Arch Toxicol (1998) 72: 141–146

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Airway hyperreactivity elicited by toluene diisocyanate (TDI)-albumin conjugate is not accompanied by airway eosinophilic infiltration in guinea pigs

Received: 2 June 1997 / Accepted: 8 October 1997

Abstract Nonspecific airway hyperresponsiveness is present in many patients with toluene diisocyanate (TDI)-induced asthma; however, the underlying pathophysiological mechanisms of this hyperresponsiveness remain controversial. In the present study, we used a guinea pig model to investigate the association of TDI-induced airway hyperresponsiveness with eosinophilic airway infiltration, which is widely considered to play a key role in the development of allergen-induced hyperresponsiveness. Guinea pigs were sensitized by i.d. injections of 10 μl TDI on day 1 and day 6. Control animals received saline injections. Two weeks after the second injection, airway reactivity to inhaled methacholine and specific airway resistance (sR_{aw}) was measured before and at several times after inhalation challenge with TDI-GSA (guinea pig serum albumin) conjugates. Eosinophils in the airways were detected using enzyme histochemistry and quantified using computer-assisted image analysis. TDI-specific IgG_1 antibodies were found in the blood of TDI-sensitized animals. An immediate increase in sR_{aw} was induced in these animals by TDI-GSA challenge; airway hyperresponsiveness to methacholine was observed at 6 h and 18 h after TDI-GSA challenge. However, TDI-GSA challenge did not result in an elevation of eosinophils in the airways, compared with control animals. The results suggest that the development of TDI-induced airway hyperresponsiveness is not dependent upon eosinophil infiltration in airways.

Key words Toluene diisocyanate · Asthma · Airway obstruction · Airway hyperreactivity · Eosinophil · Guinea pig

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Introduction

Toluene diisocyanate (TDI), a low molecular weight chemical widely used in the production of polyurethane plastics, is a leading cause of occupational asthma. Repeated airway exposures in the workplace to TDI, which contains a chemically reactive group (—N=C=O) and reacts with endogenous protein carriers to produce a complete antigen, may cause work-related allergic asthma. Nonspecific airway hyperresponsiveness (AHR) to inhaled aerosols of methacholine (MCh) or histamine is present in many patients with TDI-related asthma (Cartier et al. 1989; Burge et al. 1991; Baur et al. 1994). However, the mechanisms underlying the AHR are still controversial. An immunologically mediated mechanism has been described to be involved, based on the finding of TDI-specific antibodies and mucosal inflammation with an influx of polymorphonuclear leukocytes in some affected subjects (Cartier et al. 1989; Baur et al. 1994; Burge 1991).

While a variety of inflammatory cells have been implicated in the pathogenesis of AHR in asthma, current evidence suggests that eosinophils play a key role (Gundel et al. 1990; Corrigan and Kay 1992). This is because eosinophils are found in increased numbers in the airways of asthmatic subjects after allergen challenge, and are capable of producing mediators that can induce AHR. However, it has also been reported that AHR is not associated with eosinophil infiltration (Garssen et al. 1993; McFadden 1994).

It has been reported that in patients with a late asthmatic response induced by TDI, bronchoalveolar lavage (BAL) fluid showed an increased number of eosinophils at 8 h after inhalation challenge with TDI (Fabbri et al. 1987). An increase in eosinophils was also observed in peripheral airways of TDI-sensitized guinea pigs 6 h after TDI inhalation challenge (Mapp et al. 1996). Conversely, Satoh et al. (1995) observed no significant infiltration of polymorphonuclear cells in the lungs of TDI-sensitized mice after intranasal challenges.

In another murine model, tracheal reactivity was significantly increased in TDI-sensitized animals 24 h following TDI challenge; however, it was not associated with eosinophil accumulation in airways (Scheerens et al. 1996).

The purpose of this study was to explore further the characteristics of TDI-induced nonspecific AHR in a guinea pig model, and secondly, to clarify the relationship between TDI-induced AHR and eosinophil recruitment in airways. We used conjugates of TDI and guinea pig serum albumin (TDI-GSA) for challenge since previous reports have demonstrated that animals sensitized by inhalation of TDI (Karol 1983; Huang et al. 1993) or by dermal contact with TDI (Karol et al. 1981) responded to inhaled TDI-GSA challenge with immunologically mediated pulmonary obstructive responses.

Materials and methods

Animals

Male Dunkin-Hartley specific pathogen-free guinea pigs (Harlan Sprague Dawley, Indianapolis, Ind.) weighing 350–450 g were used in the study. All animals were acclimated to our animal housing facility for 1 week before use. Animals were housed one per cage and provided food and water ad libitum.

Preparation of TDI-GSA conjugates

TDI-GSA conjugates were prepared as previously reported (Huang et al. 1993). In brief, TDI (1 ml) was added drop-wise to 100 ml of 1% guinea pig serum albumin (GSA) in borate-buffered saline (pH 9.4), and stirred at room temperature for 60 min. After centrifugation, the supernatant was extensively dialyzed against saline and freeze dried. In the TDI-GSA conjugate thus prepared, approximately 80% of primary amino groups of GSA were found to be conjugated with TDI, as determined by the method of Snyder and Sobocinski (1975).

Sensitization with TDI and challenge with TDI-GSA conjugate

Three groups of eight guinea pigs (group I, II, III) were sensitized by i.d. injection of 10 µl TDI into each of two dorsal sites on day 1 and day 6. Control groups of eight animals (group IV, V, VI) received saline injection. Two weeks after the second injection, TDI-sensitized and saline-treated animals were placed in a double-chamber plethysmograph and challenged by exposure to aerosols of 1% TDI-GSA conjugates for 5 min. Aerosols were generated by an ultrasonic nebulizer and delivered to a 3 l plexiglass chamber in which the mass concentration was monitored by a Miniram Aerosol Monitor (model PDM-3, MIE, Bedford, Mass.) connected to a computer. The aerosol mass concentration was maintained at 10 mg/m^3, and was pumped to the head chamber of the plethysmograph. The mean aerodynamic diameter of the aerosol was 7.7 µm as measured by an Aerodynamic Particle Sizer (model no. 3300, TSI; Minneapolis, Minn.).

Measurements of airway obstruction

As an index of airway obstruction, specific airway resistance (sR_{aw}) was determined by the method of Pennock et al. (1979), using the double-chamber plethysmograph connected to a noninvasive airway mechanics analyzer (model LS-20; Buxco Electronics, Troy, Conn.). sR_{aw} was logged at 6-s intervals; the mean of 10 consecutive interval averages was calculated as the measurement at each time point. Measurements were made 1 min prior to challenge, and at 5 min, 10 min, 1 h, 2 h, 4 h, 6 h, and 18 h after challenge with TDI-GSA aerosol.

Measurements of airway responsiveness to inhaled MCh

Airway responsiveness to MCh aerosols was assessed in TDI-sensitized and control animals by measuring sR_{aw} 24 h before, and 6 h and 18 h after TDI-GSA challenge in animals of group I and IV. In a guinea pig model of asthma produced by sensitization and challenge with aerosolized ovalbumin, an airway hyperreactivity was observed at 17 h after the challenge (Hutson et al. 1988). A recent study reported the development of in vitro tracheal hyperreactivity in a TDI-treated murine model 24 h after TDI challenge, which was not found 2 and 48 h after challenge (Scheerens et al. 1996). Based on these earlier findings, 6 h and 18 h were chosen as the time points for evaluation of airway reactivity changes after challenge.

Aerosols generated from saline and MCh solutions, in increasing concentrations of MCh from 0.4 mg/ml to 51.2 mg/ml, were delivered for 3 min to the head chamber. sR_{aw} readings were taken at 6 s intervals for 1 min following the exposures. The minimum provocative concentration of MCh at which sR_{aw} exceeded 200% of the baseline value for an individual animal was calculated by linear interpolation and expressed as PC_{200} (mg/ml).

Tissue preparation

At 6 h (group II and V) and 18 h (group III and VI) after TDI-GSA challenge, guinea pigs were anesthetized i.p. with sodium pentobarbital (50 mg/kg). Blood samples were drawn by cardiac puncture for antibody analysis. The animals were then exsanguinated by severing the abdominal aorta. A 3-cm length of trachea was marked and an incision was made in the lower trachea through which a 1-cm piece of polyethylene tubing was inserted and tied. The trachea was then dissected out and stretched to 3 cm. The lungs were inflated with Histocon (Polysciences, Warrington, Pa.) at a pressure of 30 cm H_2O and removed en bloc. The trachea and right diaphragmatic and left cardiac lobes of the lung were snap-frozen in isopentane cooled by liquid nitrogen to −80 °C. Tissue was cut into sections of 6 µm in thickness (Hacker-Bright Micro Cryostat 2122, Hacker Instruments, Fairfield, N.J.) at −20 °C.

Eosinophil detection

A histochemical method for cyanide-resistant eosinophil peroxidase was employed to stain for eosinophils (Yam et al. 1971). Sections were incubated in 0.1 M TRIS-HCl buffer, pH 7.2, containing 0.5 mg/ml diaminobenzidine (DAB), 0.015% H_2O_2, and 0.01 M KCN for 5 min at room temperature, and were counterstained with Harris hematoxylin (Polysciences).

Quantitative histochemistry

Slides were coded and read in a 'blind' fashion. Eosinophils in the trachea were enumerated in different locations: the epithelium, between the lumen and the basement membrane; the lamina propria, between the basement membrane and the smooth muscle; and the submucosa, between the smooth muscle and cartilage. Eosinophils in the bronchi were enumerated in: the epithelium, between the lumen and the basement membrane; the lamina propria, between the basement membrane and the smooth muscle; and the adventitia, outside of smooth muscle. Eosinophil prevalence was quantitated with computer-assisted image analysis (Optimas Corp.; Edmonds, Wash.) by calculating the percentage of a defined area that stained positively for DAB. We designated this percentage as the eosinophil influx index.

Passive cutaneous anaphylaxis (PCA) assay

The PCA method, developed by Ovary (1986), allowed detection of both IgG_1 and IgE in blood. Diluted sera were injected intradermally in the shaved backs of naive guinea pigs. A 0.5 ml aliquot of solution containing 2.5 mg TDI-GSA conjugates and 5 mg Evans blue dye was injected intravenously either 6 h later (to detect IgG_1 antibodies) or 6 days later (to detect IgE antibodies). Antibody titers were defined as the reciprocal of the highest dilution of serum showing a blue spot of 3 mm or more in diameter 30 min following i.v. injection.

Statistics

All values are presented as mean ± SE. Changes in basal sR_{aw} and reactivity to methacholine were analyzed using one-way analysis of variance (ANOVA) with repeated measures. Differences in eosinophil influx index were determined by the unpaired *t*-test; $P < 0.05$ was considered significant.

Chemicals

Guinea pig serum albumin (fraction V) was purchased from United States Biochemical Corp. (Cleveland, Ohio). Toluene diisocyanate (2,4/2,6 isomer ratio 80:20) was purchased from Aldrich Chemical Co. (Milwaukee, Mich.). Methacholine (acetyl-β-methylcholine chloride) was purchased from Sigma Chemical Co. (St. Louis, Mo.). Sodium pentobarbital was purchased from The Butler Co. (Columbus, Ohio).

Results

Production of specific antibodies

IgG_1 antibodies to TDI were observed only in the TDI-sensitized animals. The IgG_1 titers of 16 animals, demonstrated as the reciprocal of the highest dilution of serum showing a positive response, were the following: 2^4 (two animals); 2^5 (four animals); 2^6 (three animals); 2^7 (four animals); and 2^8 (three animals). IgE antibody was not found in either TDI-sensitized or control animals.

Time-course of airway response after TDI-GSA challenge

TDI-sensitized animals had a pre-challenge basal sR_{aw} of 1.01 ± 0.24 cm $H_2O \cdot s$. Five minutes after the TDI-GSA challenge, the sR_{aw} was elevated to 3.56 ± 0.93 cm $H_2O \cdot s$, which was significantly greater than the pre-challenge value ($P < 0.01$, $n = 8$). The increase in sR_{aw} reached to a peak (5.98 ± 1.37 cm $H_2O \cdot s$, $P < 0.01$) 10 min after the challenge, and the sR_{aw} value returned to the pre-challenge level within 1 h (Fig. 1). No significant change in sR_{aw} was observed in control animals ($n = 8$) at any time-point after TDI-GSA challenge (Fig. 1).

Airway reactivity to MCh

The extent of AHR was determined by measuring the PC_{200} for MCh (mg/ml) before and after challenge. TDI-GSA challenge produced airway hyperreactivity in TDI-sensitized animals ($n = 8$). As shown in Fig. 2, the PC_{200} values were 9.76 ± 2.97 and 4.06 ± 1.72 mg/ml, respectively, at 6 h and 18 h after the challenge. The values reflect a two- and fourfold decrease in PC_{200} compared to the pre-challenge value of 19.06 ± 5.90 ($P < 0.01$ for both 6 h and 18 h). There was no change in PC_{200} values in control animals at 6 h and 18 h after TDI-GSA challenge.

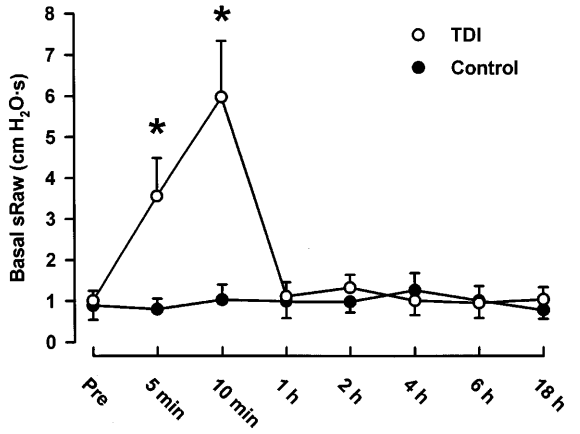

Fig. 1 Effect of toluene diisocyanate-guinea pig serum albumin conjugate (TDI-GSA) inhalation challenge on specific airway resistance (sR_{aw}) in TDI-sensitized (●; $n = 8$) and control (○; $n = 8$) animals. 'Pre', 1 min before challenge; the other time-points represent times after challenge. *$P < 0.01$, compared to Pre values

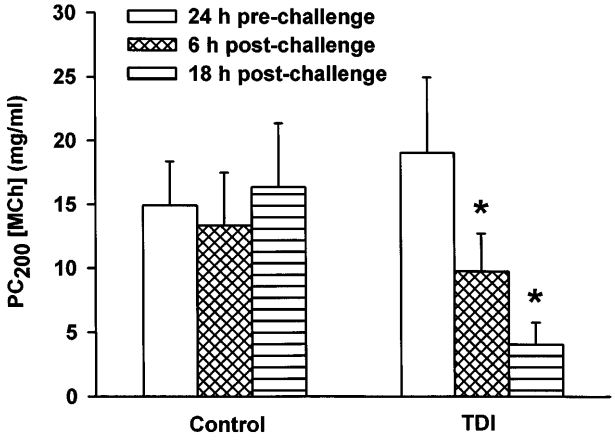

Fig. 2 Effect of TDI-GSA inhalation challenge on airway responsiveness to methacholine (MCh) in TDI-sensitized ($n = 8$) and control ($n = 8$) animals 6 h and 18 h after TDI-GSA challenge. Airway responsiveness to MCh is expressed as PC_{200} (mg/ml), the minimum provocative concentration of MCh at which sR_{aw} exceeded 200% of the baseline value of an individual animal. *$P < 0.05$, compared to pre-challenge values

Eosinophil accumulation in airways

Figure 3 shows the eosinophil prevalence in airways from TDI-sensitized and control animals 6 h and 18 h

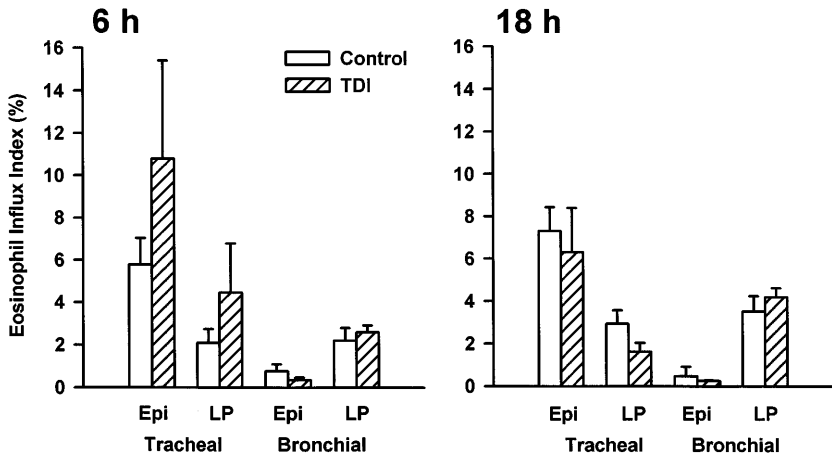

Fig. 3 Effect of TDI-GSA inhalation challenge on eosinophil influx index (%) in trachea and bronchi 6 h and 18 h after the challenge in TDI-sensitized ($n = 8$) and control ($n = 8$) animals. The results are represented as the percentage of area that was diaminobenzidine (DAB)-positive. (*Epi* Epithelium, *LP* lamina propria)

after TDI-GSA challenge. Compared to control animals ($n = 8$), TDI-sensitized animals ($n = 8$), regardless of the time points after the challenge, did not have a greater prevalence of eosinophils in the trachea and bronchi, either in the epithelium or the lamina propria ($P > 0.05$). Very few eosinophils were observed in tracheal submucosa and bronchial adventitia in either TDI-sensitized or control animals (data not shown).

Discussion

Our experiments have indicated that sensitization of guinea pigs with intradermal TDI followed by inhalation challenge with TDI-GSA conjugate produced a rapidly occurring but transient pulmonary obstruction, manifest as airway hyperresponsiveness to MCh, but not airway eosinophilia. Previous investigations have demonstrated hapten-specific antibodies in both human and animal models following repeated exposures to TDI (Cartier et al. 1989; Karol and Jin 1991; Huang et al. 1993). Our present study found that i.d. injections of pure TDI induced a production of TDI-specific IgG_1 antibodies in guinea pigs, but we found no evidence of TDI-specific IgE antibodies.

Following inhalation challenge with TDI-GSA, the animals developed an immediate obstructive response, which we believe was caused by a specific IgG_1 antibody-mediated immunological mechanism. The sR_{aw} values, which are reflective of lower airway resistance (Finney and Forsberg 1994), were returned to the pre-challenge levels within 1 h after the challenge and subsequently remained almost unchanged. This finding suggests immediately the absence of a late obstructive response in the animals. Our findings are similar to the results of other animal models of TDI-induced asthma. Karol et al. (1981) and Karol (1983) sensitized guinea pigs with dermal applications or i.d. injections of TDI, or sensitized with inhalation of TDI vapor, and then challenged them with TDI-GSA conjugates or TDI vapor. The occurrence of an immediate response was reported in these studies. On the other hand, both immediate and late obstructive responses were observed by Sugawara et al. (1993) and Niimi et al. (1996) in their guinea pig models, who utilized intranasal application of TDI for both sensitization and challenge.

Mapp et al. (1986) reported that only late, but not immediate, obstructive responses induced by TDI vapor inhalation were associated with an increase in airway responsiveness. Clearly, our findings differed from theirs. In the present study, TDI-sensitized, TDI-GSA-challenged animals demonstrated only an early asthmatic response, which disappeared within 1 h following the challenge. A late-phase obstruction did not develop in the 18 h measurement period. However, the animals still developed a marked increase in airway reactivity to MCh at 6 h (twofold) and 18 h (fourfold) after the challenge. Scheerens et al. (1996) recently reported that tracheal hyperreactivity was found 24 h after intranasal challenge with TDI in a murine model involving skin sensitization with TDI; this hyperreactivity disappeared 48 h after the challenge. However, the authors did not measure the airway obstructive response to TDI challenge per se, and did not comment on the appearance and relationships between the immediate or late asthmatic response and AHR to MCh.

Two important hallmarks of allergic asthma are airway eosinophil infiltration and AHR. These phenomena may be intimately related, but the causal relationship between eosinophilia and AHR is equivocal in the literature. A recent review revealed that an equal number of reports both support and fail to support a direct causation between airway eosinophilia and AHR (McFadden 1994). Hessel et al. (1997) found that in mice exposed to ovalbumin, AHR can occur independently of eosinophil infiltration, and vice versa, suggesting the phenomena are not causally related. There are humans who suffer from AHR without having inflammatory cells in the airways (Power et al. 1993).

There are very few reports examining the association between obstructive responses, AHR, and airway eosinophilia after TDI exposure. Niimi et al. (1996) reported that eosinophilic inflammation was involved in the late obstructive response induced by repeated in-

tranasal application of TDI in a guinea pig model, but its relationship to AHR was not explored. Mapp et al. (1996) observed that TDI challenge induced an eosinophil infiltration into airways of animals that were sensitized intradermally by TDI, but did not measure either pulmonary function changes or AHR after TDI challenge. In the present study, we found no evidence that the AHR induced by TDI was accompanied by an influx of eosinophils in airway tissues, either in trachea or bronchi at 6 or 18 h after TDI-GSA challenge, though AHR was already demonstrated at these times. Our results are similar to the observations of Scheerens et al. (1996) in a murine model that tracheal hyperreactivity after TDI challenge was not associated with airway eosinophilia.

It is difficult to reconcile the relationships between early and late obstructive responses, AHR to MCh, and eosinophilic inflammation after TDI sensitization and challenge which have been reported in the literature. In this study, we used TDI-GSA for challenge, which elicited a TDI-specific IgG_1-mediated immediate obstructive response and AHR to MCh in the animals. The use of TDI-GSA conjugates for inhalation challenge eliminated the contributions of factors such as chemical reactivity, while still providing haptenic groups to trigger the immune response. Clearly, eosinophilia is not evoked by this stimulus, and it may be that the inflammatory response is triggered by TDI itself, not a haptogenic adduct. Further experiments should be performed using reactive TDI for challenge to establish this possibility.

Lymphocytes and their derived products, such as proinflammatory cytokines, may play a role in the development of TDI-related airway hyperreactivity. Scheerens et al. (1996) reported that adoptive transfer of lymphocytes from TDI-sensitized animals to naive recipients resulted in tracheal hyperreactivity similar to that seen after active sensitization by TDI. Bronchial biopsies from some patients with TDI-induced asthma have revealed evidence of persistent activation of lymphocytes and increased expression of cytokines such as CD25, VLA-1, TNFα and IL-1β in the airways (Maestrelli et al. 1995). However, the precise roles of such cytokines in TDI-induced occupational asthma remains to be determined.

In summary, we have shown that an immediate asthmatic response was induced in TDI-sensitized animals by TDI-GSA challenge, which was mediated by TDI specific IgG_1 antibodies. Airway hyperresponsiveness was observed in these animals at 6 h and 18 h after the challenge. However, the animals did not have a greater number of eosinophils in the airways, compared with control animals. The results suggest that airway hyperresponsiveness elicited by TDI sensitization and TDI-GSA challenge was not associated with eosinophil infiltration in airways.

Acknowledgements The authors thank Dr Tracey Lawrence for her technical assistance in some experiments. This work was performed while J. Huang held a National Research Council-NIOSH Research Associateship. Mention of brand name does not constitute product endorsement.

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