tracheal bifurcation to obtain BALF. Each mouse lung was lavaged with 1.0 mL of sterile HBSS at 37°C. BALF recovery was 80% ± 5% for all animals. Cytospin preparations were fixed and stained with Diff-Quick (VWR, Pittsburgh, Pa), and differential cell counts were obtained by using light microscopic evaluation of 300 cells per slide. Total cell counts were performed with a hemocytometer.

Airway hyperresponsiveness

Airway hyperresponsiveness (AHR) to methacholine challenge was assessed 24 hours after TDI challenge by using a single-chamber whole-body plethysmograph (Buxco, Troy, NY), as previously described.[5,6] Briefly, mice were placed into the plethysmograph and exposed for 3 minutes to nebulized PBS, and breathing was followed for 5 minutes to establish baseline values. This was followed by challenge with increasing concentrations of nebulized methacholine (0-50 mg contained in 1.0 mL of PBS) for 3 minutes per

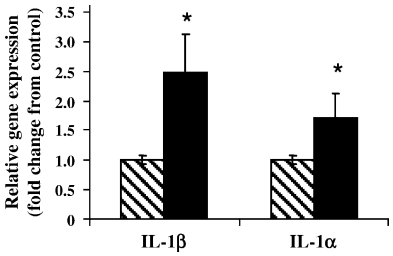

FIG 1. Effect of TDI challenge on IL-1 gene expression in the lung. Mice were exposed to 20 ppb TDI by means of inhalation for 6 weeks and challenged with 20 ppb TDI 2 weeks later, and changes in lung tissue expression of mRNA for IL-1β and IL-1α were determined 48 hours after challenge. *Hatched bars* and *filled bars* represent the control group and the sensitized-challenged group, respectively. Values are presented as means ± SEM (n = 5). *Significantly different from control group, $P < .05$.

dose with an AeroSonic ultrasonic nebulizer (DeVilbiss, Somerset, Pa). Recordings were taken for 5 minutes after each nebulization. The enhanced pause values during each 5-minutes sequence were averaged and expressed as the percentage increase over baseline values after PBS exposure for each methacholine concentration. Additional mice were placed in the plethysmograph immediately after challenge, and breathing was monitored continuously for 12 hours to determine the effects of TDI challenge on late-phase airway responses. The area under the enhanced pause versus time curve was then calculated and used to compare treatment groups.

TDI-specific IgG_1 antibody detection

TDI-specific IgG_1 antibodies were detected by using an ELISA procedure, as previously described.[5,6] The TDI–mouse serum albumin conjugate used as antigen in the ELISA was kindly provided by Dr Paul Siegel (National Institute for Occupational Safety and Health, Morgantown, WV). A cutoff OD_{405} of 0.2 (average OD_{405} of challenge only mouse serum was 0.06 ± 0.005) was used to determine the titer by means of extrapolation from individual dilution versus absorbance curves.

Real-time RT-PCR

Tissues were homogenized, and total cellular RNA was extracted with the Qiagen RNeasy kit (Qiagen, Valencia, Calif), according to the manufacturer's instructions. One microgram of RNA was reverse transcribed by using random hexamers and 60 U of Superscript II (Life Technologies, Grand Island, NY). Real-time PCR primer-probe sets for murine IL-1β, IL-1α, IL-4, IFN-γ, and 18s were purchased from Applied Biosystems (Foster City, Calif). Primers for ICAM-1 (forward, GAGTTTTACCAGCTATTTATTGAGTACCC; reverse, CTCTCACAGCATCTGCAGCAG) and VCAM-1 (forward, TTAAAGTCTGTGGATGGCTCGTAC; reverse, CTTAATTGTCAGCCAACTTCAGTCTT) were designed for Sybr green analysis by using Primer Express software (Applied Biosystems). Real-time PCR was performed with Taqman or Sybr Green Universal Master mix with Amperase in an iCycler (Bio-Rad, Hercules, Calif) for 1 cycle at 50°C for 2 minutes (degrade carryover with Amperase) and 95°C for 10 minutes, followed by 60 cycles at 95°C for 15 seconds and 60°C for 1 minute. Relative differences in mRNA expression between the control and treatment groups were determined by using the relative quantification method developed by Pfaffl.[18] This method uses gene-specific PCR efficiencies to more accurately generate relative changes on the basis of threshold cycle. Target gene expression was normalized to the housekeeping gene 18S/rRNA.

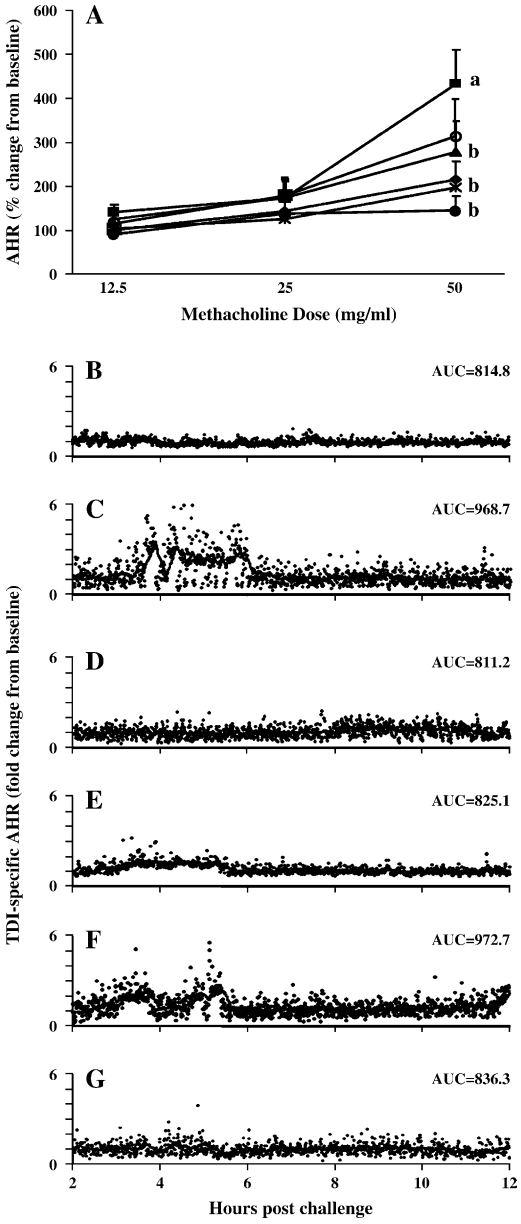

FIG 2. Influence of IL-1 depletion on TDI-specific and nonspecific AHR. Mice were exposed to 20 ppb TDI by means of inhalation for 6 weeks and challenged with 20 ppb TDI 2 weeks later. **A**, Nonspecific AHR to aerosolized methacholine (0-50 mg/mL in PBS) was determined 24 hours after TDI challenge by using whole-body plethysmography and is expressed as a percentage of baseline enhanced pause values. Changes in TDI-specific AHR were determined by using whole-body plethysmography. Mice were placed in the plethysmograph immediately after challenge, and enhanced pause was monitored continuously for 12 hours. Data are expressed in arbitrary units derived from calculating the area under the curve for enhanced pause plotted over the 12-hour monitoring period. **B-G**, Representative enhanced pause versus time curves for the control, sensitized-challenged, IL-1Rko, xIL-1β, xIL-1α, or xIL-1β/xIL-1α groups, respectively, where the *dots* represent enhanced pause readings every 30 seconds, and the *lines* are rolling averages of 10 readings. *AUC*, Area under the curve (see the Methods section). Values are presented as means ± SEM (n = 5). Significantly different from the control group *(a)* or the sensitized-challenged group *(b)*, $P < .05$. *S/C*, Sensitized-challenged group.

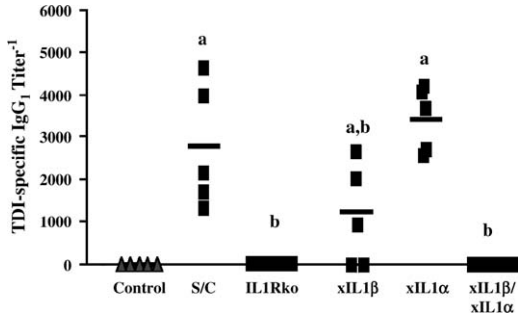

FIG 3. Effect of TDI inhalation on TDI-specific IgG_1 serum antibody levels. Mice were exposed to 20 ppb TDI by means of inhalation for 6 weeks and challenged with 20 ppb TDI 2 weeks later. Blood was drawn 48 hours after challenge, and serum was analyzed for TDI-specific IgG by using a direct ELISA procedure. Data are expressed as the reciprocal of the serum dilution, resulting in an OD_{405} value of 0.2. Each *point* represents an individual mouse, and the *bars* represent group means. Significantly different from the control group *(a)* or the sensitized-challenged group *(b)*, $P < .05$. (n = 5). *S/C*, Sensitized-challenged group.

Statistical analysis

Studies were conducted by using a complete block design. Treatment effects were determined by using 1-way ANOVA, followed by the Fisher PLSD post-hoc test.[19] Differences were considered significant at a P value of less than .05.

RESULTS

Pulmonary expression of IL-1β and IL-1α is upregulated after challenge in TDI-sensitized mice

Increased IL-1β (2.5-fold increase relative to control) and IL-1α (1.8-fold increase relative to control) gene expression was observed in the lung tissue of TDI-exposed mice after challenge (Fig 1). Mice that were subchronically exposed to TDI but did not receive TDI challenge did not show increased IL-1 gene expression (data not shown). This is consistent with earlier observations of the lack of an asthmatic phenotype in the sensitized-only mice.[5,6]

IL-1 signaling is important for nonspecific AHR and TDI-specific late-phase AHR

Increased nonspecific AHR in response to methacholine was observed at the highest dose of methacholine 24 hours after TDI challenge in mice subchronically exposed to TDI for 6 weeks (Fig 2, *A*). This methacholine dose response is similar to that seen in our previous studies.[5,6] The AHR response to TDI challenge was abrogated in the IL-1Rko mice carrying a deletion of IL-1 receptor type I. In contrast, treatment with neutralizing antibody to IL-1β resulted in significant but only partial reduction in AHR, whereas anti-IL-1α antibody administration did not significantly reduce AHR relative to the sensitized-challenged mice. However, administration of both IL-1β and IL-1α neutralizing antibodies together completely eliminated the increase in AHR, confirming the results observed for the IL-1Rko mice.

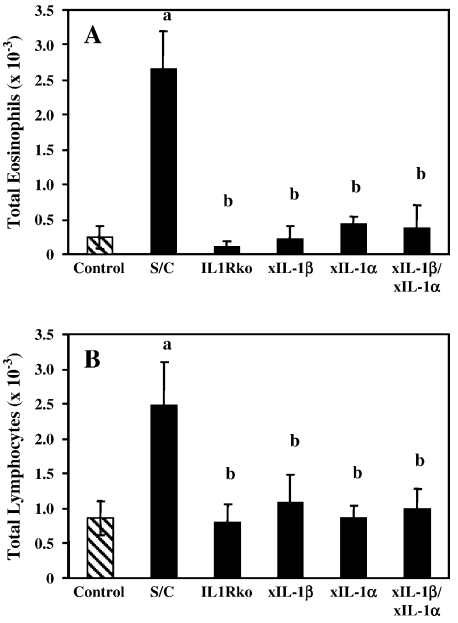

FIG 4. Effect of TDI challenge on recruitment of inflammatory cells into the BALF. Mice were exposed to 20 ppb TDI by means of inhalation for 6 weeks and challenged with 20 ppb TDI 2 weeks later. Forty-eight hours after challenge, the number of eosinophils **(A)** and lymphocytes **(B)** in the BALF were calculated from differential counts by using cytospin preparations, and total leukocyte counts were done with a hemocytometer. Values are presented as means ± SEM (n = 5). Significantly different from the control group *(a)* or the sensitized-challenged group *(b)*, $P < .05$. *S/C*, Sensitized-challenged group.

Specific airway responsiveness to suspected respiratory allergens is an important determinant of occupational asthma, and human subjects with TDI-induced asthma exhibit both immediate and late-phase changes to TDI. Because our challenge system did not allow for examination of the immediate phase, we determined the effect of TDI challenge on late-phase changes in AHR (Fig 2, *B-G*). TDI challenge induced a 20% increase in the late-phase AHR in the sensitized-challenged mice relative to the control mice. Sensitized-challenged mice showed increased AHR starting approximately 3.5 hours after challenge that lasted for 3 hours (Fig 2, *C*). Changes in late-phase AHR were attenuated in IL-1Rko mice (Fig 2, *D*) or those treated with neutralizing antibody to IL-1β (Fig 2, E) or combined anti-IL-1β and IL-1α antibodies (Fig 2, *F*), whereas treatment with neutralizing antibody to IL-1α alone (Fig 2, *G*) did not affect the generation of late-phase AHR relative to the sensitized-challenged group.

IL-1β and IL-1α are both involved in TDI-specific antibody production

Blood was collected 48 hours after TDI challenge, and serum was analyzed for TDI-specific IgG_1 antibodies. No TDI-specific IgG_1 antibodies were detected in the serum of control mice, whereas significant titers were observed in all mice that were sensitized and challenged with TDI

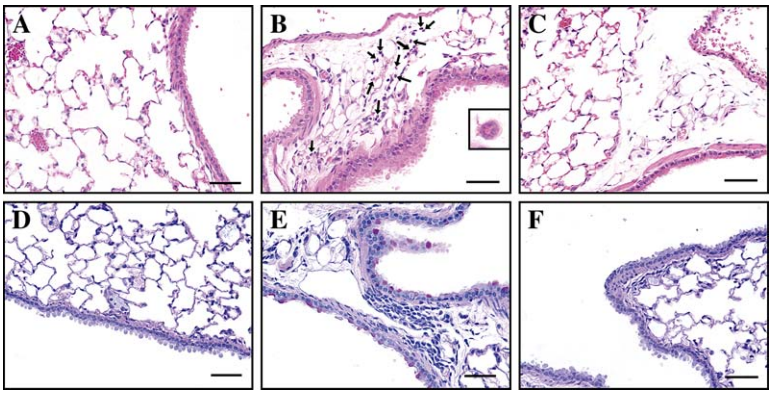

FIG 5. Lung histopathology after TDI challenge. Mice were exposed to 20 ppb TDI by means of inhalation for 6 weeks and challenged with 20 ppb TDI 2 weeks later. Lungs were removed and fixed 48 hours after challenge, and sections were prepared for histologic examination of inflammation after hematoxylin and eosin staining **(A-C)** and for evidence of goblet metaplasia after PAS staining **(D-F)**. Panels *A* and *D*, Control group; panels *B* and *E*, sensitized-challenged group; panels *C* and *F*, IL-1Rko group. *Arrows* in panel *B* represent eosinophils, and the *inset* in panel *B* is a magnified eosinophil from the same mouse. The magenta cells in the epithelium of panel *E* represent mucus-hypersecreting goblet cells. Micrographs are representative of 3 mice per group. *Bar* = 100 μm.

(Fig 3). TDI exposure did not result in specific IgG_1 production in any of the IL-1Rko mice. Antibody neutralization of IL-1β reduced the mean titer of TDI-specific IgG_1 compared with that seen in the sensitized-challenged group, with only 60% of exposed mice demonstrating detectable antibodies. Neutralization of IL-1α had no effect on TDI-specific antibody production. In contrast, neutralization of both IL-1β and IL-1α prevented IgG_1 antibody response to TDI exposure.

IL-1β and IL-1α are required for pulmonary inflammation after TDI challenge

Cellular analysis of BALF obtained 48 hours after TDI challenge showed a greater than 10-fold increase in eosinophil extravasation in the sensitized-challenged mice compared with that seen in the control mice (Fig 4, *A*). Lymphocyte numbers in the BALF were increased in sensitized-challenged mice about 2.5-fold above those in the control group (Fig 4, *B*). Total leukocyte, macrophage, and neutrophil numbers in the BALF were not changed in any group relative to the control group (data not shown). Histologic analysis of lung tissue 48 hours after TDI challenge showed peribronchial and perivascular infiltration of eosinophils and some lymphocytes (Fig 5, *B*), which was not evident in control lungs (Fig 5, *A*). The inflammatory response to TDI challenge was abrogated in mice deficient in IL-1 receptor type I (Fig 5, *C*). All other experimental manipulations of the IL-1 signaling system, including administration of neutralizing antibodies to IL-1β, IL-1α, or both IL-1β and IL-1α abrogated the invasion of eosinophils (Fig 4, *A*) and lymphocytes (Fig 4, *B*) into the lung lumen and the lung tissue (data not shown).

Histologic examination showed moderate numbers of PAS-positive goblet cells in bronchial epithelium 48 hours after TDI challenge in mice that were subchronically exposed to TDI (Fig 5, *E*), whereas PAS-positive cells were not present in the control group (Fig 5, *D*). Goblet metaplasia, although present in the sensitized-challenged group, was not evident in the IL-1Rko mice (Fig 5, *F*). Likewise, no goblet metaplasia was evident in the xIL-1β, xIL-1α, or xIL-1β/xIL-1α groups 48 hours after TDI challenge (data not shown).

Preventing IL-1 signaling reduces T_H2 cytokine and adhesion molecule expression in the lung

Cytokine and adhesion molecule gene expression in the lung were determined by using real-time PCR 48 hours after TDI challenge. The expression of IL-4 in the lungs of mice sensitized and challenged with TDI was increased by 3.4-fold relative to the control mice (Fig 6, *A*). Interfering with IL-1 signaling as in the IL-1Rko, xIL-1β, and xIL-1α groups prevented this increase in IL-4 expression, with these groups being similar to the control group. Administration of both IL-1β and IL-1α antibodies similarly reduced IL-4 expression, but this effect was not statistically significant. No significant differences were observed between the control, IL-1Rko, xIL-1β, xIL-1α, and xIL-1β/xIL-1α groups. The expression of IFN-γ did not change in any treatment group relative to the control mice (Fig 6, *B*).

Adhesion molecules, including ICAM-1 and VCAM-1, known to be modulated by IL-1,[10] are important in the recruitment of inflammatory cells to the lung. Mice in the sensitized-challenged group showed a 3.5-fold increase in ICAM-1 expression (Fig 6, *C*) and a 3.3-fold increase in VCAM-1 expression (Fig 6, *D*) in the lung within 48 hours after TDI challenge compared with the control group. Expression of ICAM-1 and VCAM-1 in the IL-1Rko, xIL-1β, xIL-1α, and xIL-1β/xIL-1α groups was not increased, being similar to that seen in the control group.

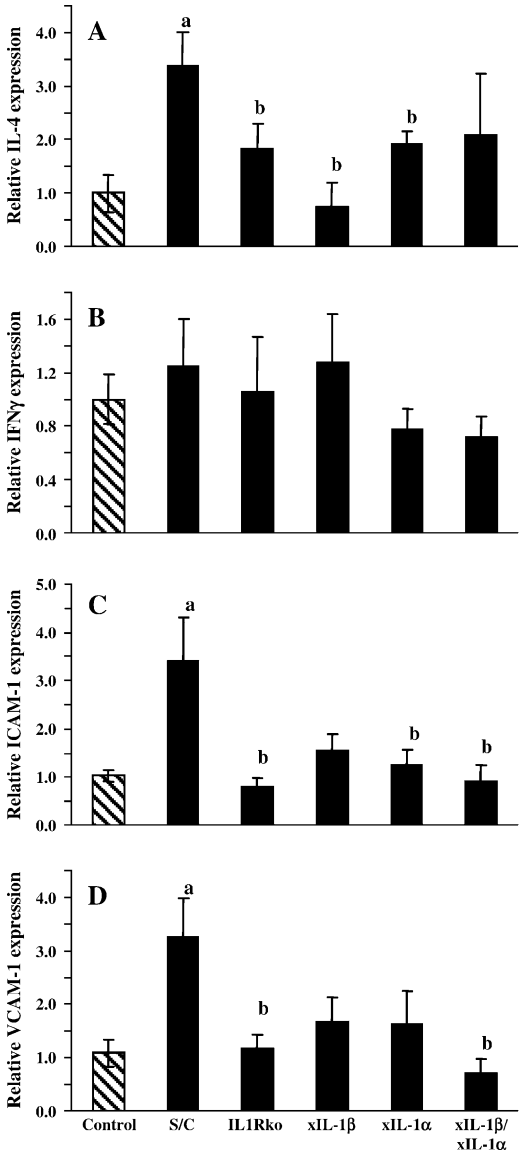

FIG 6. Expression of IL-4, IFN-γ, ICAM-1, and VCAM-1 in the lung after TDI challenge. Mice were exposed to 20 ppb TDI by means of inhalation for 6 weeks and challenged with 20 ppb TDI 2 weeks later. Lungs were removed and fixed 48 hours after challenge, and gene expression was analyzed by using real-time PCR. Changes in the relative expression of IL-4 **(A)**, IFN-γ **(B)**, ICAM-1 **(C)**, and VCAM-1 **(D)** are shown. Data are presented as fold change from control levels of gene expression (mean ± SEM [n = 5]). Significantly different from the control group *(a)* or the sensitized-challenged group *(b)*, $P < .05$. *S/C*, Sensitized-challenged group.

DISCUSSION

Human and animal studies have implicated proinflammatory cytokines as potential early mediators in the development of diisocyanate-induced asthma. The goal of the present study was to determine the role of IL-1 in disease pathogenesis. We used an occupational asthma model[5,6] that displays histopathology in common with TDI-induced asthma in human subjects and with an exposure paradigm consistent with potential workplace exposures.[20] By using this model, it was shown that IL-1 signaling is critical to TDI-specific changes in AHR, as well as changes induced by nonspecific stimuli. Both IL-1β and IL-1α play a role in AHR because blocking either cytokine alone did not completely abolish TDI-induced AHR changes, whereas blocking both cytokines prevented AHR. We also show that IL-1 signaling is integral to the recruitment of inflammatory cells to the lung. These findings suggest that IL-1 is an important mediator of TDI-induced asthma and might represent a therapeutic target.

AHR to specific and nonspecific stimuli is known to be mediated by T_H lymphocyte activation and T_H2 cytokines, including IL-4, IL-5, and IL-13.[21] In fact, numerous animal studies have demonstrated that interference with signaling that mediates T_H2 cell differentiation and activation prevents the development of asthma.[22-24] Importantly, increasing evidence suggests that IL-1 can promote the proliferation and differentiation of T_H2 cells *in vitro* and *in vivo*.[25] This effect might be mediated through the induction of costimulatory molecules on the surface of T lymphocytes in the presence of IL-1.[26] The results of the present study show that both IL-1β and IL-1α are produced in response to TDI challenge. Elimination of IL-1 signaling, as occurs in IL1Rko mice or cotreatment of wild-type mice with neutralizing antibodies to both IL-1β and IL-1α, prevented the development of TDI-induced asthma. The influence of IL-1 might be related to its ability to recruit and activate T_H2 cells because blocking IL-1 signaling eliminates TDI-induced lymphocyte infiltration into the lung, as well as IL-4 expression. Reduced T_H2 cytokine production might also be responsible for the lack of goblet metaplasia after interference with IL-1 signaling in the present study.

Neutralization of IL-1β alone resulted in a significant reduction in specific AHR after TDI challenge compared with that seen in the sensitized-challenged group, although only a partial reduction of methacholine-induced AHR was observed. In contrast, neutralization of IL-1α did not alter specific or nonspecific AHR, which remained similar to that in the sensitized-challenged group. This occurred despite inhibition of lymphocyte recruitment and IL-4 production in mice treated with anti-IL-1α. A possible explanation for the differences in the role of IL-1β and IL-1α in AHR might be related to TDI-specific IgG_1 production. Mice treated with anti-IL-1β showed significantly reduced IgG_1 antibody titers to TDI, whereas antibody titers remained increased in mice receiving anti-IL-1α, indicating a dominant role for IL-1β. This finding is consistent with previous work showing that IL-1β, but not IL-1α, is required for the production of T-cell–dependent antibody.[27] Our findings also show that preventing IL-1β and IL-1α signaling together completely prevented TDI-specific IgG_1 production, suggesting that both cytokines play a role. It is possible that IL-1β is capable of compensating for a loss of IL-1α, resulting in maintenance of antibody production, whereas IL-1α

cannot compensate as efficiently for a loss of IL-1β. Our previous studies demonstrated that passive transfer of heat-treated serum caused contact hypersensitivity in naive mice after TDI challenge, suggesting that a heat-stable (ie, non-IgE) humoral component, likely TDI-IgG antibody, was sufficient to transfer specific immunity.[6] In this respect, Macedo-Soares et al[28] recently showed that reconstitution of immunosuppressed mice with anaphylactic IgG_1 antibodies restored the ability of antigen challenge to induce nonspecific AHR. Reaginic IgG antibodies capable of inducing immediate hypersensitivity reactions have also been identified in workers[29] and experimental animals[30] exposed to diisocyanates. Thus it is possible that anaphylactic IgG_1 antibodies are produced in mice treated with neutralizing anti-IL-1α antibodies, resulting in AHR.

Lung eosinophilia is another important characteristic of TDI-induced asthma, and our results demonstrate that IL-1 is a critical mediator. Deletion of the IL-1 receptor type I or treatment with neutralizing antibodies to IL-1β, IL-1α, or both resulted in complete loss of TDI challenge–induced eosinophil recruitment. It is well established that allergen-induced adhesion molecule expression on endothelial cells is a key step in eosinophil tissue inflammation in allergic responses.[31] It is also known that IL-1 and other inflammatory cytokines induce the expression of these adhesion molecules.[10] We observed that TDI challenge resulted in increased expression of ICAM-1 and VCAM-1 in lung tissue, a finding consistent with previous models of TDI-induced asthma.[7] Interestingly, interference with IL-1 signaling prevented the effects of TDI on adhesion molecule expression. These results suggest that TDI-induced IL-1 production might regulate the expression of adhesion molecules in the vascular endothelium, facilitating the adhesion and transmigration of eosinophils into the lung tissue. This is consistent with results from an ovalbumin mouse model of lung eosinophilia in which IL-1 receptor type I–deficient mice showed reduced eosinophilia caused by interference with expression of the adhesion molecules L-selectin and very late antigen 4.[10]

In summary, our studies indicate that IL-1β and IL-1α are upregulated in TDI-sensitized and TDI-challenged mice and that these cytokines are critical in orchestrating the pathology associated with TDI-induced asthma. IL-1β and IL-1α were both integral to the recruitment and activation of inflammatory cells, including lymphocytes and eosinophils. In contrast, IL-1β, but not IL-1α, was involved in TDI-induced AHR, and this might be related to differences in the effects of these cytokines on TDI-specific IgG_1 production. The role of IL-1 in TDI-induced asthma might be mediated through regulation of endothelial cell adhesion molecules because their expression in the vascular endothelium is an early event in the recruitment of inflammatory cells to the lung.

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