Allergic Skin Inflammation Induced by Chemical Sensitizers Is Controlled by the Transcription Factor Nrf2

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Allergic contact dermatitis (ACD) is induced by low–molecular weight electrophilic chemicals and metal ions. Chemical contact sensitizers trigger reactive oxygen species production and provoke electrophilic stress, leading to the accumulation of the transcription factor nuclear-related factor 2 (Nrf2) in innate immune cell types. The objective of this work was to identify the role of Nrf2 in the regulation of ACD. We used the local lymph node assay (LLNA) and the mouse ear swelling test (MEST) to study the role of Nrf2 in both the sensitization and elicitation phase in nrf2 knockout (nrf2−/−) and wild-type (nrf2+/+) mice. Five chemicals were used: two compounds known to react with cysteine residues, 2,4-dinitrochlorobenzene (DNCB) and cinnamaldehyde (CinA); one sensitizer known to exhibit mixed reactivity to cysteine and lysine residues, isophorone diisocyanate; and one reacting specifically with lysine residues, trimellitic anhydride and croton oil, a well-known irritant. In the MEST assay, DNCB (1 and 2%) induced a significant increase in ear thickness in nrf2−/− compared with nrf2+/+ mice, suggesting a role for Nrf2 in the control of the inflammatory process. When DNCB was used at 0.25 and 0.5% or when mice were treated with CinA, inflammation was found only in nrf2−/− mice. In the LLNA, all chemical sensitizers induced an increase of lymphocyte proliferation in nrf2−/− compared with nrf2+/+ mice for the same chemical concentration. These results reveal an important role for Nrf2 in controlling ACD and lymphocyte proliferation in response to sensitizers.

Key Words: Nrf2; contact hypersensitivity; chemicals sensitizers; irritation; hapten.

Allergic contact dermatitis (ACD) is a common inflammatory skin disease, with a prevalence of 15–20% in the general population (Thyssen et al., 2007). ACD is classified as a delayed hypersensitivity reaction and is composed of two phases: the clinically silent sensitization phase and the inflammatory elicitation phase. Innate immune cells including dendritic cells (DC) play a major role in the initiation of the antigen-specific primary immune response during the initial phase of sensitization (Martin, 2012).

The development of ACD is promoted by skin contact irritation (Ueter et al., 2005), leading to activation of the innate immune system (Kaplan et al., 2012; Martin et al., 2011). Skin irritation has the potential to impact on both the induction and the elicitation phase of ACD. Few decades ago, it has been concluded that an adequate level of skin irritation was critical to the optimal development of skin sensitization (Magnuson, 1969).

Chemicals inducing ACD are small compounds called haptons, which have a molecular weight below 500 Da. Covalent interactions with proteins are required for chemicals to become immunogenic and to elicit a specific immune response (Lepoittevin, 2006). Full activation of DC is essential for the establishment of sensitization to haptons. Electrophilic and oxidative stress may be involved in this process (Ade et al., 2009; Mizuashi et al., 2005). Indeed, reactive oxygen species (ROS) have been found to be produced following treatment with contact sensitizers such as 2,4-dinitrochlorobenzene (DNCB) in cells like DC (Byamba et al., 2010; Migdal et al., 2010). In a mouse model of contact hypersensitivity (CHS), ROS are rapidly produced in the skin following contact sensitizer treatment and play a role in the oxidative and enzymatic degradation of the extracellular matrix component hyaluronic acid (HA) (Esser et al., 2012). Fragmentation of HA has been shown to be required for full activation of DC, and antioxidant treatment of mouse skin prevented 2,4,6-trinitrochlorobenzene-induced CHS. These findings underline the role of electrophilic/oxidative stress for the induction of skin inflammation in ACD and modulation of innate immunity.

To prevent an excess accumulation of ROS and electrophilic stress, several phase II detoxification enzymes such as glutathione-S-transferases, NADP(H):quinone oxidoreductase,
glutathione peroxidases, and heme oxygenase are mobilized in cells and mainly regulated by the transcription factor Nrf2.

Under basal conditions, Nrf2 is sequestered in the cytoplasm by its inhibitor, the Kelch-like ECH-associated protein 1 (Keap1), then ubiquitylated, and constantly degraded by the proteasome. In the presence of oxidative and/or electrophilic stress, Keap1 releases Nrf2, which migrates to the nucleus, and binds to the antioxidant-responsive element sequence, inducing gene transcription (Kensler et al., 2007). Keap1 is a thiol-rich protein having three cysteines known to play a role in Nrf2 activation (Yamamoto et al., 2008). We recently reported that contact sensitizers induced Nrf2 protein accumulation in the THP1 cell line and in human DC (CD34-DC). Nrf2 protein accumulation was inhibited in cells pretreated with the antioxidant N-acetylcysteine (Adé et al., 2009). Ryan et al. (2004) showed an upregulation of Nrf2-inducible genes in microarray studies using peripheral blood–derived DC treated with dinitrobenzenesulfonic acid for 24 h. All these results have led to the KeratinoSens assay development based on Nrf2 transcriptional activity for in vitro detection of chemical sensitizers (Emter et al., 2010).

nrf2-Deficient (nrf2−/−) mice have been shown to be more susceptible to sepsis, in association with an augmented expression of several innate immune response genes (Thimmulappa et al., 2006). A recent study showed that the allergen ragweed extract (RWE) provoked oxidative stress in DC that was significantly higher in nrf2−/− DC compared with nrf2 wild-type (WT, nrf2+/+) DC (Rangasamy et al., 2010). The oxidative stress induced by RWE was accompanied by an upregulation of CD80, CD86, and HLA-DR expression in nrf2−/− DC compared with nrf2+/+ DC. Therefore, Nrf2 deficiency may enhance the “innate allergic” immune response.

The objective of this work was to address the role of Nrf2 in ACD. In the MEST assay, a mouse model of CHS, Nrf2 disruption led to an increased inflammatory response to DNCB and allowed a positive response to cinnamaldehyde (CinA). In nrf2−/− mice, nonsensitizing doses of DNCB (0.25 and 0.5%) in WT mice efficiently induced CHS. However, the lowest concentration of DNCB (0.1%) did not induce any response in WT mice. The local lymph node assay (LLNA) performed with chemicals reacting specifically to cysteine and/or to lysine showed that Nrf2 can control the sensitization phase of ACD.

MATERIALS AND METHODS

Mice. nrf2−/− Mice (Itoh et al., 1997) were provided by the RIKEN BRC according to a MTA to Prof S. Kerdine-Römer. Wild-type (nrf2+/+) and nrf2−/− mice, investigated in this study, were generated from inbred C57BL/6J background nrf2 heterozygous mice. The donating investigator reported that these mice were backcrossed to C57BL/6J for at least 10 generations. Mice were housed in a pathogen-free facility and handled in accordance with the principles and procedures outlined in Council Directive 86/609/EEC. Genotyping was performed by PCR using genomic DNA that was isolated from tail snips as described (Itoh et al., 1997).

Measurement of ear irritation. Naive mice were treated at day 0 by a single application of different concentrations of DNCB, CinA, trimellitic anhydride (TMA), isophorone disiocyanate (IPDI), or croton oil (Sigma-Aldrich, Saint-Quentin Fallavier, France) applied on both ears. Ear thickness was measured both before and at different time points after treatment with a spring-loaded micrometer (Mitutoyo, Kanagawa, Japan). Each ear was measured in triplicate, and the mean of these values was calculated. The percentage of ear thickness increase was then calculated as following: ([mean thickness of both ears] − [mean thickness of ears measured before treatment]) × 1000.

Assay for CHS: the mouse ear swelling test. For DNCB, CHS was induced as described (Martin et al., 2008). Briefly, mice were sensitized by one application of 100 µl DNCB solution (0.1, 0.25, 0.5, 1, or 2%, wt/vol dissolved in acetone) or solvent control on the shaved abdomen. Ear thickness was measured on day 5, and mice were then challenged by application of 20 µl DNCB (0.5%) on both ears.

For the moderate hapten CinA, mice were sensitized for 3 consecutive days (day 0, day 1, day 2) by application of 100 µl CinA solution (50 or 75% wt/vol dissolved in acetone) on the shaved abdomen and challenged 5 days after the last sensitization dose with 20 µl CinA (25 or 40% wt/vol dissolved in acetone) on the ears. Increase of ear thickness was measured as described above.

All mouse ear swelling test (MEST) experiments done and termed “vehicle” have been performed as follows: at day 0, the mice received only the solvent (100 µl of acetone) on the shaved abdomen. Five days later, 20 µl of DNCB or CinA was applied on both ears. The group “vehicle” therefore represents the vehicle control group.

LLNA and measurement of lymphocyte proliferation. Standard LLNA protocols were conducted according to GLP procedures (CIToxLAB, Evreux). Briefly, nrf2−/− and nrf2+/+ mice received topical applications (25 µl per ear), once a day for 3 consecutive days of DNCB (0.01, 0.05, or 0.1%), CinA (2.5, 5, or 10%), IPDI (0.025, 0.05, or 0.1%), or TMA (0.1, 0.25, or 0.5%) or the vehicle (4/1 acetone/olive oil) on the dorsal of both ears. Five days following the initiation of exposure, all mice received an iv injection of [3H]-labeled thymidine ([3H]TdR (PerkinElmer, Courtaboeuf, France)) into their tail vein. Six hours later, animals were sacrificed, and draining auricular lymph nodes (LNC) were excised. A single cell suspension of LNC was prepared by gentle mechanical disaggregation, and the cells were washed and resuspending in trichloroacetic acid (TCA, Sigma-Aldrich) for at least 12 at 4°C. Precipitates were resuspended in TCA and transferred to an appropriate scintillation fluid (Ultima Gold XR scintillation fluid, PerkinElmer). The incorporation of [3H] TdR by draining LNC was measured by β-scintillation counting and recorded as mean dpm. The stimulation index (SI) for each group was determined by dividing the mean dpm value for the treated group by the mean dpm value for the vehicle control group.

Histological examination. Ears were fixed in 4% buffered paraformaldehyde (Sigma-Aldrich) overnight, embedded in paraffin, and cut into 7-µm-thick sections. Sections were first deparaffinized, rehydrated, and stained with hematoxylin-eosin (H/E) (Sigma-Aldrich). Mounting was done using a nonaqueous medium (Fisher Bioblock, Illkirch, France). Images were acquired on a Leica DMLB microscope (Leica Microsystems, Nanterre, France) equipped with standard optic objectives and digitized directly with a Sony 3CCD color video camera (Sony, Paris, France), original magnification x1000.

Statistics. As the data were not normally distributed along the mean to compare the two groups, the Mann-Whitney U-test was used. All data are presented as mean values ± SEM. Measurements were considered significant at a p value ≤ 0.05.
RESULTS

ACD Induced by a Strong Contact Sensitizer (DNCB) Is Nrf2 Dependent

Because skin irritation is known to play an important role in ACD, we first defined the minimal irritating concentration (MIC) and the maximal nonirritating concentration (MNC) for DNCB in the MEST. MIC and MNC were found to be 1 and 0.5%, respectively. Wild-type mice (nrf2+/+) and nrf2 knockout mice (nrf2−/−) were treated with both concentrations (0.5 or 1% DNCB), the two lower concentrations of MNC (0.1 or 0.25%) and with a higher concentration of MNC (2%) on the shaved abdomen. Five days later, mice were challenged on both ears with 0.5% DNCB for elicitation. The ear thickness increase was measured 24 h postchallenge and every consecutive 24 h during 4 days (Fig. 1a). The results were expressed as % of ear thickness increase. Results showed that when 1 or 2% of DNCB was applied during sensitization, a significantly higher increase of ear thickness was observed in nrf2−/− mice compared with nrf2+/+ mice. In nrf2−/− mice, the % of increase reached 40% compared with 20% in nrf2+/+ mice for 1% of DNCB and 60% compared with 30% in nrf2+/+ mice for 2% at 48 h postchallenge (Fig. 1b).

When mice were sensitized with the MNC (0.5%) or with lower concentrations (0.1 or 0.25%), no increase of ear thickness was observed in nrf2+/+ mice compared with vehicle-treated mice (Fig. 1b). In contrast, in nrf2−/− mice, 0.25 and 0.5% DNCB induced a positive MEST and provoked a significant increase of ear thickness compared with nrf2 WT mice. However, the lowest concentration of DNCB (0.1%) did not induce any increase in both genotypes compared with vehicle. In nrf2−/− mice, the maximal % of increase was obtained 48 h postchallenge and then reached a plateau (Fig. 1b).

Moreover, the results clearly show that a single application of 0.5% DNCB at the elicitation phase and without prior sensitization (vehicle groups) is irritating only in nrf2−/− mice, indicating that Nrf2 impacts DNCB irritation potency (Fig. 1b).

Histology of ear skin using H/E staining showed that the ears of nrf2−/− mice were thicker in response to DNCB due to dermal edema and cell infiltration in the dermis, but not found in the ears of nrf2+/+ mice (Fig. 2a). These data provided evidence for the role of Nrf2 in the control of ACD induction and underline the role of Nrf2 in controlling the inflammatory response induced by a strong sensitizer such as DNCB.

Nrf2 Controls the Allergic Inflammation Induced by a Moderate Sensitizer

Next, we decided to address the role of Nrf2 using a moderate sensitizer known to induce a moderate irritation. CinA was chosen due to its effect on Nrf2 activation in vitro (Ade et al., 2009). We then performed a MEST in response to CinA using the same protocol as for DNCB. The results showed no increase of ear thickness (data not shown). Therefore, we used a modified MEST (Vocanson et al., 2006) (Fig. 2a). Briefly, mice were sensitized daily on 3 consecutive days with 50 or 75% of CinA and were challenged 5 days later with 25 or 50% of CinA, respectively. No CHS was induced by CinA in nrf2−/− mice at all concentrations tested. In contrast, in nrf2−/− mice, CinA induced an increase of ear thickness depending on the concentration used during sensitization. The % of ear thickness increase obtained 72 h postchallenge reached 30% in nrf2−/− sensitized with 75% CinA compared with 12% in nrf2−/− sensitized with 50% CinA (Fig. 2b).

Interestingly, both doses of CinA are strongly irritating without prior sensitization (vehicle groups) in nrf2−/− mice, whereas no irritation was found in nrf2+/+ mice. This indicates that, as observed for DNCB, Nrf2 also impacts CinA irritation potency (Fig. 2b).

Histology of ear skin using H/E staining showed that the ear of nrf2−/− mice in response to CinA was thicker due to dermal edema and cell infiltration in the dermis, but not found in the ears of nrf2+/+ mice (Fig. 2c). Ears from mice sensitized with CinA (75%) presented a very prominent dermal edema and cell infiltration compared with CinA (50%) showing a concentration-dependent effect (Fig. 2c).

Nrf2 Controls the Proliferation of Lymph Node Cells During the Sensitization Phase of ACD

To study the role of Nrf2 in the control of the sensitization phase of ACD in response to cysteine-reactive sensitizers and non-cysteine-reactive sensitizers, LLNA experiments were performed to determine lymphocyte proliferation. Four compounds were used: DNCB and CinA known for their cysteine specific reactivity, IPDI known to be reactive with cysteine and lysine residues, and TMA known to react specifically with lysine. Concentrations to be tested were first determined in C57BL/6 mice (data not shown). In response to DNCB, we showed that the SI was higher in nrf2−/− mice for all the concentrations used in comparison to nrf2+/+ mice (Fig. 3a). For CinA, the SI was also higher in nrf2−/− mice at 2.5 and 5% CinA. At 10% of CinA, no statistical difference between the SI of nrf2−/− and nrf2+/+ mice was found (Fig. 3b). However, the proliferation in response to CinA tended to be higher in nrf2−/− compared with nrf2+/+ (3 mice out of 4) for this concentration (data not shown). For IPDI, for all concentrations used, the SI was considerably higher in nrf2 KO mice (Fig. 3c). In response to TMA, a well-known respiratory sensitizer, we showed a significant SI increase at 0.25 and 0.5% (Fig. 3d). These results demonstrated that Nrf2 controls lymphocyte proliferation in response to sensitizers reacting specifically with cysteine residues (DNCB and CinA) but also in response to chemicals specific for lysine such as TMA and to mixed reactive compound such as IPDI.

Skin Irritation Is Exacerbated in the Absence of Nrf2

We next studied the role of Nrf2 in response to the irritant croton oil, a well-known irritant not described to induce ACD. nrf2+/+ and nrf2−/− mice were treated with different concentrations of croton oil (1, 5, and 10%), and the ear thickness increase was measured every 24 h postapplication during 4 days. The % of ear thickness increase was very low in response to 1%
FIG. 1. DNCB increased inflammation in nrf2-deficient mice. (a) Experimental protocol. nrf2<sup>+/+</sup> and nrf2<sup>−/−</sup> mice were sensitized at day 0 with DNCB (0.1, 0.25, 0.5, 1, or 2%) in acetone on the shaved abdomen and were challenged 5 days later by application of DNCB (0.5%) on both sides of both ears. (b) The ear thickness increase was measured at 24, 48, 72, and 96 h postchallenge. As a control, vehicle-treated mice were only challenged with DNCB (0.5%). Results are expressed as the % of ear thickness increase = ([mean thickness of both ears [24, 48, 72, or 96 h post challenge]] − [mean thickness of ears measured before challenge])/[mean thickness of ears measured before challenge] × 100). Data represent the mean increase of ear thickness ± SEM (mice number n = 9),*p ≤ 0.05. (c) Representative images of H/E staining of ear skin of mice sensitized and challenged with DNCB (0.1, 0.25, 0.5, 1, or 2%) or vehicle, magnification ×1000.
croton oil in $nrf2^{+/+}$ and increased at higher concentrations (5 and 10%). In $nrf2^{+/+}$ mice, the maximum response was reached 24h postapplication, and the % of ear thickness declined later on (Fig. 4). In $nrf2^{-/-}$ mice, the tendency of the response was similar but the magnitude of the response was significantly higher (Fig. 4). Also, the % of ear thickness increased in $nrf2^{-/-}$ mice and then reached a plateau 24h postapplication with 5 or 10% of croton oil compared with that of $nrf2^{+/+}$ mice. These data demonstrate that in the absence of Nrf2, irritant contact dermatitis (ICD) was increased and maintained at a high level.

**DISCUSSION**

Skin inflammation is essential for sensitization to contact allergens and also plays an important role in the recruitment of cells in the elicitation phase (Kaplan et al., 2012; Martin, 2012).
FIG. 2. CinA induced an ear thickness increase in nrf2−/− mice in contrast to nrf2+/+. (a) Experimental protocol. nrf2+/+ and nrf2−/− mice were sensitized at day 0, day 1, and day 2 with CinA (50 or 75%) in acetone on the shaved abdomen and were challenged 5 days after the last sensitization by application of CinA (25 or 40%) on both sides of both ears. (b) The ear thickness increase was measured at 24, 48, 72, and 96h postchallenge. As a control, vehicle-treated mice were only challenged with CinA (25 or 40%). Results are expressed as the % of ear thickness increase as described in Figure 1. Data represent the mean increase of ear thickness ± SEM (mice number = 7), *p ≤ 0.05. (c) Representative images of H/E staining of ear skin of mice sensitized and challenged with CinA (50 or 75%) or vehicle, magnification ×1000.
During the sensitization phase, DC are activated, migrate to the draining lymph nodes, and induce the priming of hapten-specific T cells (Kissenpfennig et al., 2005). Direct modifications of the cellular microenvironment by chemical sensitizers are mandatory for DC maturation and migration (Ainscough et al., 2012; Martin, 2012). Nrf2 is induced in response to contact sensitizers in DC (Ade et al., 2009) and also in keratinocytes (Emter et al., 2010) and is probably involved in the regulated cellular response to pro-oxidant and electrophilic stress in these cells (Divkovic et al., 2005; Kang et al., 2005). Nrf2 has also been reported to control airway inflammation in response to ovalbumin in mice (Rangasamy et al., 2005).

In our study, the results show that nrf2-deficient mice (nrf2−/−) exhibit an increased CHS response to both strong and moderate contact sensitizers known to react with cysteine residues. We show that DNCB (1 and 2%), a strong contact sensitizer, induces a higher ear swelling response in nrf2−/− mice compared with nrf2+/+ mice. These results suggest that Nrf2 controls inflammation during the elicitation phase. In response to lower concentrations of DNCB (0.25 and 0.5%), nrf2−/− mice developed a positive MEST, whereas in nrf2+/+ mice, inflammation was not observed. Furthermore, the MEST was negative in both genotypes in response to 0.1% of DNCB, suggesting that nrf2 KO mice are not predisposed to an enhanced immune response to chemical allergens. This observation shows that the threshold for an inflammatory responses in the skin in nrf2−/− mice needed to elicit CHS is lower compared with nrf2+/+ mice.

The results obtained in response to croton oil confirm that nrf2−/− mice are more susceptible to ICD. Bonneville et al. (2007) have shown in C57BL/6 and Balb/c mice that the magnitude of ICD was correlated with the intensity of ACD and related to the concentration of the chemicals. Furthermore, the increased ROS production in nrf2-deficient mice (Yeang et al., 2012) might contribute to a stronger activation of DC (Bruchhausen et al., 2003). Because the strength of the inflammatory response, i.e., of the irritant effect of the sensitizer, determines whether constitutive immunoregulation can be overcome, our findings indicate that Nrf2 critically regulates the threshold in this process. Thus, the activation of Nrf2 by chemical sensitizers may play the role of a sensor controlling and limiting the inflammatory response and consequently the magnitude of ACD. Interestingly, in old mice (20 months and older), CHS in response to dinitrofluorobenzene was attenuated compared with younger mice (6 months old), and old nrf2−/− mice had an even lower ear swelling response than old nrf2+/+ mice (Kim et al., 2008). This was attributed to accentuated age-related cumulative oxidative stress, which impairs Th1 immunity.

In response to CinA, a moderate sensitizer, no positive MEST was observed in nrf2+/+ mice. The modified MEST, according to Vocanson et al. (2006), allowed a
positive CHS in nrf2−/− mice in which the ear swelling was induced. Recent studies have described that CHS reactions in response to CinA or other weak sensitizers occurred in mice depleted of CD4+ cells including regulatory lymphocytes (Treg) (Lass et al., 2008; Vocanson et al., 2006). Other studies have described a correlation between the activation of Nrf2 induced by chemicals such as antroquinonol and the enhancement of Treg cells activity (Tsai et al., 2012). There is also a hypothesis involving the DC because DC deficient for nrf2 have an activated phenotype (data not shown) and an increased antigen-specific CD8 T-cell stimulation capacity (Yeang et al., 2012). In the absence of Nrf2, DC are more sensitive to a danger signal (LPS) and produce more ROS (Yeang et al., 2012). Furthermore, it has been shown that ROS production plays a role in the upregulation of surface molecules such as CD86, CD40, and HLA-DR known to be important for DC function in response to DNCB (Byamba et al., 2010; Mizuashi et al., 2005).

Moreover, DC derived from bone marrow of nrf2 KO mice did show lower basal glutathione (GSH) levels (Yeang et al., 2012). DNCB and CinA, known to be detoxified by GSH, would be less trapped in such cells. The consequence would be an increase of the concentration of chemicals available in nrf2 KO cells, leading to a higher CHS.

**FIG. 4.** Croton oil induced a dose-dependent higher irritation in nrf2-deficient mice compared with WT mice. nrf2+/* and nrf2−/* mice received at day 0 on both sides of both ears croton oil 1, 5, and 10% in acetone/olive oil (4:1). The ear thickness increase was measured 24, 48, 72, and 96 h postapplication. As control, vehicle mice received acetone/olive oil on both ears. Results are expressed as the % of ear thickness increase. Data represent the mean increase of ear thickness ± SEM (n = 7 mice), representative of two independent experiments. *p ≤ 0.05.
Following our hypothesis that Nrf2 may affect the sensitization phase by modifying the cellular microenvironment and/or DC phenotype, we addressed the effect of Nrf2 on lymphocyte proliferation during the sensitization phase. We used the LLNA, a model currently used to measure the potential for a chemical to be a sensitizer.

Our LLNA data show that lymphocyte proliferation is higher in nrf2−/− mice in response to DNCB, CinA, IPDI, and TMA. The concentrations used for all chemicals tested were in the range of the EC3 defined previously in CBA/J mice (Loveless et al., 2010). EC3 is the concentration defined as the amount of chemical that is required to induce (1) a response of a magnitude that defines skin sensitizing potential and (2) an accepted arbitrary augmentation of lymphocyte proliferation proving an effect of the chemical. Working close to the EC3 is important because it is a concentration that defines an effect in the LLNA and probably close to the threshold of stimulation. Our study shows, for the first time, that the absence of Nrf2 affects sensitization to both cysteine- and/or lysine-reactive compounds. Moreover, our data concerning DNCB confirm and expand on the observation made by Van der Veen et al. (2012) even if the concentrations of DNCB used in this study were higher with regard to the EC3 of DNCB (~0.08%).

Our study highlights that Nrf2 plays a major role in the control of both phases of ACD: sensitization and elicitation. Nrf2 crucially impacts the irritation potency of the contact sensitizers, suggesting a control of the inflammatory process that may play the role of danger signal during the sensitization phase. However, nrf2 deficiency and the concomitant higher ROS production in nrf2−/− mice could also contribute to a higher basal activation level of innate cells such as DC, augmenting the capacity of these cells to elicit an adaptive immune response. Finally, Nrf2 deficiency leads to an increased inflammatory response during the elicitation phase, suggesting that Nrf2 may also play a role in the regulation of inflammation through a defect in Treg function (Josefowicz et al., 2012; Krishnamoorthy et al., 2012; Saadoun et al., 2011).

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