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Use of Tumor Necrosis Factor Receptor (TNFR)-Knockout Mice to Probe the Mechanism of Chemically-Induced Asthma

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ABSTRACT *: Toluene diisocyanate (TDI) is widely used in the manufacture of polyurethanes and is a recognized cause of occupational asthma. Although extensive investigations have been undertaken, the molecular mechanism(s) of the disease is still unclear. We hypothesized that inflammatory cytokines are required during both the sensitization and elicitation phases of the disease and have utilized TNF-R knock-out (KO) mice to address the hypothesis. Black C57 TNFR knock-out mice were exposed to TDI by sc injection and challenged by inhalation of 100 ppb TDI vapor. Control animals included: wild type C57 animals, sham-exposed animals that were challenged with TDI, and animals that were injected with anti-TNF antibodies prior to sensitization and again prior to challenge. Total IgE was increased in the knock-out animals compared with the wild type sensitized and challenged animals whereas TDI-specific IgG antibodies did not differ significantly in KO and wild type animals. There was less inflammation in the nares and trachea in KO animals compared with the wild type animals exposed to TDI as well as less goblet cell hyperplasia and epithelial damage. Airway reactivity was assessed in animals treated with anti-TNFα antibody and found to be substantially reduced compared with that in sensitized and challenged animals. These results indicate that TNFa plays a role in the immunologic and physiologic responses and in airways inflammation in this animal model and suggests a role for TNF in occupational asthma due to TDI.*

I. INTRODUCTION

Toluene diisocyanate (TDI) is a highly reactive chemical used in the manufacture of polyurethanes and plastics for numerous consumer and industrial products including paints, moldings, foams for mattresses, bedding, and insulation. It is the most prevalent cause of occupational asthma in the USA. Symptoms are similar to allergic reactions to environmental agents such as pollens, danders and weeds, and consist of wheeze, chest tightness, sneeze, cough, and difficulty in breathing. Although extensive research has been undertaken, the mechanism of disease remains unknown. Atopy is not a risk factor for disease and IgE antibodies are infrequently detected in symptomatic individuals (Mapp *et al.*, 1994).

Tumor necrosis factor (TNF)α is a polypeptide with numerous biological activities (Askenazi and Dixit, 1998). It was first named in 1975 because of its effects on tumors where it causes hemorrhage of the vasculature leading to starvation of the tumors. TNF regulates genes that code for other inflammatory mediators such as IL-1, IL-6, IL-8, GM-CSF, ICAM-1, ECAM-1. It enhances local vascular permeability and stimulates expression of adhesion molecules on endothelial cells. Sources of TNF include activated mononuclear cells, antigen-activated T cells, NK cells and mast cells.

Receptors for TNFα are transmembranal having an intracellular amino terminus and extracellular carboxy terminus. The 55 kDa TNF-R1 receptor activates pathways leading to apoptosis and the induction of genes controlled by transcription factor NF-kB. The 75 kDa TNF-R2 receptor can lead to NF-kB activation, but does not result in cell death. In view of the ability of TNFα to regulate immune responses, the current research was designed to investigate the importance of immunologic factors in sensitization to a potent chemical allergen by controlling the effects from TNFα.

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II. MATERIALS AND METHODS

1. Mice

Groups of mice consisted of wild-type C57, TNF-R1 knock-out, TNF-R2 knock-out, TNF-R1R2 double knock-out, and C57 wild type mice that received anti-TNF antiserum i.v. prior to sensitization and challenge.

2. TDI exposure

Animals were sensitized by subcutaneous injection of TDI on days 1, 4 and 11 with doses as indicated in Fig. 1. Responses were elicited by inhalation challenge for 1 hr in a chamber containing 100 ppb TDI vapor.

3. Tissue and blood collection

Twenty-four hours after the last challenge, animals were sacrificed and blood, lungs, trachea and lymph nodes were collected. Tissues were immersed in 10% neutral buffered formalin and stained with hematoxylin and eosin for histologic evaluation.

4. Antibody determination

Total IgE was assessed using a sandwich ELISA (Satoh *et al.*, 1995). TDI-specific IgG was measured using ELISA in which microtiter plates were coated with 50 ug/ml TDI-mouse serum albumin (Satoh *et al.*, 1995).

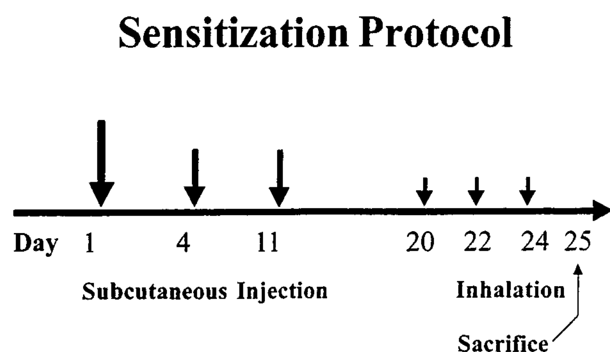

Fig. 1. Protocol for sensitization to TDI and challenge. Mice received 20 μl TDI on day 1 and 5 μl on days 4 and 11. Control animals were treated identically but received vehicle in place of TDI.

III. RESULTS

1. Inflammation

Airway inflammation was observed in histologic sections of the upper airways. Lesions consisted of goblet cell metaplasia, loss of cilia, and a cellular infiltrate including an influx of neutrophils. The trachea and nares of knock-out animals and mice that received anti-TNF neutralizing antibodies had reduced inflammation compared with the sensitized wild type animals. Responses in the single knock-out mice were similar to those in the double knock-out group. In all groups, the lungs and lymph nodes showed a mild inflammation.

2. Antibodies

Both TDI-specific antibodies (IgG) and total IgE were examined. Circulating IgG antibodies to TDI were detected in all groups that were injected with TDI. Titers were similar in wild type animals, knock-outs and animals pretreated with anti-TNF antiserum (Table 1). No antibodies were detected in sham-exposed animals.

Circulating total IgE was elevated in all animals exposed to TDI. Knock-out animals tended to have greater levels of total IgE than did both wild type ani-

Table 1. Immunoglobulin Response to TDI in Wild Type, Knock-out and Anti-TNF Treated Animals

Group	N	Total IgE		Specific IgG	
		Mean (μg/ml)	SEM	Mean Titer*	SEM
WT Control	4	0.18	0.01	ND	---
WT Sensitized	6	1.56[a]	0.37	693	0.6
WT Anti-TNF	6	0.88[a]	0.13	772	0.6
TNF-R1 Control	4	0.22	0.03	ND	---
TNF-R1Sensitized	5	3.75[b]	1.17	706	0.6
TNF-R2 Control	4	0.89	0.34	ND	---
TNF-R2 Sensitized	6	9.75[c,e]	3.33	926	0.9
TNF-R1R2 Control	4	1.43	1.11	ND	---
TNF-R1R2 Sensitized	4	8.12[d,e]	0.96	1831	1.6

Abbreviations: WT, wild type animals; TNF-RX, TNF receptor (X) knock-out animals; Anti-TNF, animals treated with antiserum to TNFa prior to TDI exposures; ND, not detected.
*Titer, highest serum dilution at which there is a significant difference from control animals.
[a,b,c,d] Value significantly different from corresponding control group.
[e]Value significantly different from wild type sensitized group.

mals and animals treated with anti-TNF antiserum (Table 1).

IV. DISCUSSION

Tumor necrosis factor is recognized for having diverse activities including critical regulation of the immune response (Ashkenazi and Dixit, 1998). We hypothesized that increased mechanistic understanding of the pathogenesis of TDI asthma would be gained from use of TNFR knock-out animals. The mouse model employed here has been shown to replicate the airway inflammation and hyperreactivity associated with the clinical disease (Matheson *et al.*, 1999).

In the mouse model utilized here, TDI-induced inflammation was found predominantly in the upper airways. The TNFR knock-out animals displayed only mild epithelial changes and cellular infiltrate compared with wild type animals indicating a prominent role of TNF in initiating the cellular injury associated with the disease. Comparison of the responses in knock-out animals with those in intact animals indicated the central role of TNF in airway inflammation and hyperreactivity, the two hallmarks of asthma.

By contrast, TNF did not appear to have a significant role in the antibody response to TDI. TNFR null mice or animals given anti-TNF antisera did not demonstrate reduced IgG or IgE titers when compared with wild type mice. Further, in this model, antibodies were unrelated to the symptoms of TDI asthma. Results of this study indicates the importance of the immune system in TDI asthma, and suggest that cellular, rather than humoral immunity, underlies the pathogenesis of the disease.

REFERENCES

Ashkenazi, A. and Dixit, V.M. (1998): Death receptors. *Science*, **281**, 1305-1308.

Mapp, C.E., Saetta, P., Maestrelli, P., Di Stefano, P., Chitano, P., Boschetto, P., Ciaccia, A. and Fabbri, L.M. (1994): Mechanisms and pathology of occupational asthma. *Eur. Respir. J.*, **7**, 153-160.

Matheson, J.M., Lange, R.W., Lemus, R., Karol, M.H. and Luster, M.I. (1999): The role of_tumor necrosis factor alpha (TNF) in lung reactivity to TDI. *FASEB.*, **13**(4), A319.

Satoh, T., Kramarik, J.A., Tollerud, D.J. and Karol, M.H. (1995): A murine model for assessing the respiratory hypersensitivity potential of chemical allergens. *Toxicol. Lett.*, **78**, 57-66.