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The contact allergen dinitrochlorobenzene (DNCB) and respiratory allergy in the Th2-prone Brown Norway rat

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Abstract

All LMW respiratory allergens known to date can also induce skin allergy in test animals. The question here was if in turn skin allergens can induce allergy in the respiratory tract. Respiratory allergy was tested in Th2-prone Brown Norway (BN) rats by dermal sensitization with the contact allergen dinitrochlorobenzene (DNCB; 1%, day 0; 0.5%, day 7) and a head/nose-only inhalation challenge of 27 mg/m^3 of DNCB (15 min, day 21), using a protocol that successfully identified chemical respiratory allergens. Skin allergy to DNCB was examined in BN rats and Th1-prone Wistar rats in a local lymph node assay followed by a topical patch challenge of 0.1% DNCB. Sensitization of BN rats via the skin induced DNCB-specific IgG in serum, but not in all animals, and an increased number of CD4$^+$ cells in the lung parenchyma. Subsequent inhalation challenge with DNCB did not provoke apneas or allergic inflammation (signs of respiratory allergy) in the BN rats. However, microarray analysis of mRNA isolated from the lung revealed upregulation of the genes for Ccl2 (MCP-1), Ccl4 (MIP-1beta), Ccl7 and Ccl17. Skin challenge induced considerably less skin irritation and allergic dermatitis in the BN rat than in the Wistar rat. In conclusion, the Th2-prone BN rat appeared less sensitive to DNCB than the Wistar rat; nevertheless, DNCB induced allergic inflammation in the skin of BN rats but even a relatively high challenge concentration did not induce allergy in the respiratory tract, although genes associated with allergy were upregulated in lung tissue.

Keywords: MIP1-beta; MCP-1; LLNA; Respiratory allergy; Skin allergy; Dinitrochlorobenzene

1. Introduction

Many low molecular weight (LMW) chemicals cause contact allergy in the skin, but only a limited number of chemicals are known to cause respiratory allergy. Interestingly, the respiratory allergens tested were all positive in the local lymph node assay (LLNA) and were able to induce skin allergy in test animals (Marignac et al., 1977; Basketter and Scholes, 1992; Kimber et al., 2007). The question here was if it is also the other way around: have skin allergens the potential to induce allergy in the respiratory tract?

Immune responses may be polarized toward either Thelper1 (Th1) or Thelper2 (Th2) production. Allergic contact dermatitis (mainly Th1) is the most common allergic disorder in the skin. Asthma and allergic rhinitis (mainly Th2) are most frequently encountered in the respiratory tract; asthma being so prominent that respiratory allergy has become almost synonymous with asthma. Thus, based on human evidence, the skin appears more prone to Th1 and the respiratory tract more prone to Th2 allergic disorders. This concept is in use to test chemicals for their potential to cause skin and/or respiratory allergy, although it is recognized as an oversimplification. Skin allergy also includes atopic dermatitis (mainly Th2) and respiratory allergy includes allergic alveolitis (hypersensitivity pneumonitis; mainly Th1; Belenky and Fuhrman, 2006), which is a serious and often insidious respiratory disease. Moreover, most LMW allergens examined today can activate both Th1- and Th2-cells, on the understanding that some of them preferentially induce either

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Th1 or Th2, whereas others do both almost equally well (Ulrich et al., 2001; Van Och et al., 2002; Dearman et al., 2003). Their action may depend on tissue factors, e.g. different manners of antigen presentation during sensitization. However, if Th1–Th2 divergence is tissue-dependent it is not so easy to understand why, in test animals, the skin can be such an effective route to sensitize the respiratory tract for Th2-mediated allergic reactions by LMW allergens (Botham et al., 1989; Warbrick et al., 2002a; Arts and Kuper, 2003; Johnson et al., 2004).

There is ample experimental and epidemiological evidence that respiratory allergens like isocyanates and acid anhydrides can induce Th1-type respiratory allergy and that some aspects of asthma are actually Th1-dependent (Bauer, 1995; Grammer, 1999; Merget et al., 2002; Arts et al., 2004; Matheson et al., 2005). Evidence that skin allergens can induce allergic reactions in the respiratory tract is only limited: Typical skin allergens like dinitrochlorobenzene (DNCB), dinitrofluorobenzene (DNFB) and trinitrochlorobenzene (TNCB) induced a slight mononuclear cell infiltrate in the larynx or lungs of sensitized Wistar rats and BALB/c mice but no changes in breathing parameters (Garssen et al., 1991; Zwart et al., 1994; Satoh et al., 1995; Arts et al., 1998). A challenge with 7.5 mg/m^3 DNCB did not induce respiratory allergy (apneus and/or allergic inflammation) in sensitized BN rats (Arts et al., 1998), nor were changes in breathing frequency, associated with allergy, observed in guinea pigs following a challenge of 10 mg/m^3 DNCB (Botham et al., 1989).

Th2-prone animals like the guinea pig and the BN rat are generally used for investigating respiratory allergy, but they may not be very sensitive to Th1-type skin allergens. Therefore, a challenge concentration (27 mg/m^3) of the skin allergen DNCB was used in DNCB-sensitized BN rats, using a protocol that successfully identified chemical and protein respiratory allergens (Saloca et al., 1994; Pauluhn et al., 2002; Arts and Kuper, 2003; Zhang et al., 2004). The challenge concentration was chosen to ascertain that enough of the material reached the lungs (Arts et al., 1998). The concentration was relatively high when compared to the 10 mg/m^3 used by Botham et al. (1989) in guinea pigs, but it was not unphysiologically high because it induced only minimal pulmonary irritation. A whole genome analysis (microarrays) of lung tissue was included, to provide unbiased insight into potential allergy pathogenesis (Zimmerman et al., 2004). The relative sensitivity of BN rats to the allergic properties of DNCB was tested in a skin allergy test (a LLNA followed by a topical patch challenge) by comparing the skin response in BN rats with that of the Th1-prone Wistar rats.

2. Material and methods

2.1. Animals and maintenance

Female and male, 7–8-week-old, inbred Brown Norway (BN) rats and male Wistar WU (Crl:WI/WU, random-bred) were purchased from a colony maintained under SPF conditions at Charles River Deutschland GmbH (Sulzfeld, Germany). The animals were acclimatized for at least 5 days before the start of the study. They were kept under conventional laboratory conditions and received the Institute's grain-based open-formula diet and unfluoridated tap water ad libitum. All animal procedures were approved by the TNO Commission of Animal Welfare.

2.2. Study design

2.2.1. Respiratory allergy

The study was conducted with four groups of rats and according to the following scheme (Table 1): Blood was collected at 1 day before the start of the study. Female BN rats received 150 μl of 1% (w/v) DNCB (purity 97%; Sigma, St. Louis, MO) in a 4:1 (v/v) mixture of acetone (Merck; Darmstadt, Germany) and raffinated olive oil (AOO) (Sigma Diagnostics Inc. St. Louis, USA) as the vehicle on each flank (approximately 12 cm^2 each) which had been shaved with an electrical razor at least 2–3 days earlier. Seven days after the first sensitization, they received 75 μl of 0.5% (w/v) DNCB on the dorsum of each of both ears. Controls received vehicle AOO. On day 21, basal lung function (breathing frequency, tidal volume and breathing pattern) was assessed, followed by DNCB inhalation challenge and assessment of lung function during and post challenge. Animals were challenged by inhalation of a slightly (based on breathing frequency and pattern) to moderately irritating target concentration of 27 mg/m^3 of DNCB for 15 min (calculated total dose is 70 μg, based on 180 ml air per minute during 15 min × 26.7 mg/m^3). At day 22, lung function was assessed again, where after necropsy was performed (blood sampling, bronchoalveolar lavage (BAL); weighing and collection of organs and tissues).

Table 1
Treatment schedule of the respiratory and skin allergy tests against DNCB

Group designation: Skin allergy[a]	Sensitization Day 0: 300 μl on flanks Day 7: 150 μl on ears	Challenge Day 21: 15 min inhalation	Day 18: Necropsy
−/− unsensitized/unchallenged	–	–	- Lung function[b] - Serum Ig
+/− sensitized/not challenged	1% DNCB–0.5% DNCB	–	- Liver, kidneys, left lung weights
−/+ unsensitized/challenged	Vehicle	27 mg/m^3 DNCB	- Nasal passages, larynx, trachea fixed in formalin; left lung snap-frozen;
+/+ sensitized/challenged	1% DNCB–0.5% DNCB	27 mg/m^3 DNCB	- BAL of right lung lobes
Group designation: Skin allergy[c]	Sensitization days 0, 1 and 2: 75 μl on ears	Challenge day 21: 15 min inhalation	Day 18: Necropsy
−/+ unsensitized/challenged	Vehicle	0.1% DNCB	- Skin application site: macroscopic examination and fixation in formalin for microscopy
+/+ sensitized/challenged	1% DNCB	0.1% DNCB	

[a] Six female BN rats per group.
[b] Lung function parameters were determined before, during and after challenge.
[c] Five male BN and Wistar rats per control (−/+) group; ten male BN and Wistar rats per test (+/+) group. Groups E and G: BN rats; groups F and H: Wistar rats.