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Dendritic cells' death induced by contact sensitizers is controlled by Nrf2 and depends on glutathione levels☆

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ABSTRACT

Dendritic cells (DC) are known to play a major role during contact allergy induced by contact sensitizers (CS). Our previous studies showed that Nrf2 was induced in DC and controlled allergic skin inflammation in mice in response to chemicals. In this work, we raised the question of the role of Nrf2 in response to a stress provoked by chemical sensitizers in DC. We used two well-described chemical sensitizers, dinitrochlorobenzene (DNCB) and cinnamaldehyde (CinA), known to have different chemical reactivity and mechanism of action. First, we performed a RT-qPCR array showing that CinA was a higher inducer of immune and detoxification genes compared to DNCB. Interestingly, in the absence of Nrf2, gene expression was dramatically affected in response to DNCB but was slightly affected in response to CinA. These observations prompted us to study DC's cell death in response to both chemicals. DNCB and CinA increased apoptotic cells and decreased living cells in the absence of Nrf2. The characterization of DC apoptosis induced by both CS involved the mitochondrial-dependent caspase pathway and was regulated *via* Nrf2 in response to both chemicals. Oxidative stress induced by DNCB, and leading to cell death, was regulated by Nrf2. Unlike CinA, DNCB treatment provoked a significant reduction of intracellular GSH levels and up-regulated *bcl-2* gene expression, under the control of Nrf2. This work underlies that chemical reactivity may control Nrf2-dependent gene expression leading to different cytoprotective mechanisms in DC.

1. Introduction

Allergic contact dermatitis (ACD) is a common skin disease known to be induced by contact sensitizers (CS) and involving dendritic cells (DC) (Martin et al., 2011). CS are low molecular weight compounds (<500 Da) having electrophilic properties and able to bind covalently to nucleophilic residues from cutaneous proteins leading to the formation of a hapten-carrier complex with immunogenic properties (Christensen and Haase, 2012).

The hypothesis that CS can be perceived as a danger signal by DC has been also proposed based on signaling pathways (MAPK, NFκB) identified upon CS treatments and known to be triggered by danger signals such as toll-like receptor agonists (Ade et al., 2007; Boisleve et al., 2004). Such chemicals can also alter the redox glutathione (GSH/GSSG) balance in human DC derived from monocytes (MoDC) (Mizuashi et al., 2005). DNCB and other CS like dinitrofluorobenzene (DNFB), trinitrochlorobenzene (TNCB) can lead to the production of reactive species (RS) in BMDC (Esser et al., 2012) or MoDC (Byamba et al., 2010).

The main pathway responsible for cell defense against oxidative stress and maintaining the cellular redox balance at physiological levels is the nuclear factor-erythroid 2-related-factor 2 (Nrf2) pathway (Stepkowski and Kruszewski, 2011). Under homeostatic conditions, Nrf2 is sequestered in the cytosol and binds to Kelch-like ECH-associated protein 1 (Keap1) (Lee et al., 2007) allowing its proteasomal degradation (Lo et al., 2006). In the presence of a chemical stress, oxidants or electrophiles, Keap1's conformation is modified leading to the release of Nrf2 and its nuclear translocation. In the nucleus, Nrf2 binds to the consensus sequence ARE (Antioxidant Response Element) allowing the transcription of many target genes [*heme oxygenase-1* (*hmox1 or ho-1*), *NAD(P)H: quinone oxidoreductase 1* (*nqo1*), *glutathione-S-transferase* (*gst*)] (Watai et al., 2007). Many studies showed that

Abbreviations: ACD, allergic contact dermatitis; BMDC, bone marrow dendritic cells; CinA, cinnamaldehyde; CHS, contact hypersensitivity; CS, contact sensitizer; DNCB, 2,4-dinitrochlorobenzene; GSH, glutathione; GPX1, Glutathione peroxidase 1; GSR, Glutathione-S-reductase; GST, Glutathione-S-transferase; IL, interleukine; HO-1, heme oxygenase 1; Keap1, Kelch-like ECH-associated protein 1; NQO1, NADPH- quinone oxidoreductase 1; MAPK, mitogen activated protein kinase; MoDC, dendritic cells derived from monocytes; Nrf2, nuclear factor 2 related factor 2; NOS2, nitric oxide synthase 2; RS, reactive species; TNCB, trinitrochlorobenzene.

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the decreased levels of phase II detoxification enzymes and antioxidant proteins make *nrf2*$^{-/-}$ mice highly sensitive to cytotoxic electrophiles compared to *nrf2*$^{+/+}$ (wild-type) mice (Lee and Johnson, 2004; Ma et al., 2006; Talalay et al., 2003). Indeed, Murine Embryonic Fibroblasts (MEF) isolated from *nrf2*$^{-/-}$ mice showed higher level of cell death in response to the redox-cycling RS generator menandione and the GSH-depleting anticancer agent cisplatin (Jung and Kwak, 2010). Thus, Nrf2-mediated antioxidant response represents a critically important cellular redox homeostasis and limits oxidative damage (Aw Yeang et al., 2012). We have previously demonstrated that a strong CS such as DNCB or a moderate CS such as CinA, both induced Nrf2 in DC (Ade et al., 2009; Migdal et al., 2013). Recently, we showed that contact hypersensitivity (CHS) induced with DNCB was exacerbated in *nrf2*$^{-/-}$ mice compared to *nrf2*$^{+/+}$ mice while in response to CinA, CHS was only observed in *nrf2*$^{-/-}$ mice (El Ali et al., 2013).

A key antioxidant molecule in the regulation of the redox state, which also plays a role in cytoprotection, is glutathione (GSH). GSH is the most abundant intracellular low molecular weight thiol, and plays a major role in detoxification processes that maintain cellular redox homeostasis. Its protective action is based on oxidation of the thiol group of its cysteine residue, resulting in the formation of oxidized glutathione (GSSG); this in turn is catalytically reversed to GSH by GSH reductase (Franco and Cidlowski, 2012). GSH depletion is an early hallmark in the progression of cell death in response to different apoptotic stimuli (Franco and Cidlowski, 2006).

Since DNCB has been found to be a stronger sensitizer compared to CinA, we investigated the difference of response between DNCB and CinA to underlie differences of mechanisms in DC activation in response to these molecules. A RT-qPCR array was performed in *nrf2*$^{+/+}$ and *nrf2*$^{-/-}$ DC that allowed us to show that CinA was a stronger inducer of genes compared to DNCB. Then, we investigated Nrf2's role in DC survival upon treatment with DNCB and CinA by studying the mechanism triggering apoptosis. Our results showed that Nrf2 controlled apoptosis induced by DNCB through oxidative stress and *bcl-2* gene expression while in response to CinA, Nrf2 controlled apoptosis independently of GSH and probably through a higher expression of detoxification genes, particularly HO-1.

2. Results

2.1. Expression of detoxification genes by Nrf2 is chemical-dependent

Nrf2 is known as a multiorgan protector against xenobiotic stress (Lee et al., 2005) and has been shown to be a critical regulator of the innate immune response after LPS treatment (Thimmulappa et al., 2006). Its target genes have been found to be involved in several key survival pathways (Banerjee et al., 2012; Kawamoto et al., 2011; Radjendirane et al., 1998). We thus conducted a RT-qPCR array designed for 43 target genes in BMDC to address the question whether gene expression regulated by Nrf2 could be altered differently depending on the type of CS. For this purpose, *nrf2*$^{+/+}$ and *nrf2*$^{-/-}$ BMDC were treated with DNCB or CinA or DMSO (vehicle) at different time of stimulation (4 h, 8 h and 24 h) (Fig. 1A).

Based on the ANOVA model, we showed a significant interaction between the three factors: groups of genes, CS and genotype (p-value = 0.02). We measured a higher induction of detoxification genes compared to immune genes in *nrf2*$^{+/+}$ BMDC in the presence of both CS (Fig. 1B).

Nrf2 deficiency markedly blocked the up-regulation of antioxidant genes after 4 h and 8 h of treatment with both CS (Fig. 1A). At 24 h, genes expression was markedly delayed in *nrf2*$^{-/-}$ leading to a significant up-regulation of genes in *nrf2*$^{-/-}$ than in *nrf2*$^{+/+}$ BMDC with both CS. In our hands, Nrf2 positively controlled antioxidant genes like *glutathiones reductase* (*gsr*), *catalase*, *glutathione peroxydase* (*gpx*) *and*

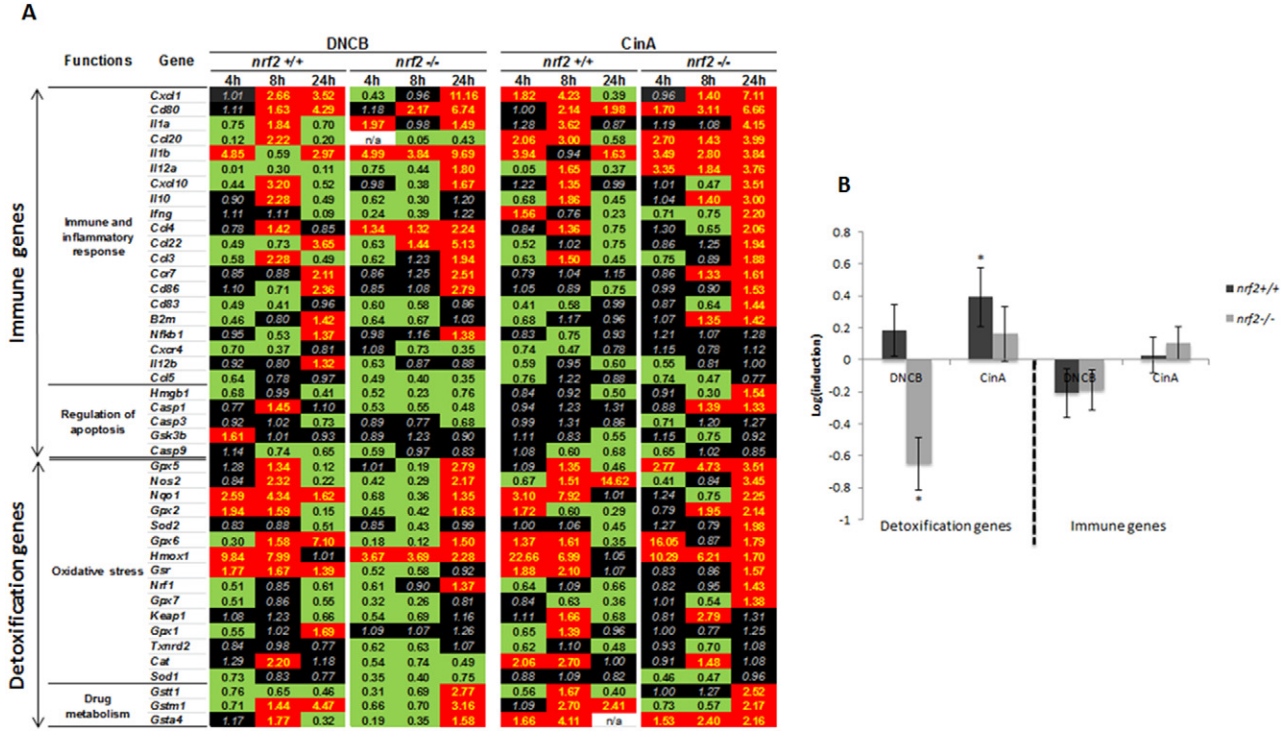

Fig. 1. Antioxidant and immune-related genes are regulated by Nrf2 in DC in response to contact sensitizers. **(A)** Expression of 19 antioxidant genes and 23 immune-related genes in DC screened by qPCR array in response to contact sensitizers. BMDC from *nrf2*$^{+/+}$ and *nrf2*$^{-/-}$ mice were treated with DNCB (10 μM), CinA (100 μM) or DMSO for 4 h, 8 h and 24 h. Gene expression of the 43 target genes was measured using RT-qPCR arrays. Results were expressed as mRNA fold change in compound-treated *vs* DMSO-treated cells, either up- (red), down- (green) regulated or unchanged (black). **(B)** Induction of detoxification and immune genes in response to DNCB or CinA in both Nrf2 genotypes. Log transformation of fold induction [*i.e.*, Log (Induction)] was applied in order to achieve the required normality assumption. Results are expressed as log (Induction) for detoxification and immune genes in response to DNCB or CinA. *: DNCB compared to CinA in *nrf2*$^{+/+}$ or *nrf2*$^{-/-}$, * $p < 0.05$, ANOVA test.