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TDI can induce respiratory allergy with Th2-dominated response in mice

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Abstract

Toluene diisocyanate (TDI), a highly reactive industrial chemical is one of the leading causes of occupation-related asthma in industrialized countries. The pathophysiology of TDI-induced asthma, however, remains poorly understood, in part due to a lack of appropriate animal models. In this study, four models of TDI-sensitised mice were investigated. In model number 1, the mice were sensitised for 4 h/day on four consecutive days to 3 ppm inhaled TDI and challenged twice for 4 h each time with 0.3 ppm inhaled TDI. In model number 2, the sensitising condition was similar to that of model 1, but the challenge conditions involved an initial inhalation of 2 ppmTDI for 4 h and then tracheal instillation with 50 μg/mouse albumin-TDI. In model number 3, the mice were sensitised first to 25% TDI (sc) and then three times for 4 h each time to 1 ppm inhaled TDI and challenged twice for 4 h each time with 0.1 ppm inhalated TDI. In model number 4, the mice were first sensitised to 1% TDI by skin application and then with 0.2% TDI by tracheal instillation and challenged tree times by tracheal instillation of 0.1% TDI. In model number 4, skin application followed by tracheal instillations of TDI led to local and systemic Th2-dominated immune responses that were characterized: (1) in the lung-associated lymph nodes by a decrease in Th1 cytokine (IFN-γ) production associated with an increase in Th2 cytokine (IL-4, IL-5, IL-3) production; (2) in the lungs by an allergic inflammation throughout the conducting airways: goblet cell proliferation and eosinophil influx and; (3) in the serums by increased total and specific IgE levels, 17.5- and 3.5-fold higher than that of the controls, respectively. The conditions used for sensitisation in the other models, i.e. inhalation or subcutaneous administration plus inhalation, failed to induce a strong Th2 response like that observed in model number 4. The findings indicate that TDI can induce a Th2-dominated response in mice when administered by topical application plus tracheal instillation for sensitisation and by intratracheal instillation for challenge (model number 4). This mouse Th2 model of TDI-induced airway allergy can, in several aspects, mimic occupational TDI asthma in humans and may prove to be useful in determining the mechanistic basis behind this disease.

Keywords: Toluene diisocyanate; Occupational asthma; Mouse asthma model; Allergic Th2 type response

1. Introduction

Toluene diisocyanate (TDI), increasingly used for the manufacture of polyurethane foams, paints, coatings, elastomers, adhesives and many other products (WHO, 1987) is a recognized human irritant and one of the leading causes of occupational asthma (OA) in industrialized countries (Chan-Yeung and Lam, 1986). It is estimated that 5–20% of chronically exposed workers exhibit symptoms of asthma. (Bernstein, 1996). However, the pathogenesis of diisocyanate-induced asthma is still largely unknown (Baur et al., 1994; Bolognesi

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et al., 2001). Although several animal models, in guinea pigs, rats, and mice, have been investigated in an attempt to better understand the mechanisms of diisocyanate-induced OA, there is, to our knowledge, no validated mouse model of TDI-induced OA. There is a need for the development of appropriate animal models.

OA induced by TDI shares several features with allergic asthma, including elevated total and specific IgE serum levels, airway inflammation characterized by activated CD4+ T cells, eosinophils and mast cells, airway remodelling, and increased levels of IL-4 and IL-5 (Mapp et al., 1999; Wisnewski and Redlich, 2001; Maestrelli et al., 1997). TDI, like other low-molecular weight chemicals, is not antigenic per se (hapten) but is spontaneously reactive with a variety of proteins, such as albumin, keratin, hemoglobulin, ciliated cell tubulin, to form immune conjugate(s) (Jin et al., 1993; Day et al., 1996; Lange et al., 1999). The latter may be recognised, and taken up by professional antigen presenting cells (e.g. dendritic cells in the airway epithelium) before being transported and presented later to the naïve T cells in the lung-associated lymph nodes (Ban et al., 1997). TDI is capable of inducing different types of immune reactions, depending on the polarization of the T cells toward the helper T type 1 (Th1) or helper type 2 (Th2) cells. Th1 cells promote cell-mediated immunity (delayed hypersensitivity) and are defined by their secretion of cytokines, mainly interferon gamma (IFN-γ). Th2 cells are recognized by their secretion of interleukins, mainly IL-4, IL-5, and IL-13, that support humoral immune response (immediate-IgE mediated hypersensitivity) (Mosmann and Coffman, 1989). In experimental animal studies, Dearman et al. (1996) and Vanoirbeek et al. (2003) showed that topical TDI exposure leads to increased total serum IgE levels and a Th2 cytokine secretion pattern in mice. However, on the other hand, Vanderbriel et al. (2000) showed an elevation in IFN-γ (Th1 cytokine) following dermal TDI exposure. In human beings, increased levels of Th2 cytokines have been detected in the airways and the bronchial mucosa of TDI asthmatics (Maestrelli et al., 1997). However, some authors (Fabbri et al., 1987; Sastre et al., 1992; Lummus et al., 1998) observed Th1-like responses characterized by an increase in the number of neutrophils and the levels of IFN-γ and IL-8. So far, the results published remain controversial regarding the TDI-induced Th1or Th2 type responses. It is possible that the divergence in these results might be due partly to differing animal experimentation conditions, e.g. sensitising route and doses of TDI used to sensitise and challenge.

Animal models of asthma, using ovalbumin in association with adjuvant to develop a full Th2 phenotype response in mice, have been carried out by several investigators (Sakai et al., 1999; Jember et al., 2001). However, a limited number of research groups have attempted to produce animal models of chemical-induced occupational asthma in mice (Herrick et al., 2002; Matheson et al., 2005; Redlich et al., 2002; Scheerens et al., 1999). Nevertheless, information about TDI-induced asthma mechanisms are needed to make the diagnosis of diseases certain.

In this study, based on certain similarities between atopic asthma and TDI-induced asthma (Bentley et al., 1992; Saetta et al., 1992), it is reasonable to hypothesize that TDI can induce a Th2-dominated response in either humans or experimental animals depending on the exposure conditions. To support this hypothesis, four TDI-induced asthma models with different exposure conditions (inhalation, instillation, topical application, subcutaneous injection) to sensitise and challenge the mice were investigated, with a view to studying the lung-associated lymph node Th1 and Th2 cytokines in conjunction with the histological examination of the lungs, and examining the cellular composition in the lavage bronchoalveolar fluids and the total and specific IgE levels in sera.

2. Materials and methods

2.1. Materials

TDI was supplied by Merck Schuchard, France, in the form of a mixture containing 80% 2,4-TDI, and 20% 2,6-TDI. TDI-Human serum albumin conjugate (TDI-HSA) was provided by Dr. Peltre from Institute Pasteur (Paris, France). IFN-γ, IL-4, and IL-13 DuoSet were purchased from R&D Systems and Opt EIA Sets for IL-5 and mouse IgE from BD Biosciences. Diff-Quik was obtained from Dade Behring AG, Switzerland.

2.2. Mice

Female, 8–10-week-old BALB/c mice were obtained from Charles River (Italy). The animals were housed in an environmentally controlled animal facility maintained at 22 °C with a 12 h/12 h light/dark cycle. They were provided with food and water ad libitum for the duration of the experiment, except for the exposure periods in inhalation chambers for sensitisation and provocation. For each experimental designs described below, the mice were divided into four groups, 6–8 mice per group for three experiments and 12 mice per group for one experiment.