

Inflammatory and immune mechanisms in contact hypersensitivity (CHS) in rats

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Abstract Contact hypersensitivity (CHS) is a T-cell-mediated skin inflammatory reaction to cutaneous exposure to small sensitizing chemicals, haptens. Majority of CHS studies were conducted in mice and there is paucity of data in other experimental animals. In this review, after a brief survey of murine CHS, hitherto known characteristics of CHS in rats were presented including inflammatory and immune mechanisms of both sensitization and elicitation phases. Survey of literature of rat CHS is presented, with our data concerning the importance of genetic background both in the induction and in the expression of reaction to dinitrochlorobenzene. The knowledge of CHS in rats, preferred animal in immunopharmacological studies, might help development of immunomodulatory intervention in contact allergy.

Keywords Contact hypersensitivity (CHS) · Rats · Dinitrochlorobenzene (DNCB) · Ear swelling response · Draining lymph node response · Peripheral blood granulocytes

Introduction

Allergic contact dermatitis (ACD) is a common skin inflammatory disease in humans, which develops at site of skin re-exposure to contact allergen, called hapten, in individuals previously sensitized with the same, or a structurally closely related, hapten. ACD is the second most commonly reported occupational illness, accounting for 10–15% of all occupational diseases [1]. It is characterized by redness, papules and vesicles, followed by scaling and dry skin [2]. Pathophysiology of ACD is studied on animal, mostly murine, models referred as contact hypersensitivity (CHS).

General characteristics of murine contact hypersensitivity

Contact hypersensitivity is a T-cell-mediated skin inflammatory reaction to cutaneous exposure to small sensitizing chemicals, haptens. As shown in murine models of CHS to strong haptens (as dinitrohalobenzenes and oxazolone), called “experimental haptens” since not encountered in environment, two distinct phases are necessary to achieve optimal CHS reaction (Fig. 1). The sensitization phase (known as afferent or induction phase) starts with the first contact of the skin with the hapten, after its topical application to animal’s abdominal or dorsal area. Owing to their lipophilic nature, haptens get into the skin and their chemical reactivity enable them to bind to skin proteins [3]. Epidermal and dermal dendritic cells in skin uptake hapten or haptenated proteins and migrate to skin-draining lymph nodes under the influence of inflammatory signals such as interleukin (IL)-1 and tumor necrosis factor (TNF), generated in the skin by hapten application [4]. In the regional lymph nodes, hapten-loaded dendritic cells present haptenated peptides bound to major histocompatibility complex (MHC) class I and II molecules to naïve hapten-specific

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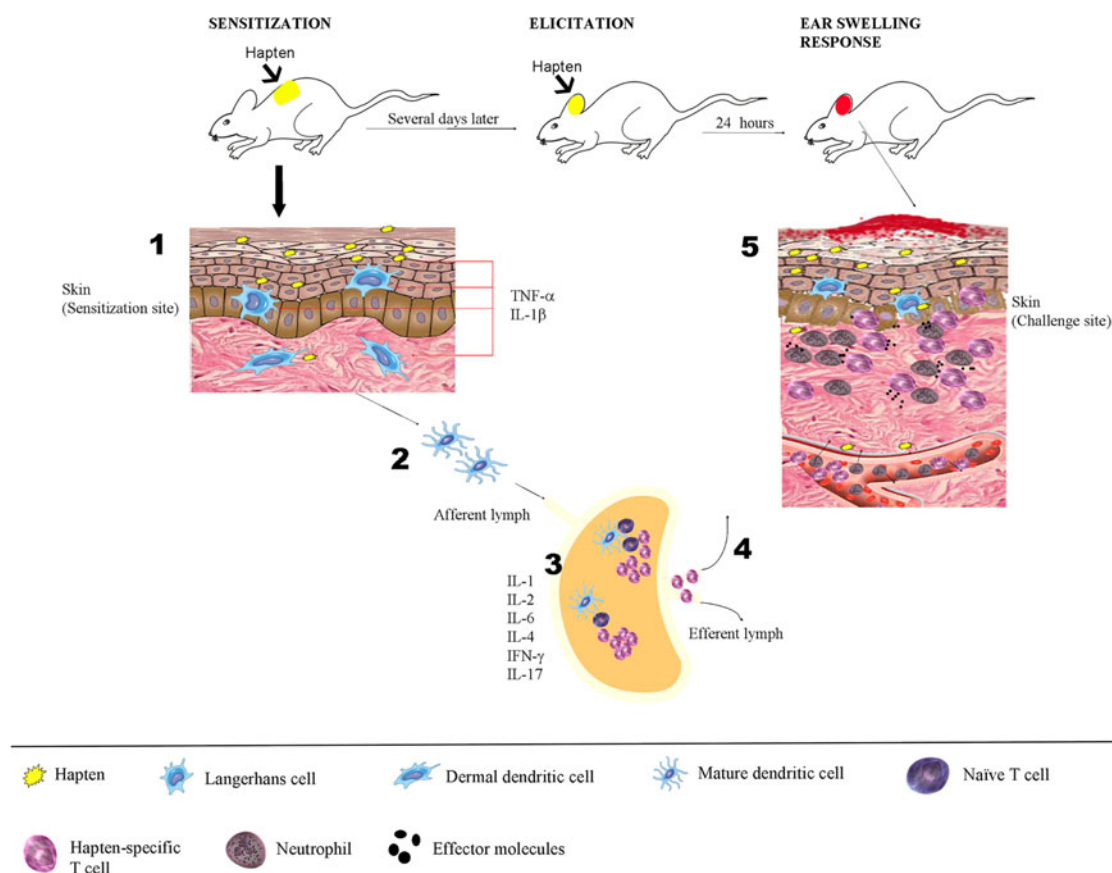


Fig. 1 CHS scheme. Sensitization phase starts after hapten topical application to the animal's skin. Hapten penetration into the skin results in the production of proinflammatory cytokines (TNF- α , IL-1 β) and chemokines (1), what induce activation and migration of hapten-loaded epidermal and dermal skin dendritic cells through afferent lymph to the draining lymph nodes (2). In draining lymph nodes, hapten presentation results in proliferation, activation and subsequent differentiation of hapten-specific T cells (3). Secretion of cytokines in draining lymph nodes during sensitization phase contributes to efficient specific T cells activation and proliferation (IL-1, IL-2, IL-6) and differentiation (IFN- γ , IL-17, IL-4). At the end

of this phase, primed specific T cells emigrate out of the lymph nodes to the blood beginning their recirculation between lymphoid organs and peripheral tissues, especially skin (4). In elicitation phase, subsequent application of the same hapten on, usually, animal's ears, leads to hapten uptake by cells in skin (Langerhans cells, keratinocytes, endothelial cells) and its presentation to recirculating hapten-specific T cells what induce production of inflammatory cytokines and chemokines in challenged skin leading to neutrophil infiltration. Dynamic interaction of adaptive and innate immune cells in challenged skin, which display their effector activities, results in the development of characteristic skin lesions (5)

CD8⁺ and CD4⁺ T cells, respectively, stimulating their activation and proliferation [5]. Production of IL-2 and IL-6 during sensitization phase are considered important for, respectively, hapten-specific and non-specific proliferation of draining lymph node cells during sensitization phase [6–8], although there are data that challenged association between IL-6 and proliferative responses during sensitization with some haptens [4]. During sensitization phase, primed (sensitized) specific T cells leave lymph nodes and enter circulation. Some of sensitized cells infiltrate skin as well, but with no clinically evident consequences [2]. Data obtained from investigations of sensitization phase resulted in the development of local lymph node assay (LLNA), a screening system for identification of contact sensitizing potency of chemicals, in which increase in draining (auricular/ facial) lymph node weight, and numbers of

dividing cells are measured following primary sensitization of ear skin with the chemical in question [9]. By analyzing changes in the dynamics as well as the type of leukocyte accumulation in LLNA assay [10], the effect of contact allergens on various cell types could be evaluated.

The elicitation phase (efferent or challenge phase) occurs following subsequent application of hapten to skin, usually ears of sensitized animals, several days later. Hapten application provokes proinflammatory innate immune cell activity and activity of skin antigen-presenting cells in challenged skin [11, 12], resulting in infiltration of hapten-primed T cells in epidermis and dermis [13]. Activation of hapten-specific effector T cells brings to the production of effector cytokines involved in recruitment of innate immunity leukocytes to the challenged skin, and further recruitment of effector hapten-specific T cells, what

mediate the characteristic tissue edema and lesions associated with ear swelling response [14]. Interferon- γ (IFN- γ) producing CD8⁺ T cells are considered as the primary cutaneous effector cells [15, 16], but the identity of effector T cells relevant for the expression of CHS is a matter of debate, as the relevance of both CD8⁺ and CD4⁺ cells as effector IFN- γ producing T cells in CHS was shown [17, 18] and even lower production of this cytokine by CD8⁺ cells was noted in mouse CHS to dinitrochlorobenzene (DNCB) [19]. IFN- γ as effector cytokine is generally accepted as an absolute requirement for CHS expression [20]; however, there are studies that showed a minor role of this cytokine in CHS [21, 22]. There is also some controversy concerning the nature/type of the CHS response. According to a dominant concept, CHS to majority of contact allergens is associated with the activation of T helper type 1 (Th1) or T cytotoxic type 1 (Tc1) cells [18], while IL-4/IL-10 producing CD4⁺ T cells are considered to have predominantly regulatory role in CHS [16]. However, although down-regulatory role of Th2 cytokine IL-4 was shown in sensitization [16] and elicitation phase [23], the importance of this cytokine in CHS expression was shown as well, as impairment of CHS was demonstrated in IL-4 deficient mice [24, 25]. According to some opinions, both Th1 and Th2 cells and their signature cytokines play a role in CHS [26].

The involvement of cells that produce IL-17 (T_{IL17} cells) was shown in murine CHS [27]. Production of IFN- γ and IL-17 by hapten-primed CD4⁺ and CD8⁺ draining lymph node cells was shown in DNFB-sensitized mice implying their role in the development of effector cells during afferent phase of CHS [28]. In addition, it was shown that both IFN- γ - and IL-17-producing CD8⁺ cells initiate immune response to hapten challenge in sensitized mice [29] and that both cytokines are necessary for optimal CHS response [28].

Contact hypersensitivity studies in rats

Despite numerous data from mouse CHS studies, the knowledge of contact allergy mechanisms is still far from complete. Data about CHS in other experimental animals might contribute to the understanding mechanisms of this reaction, which is important aspect in translational studies. Beside mice, rats are used in studying contact allergy, although there are far less data gathered since the early work in which rat CHS was suggested as an experimental model for study of human contact allergic dermatitis [30]. Rat is preferred species in pharmacological/toxicological studies of new compounds with immunomodulating potential what might help bridging variety of investigations. In this review, the survey of literature data

concerning rat CHS is presented, with the significant number of data obtained in our model of contact sensitivity to DNCB, which is employed to explore the impact of genetic background on inflammatory/immune mechanisms of this reaction.

Ear swelling response in rats

Early experiments in Th1-prone Lewis (Le) rats suggested the involvement of IFN- γ in the effector phase of rat CHS to dinitrofluorobenzene (DNFB) [31, 32]. However, the role of this cytokine in elicitation of CHS turned out controversial, as its neutralization resulted in reduction of its effects on keratinocytes (in terms of increase in MHC class II molecules), but with enhancement of ear swelling [31]. Moreover, administration of recombinant IFN- γ during elicitation of CHS was shown to reduce intensity of reaction to DNFB [32]. Comparative study of CHS in Th1-prone Lewis rats and Th-2 prone Brown Norway (BN) rats showed more intense ear swelling response in challenged Le rats, what suggested the importance of Th1 response in CHS expression in rats [33]. By measuring different aspects (ear thickness and leukocyte infiltration) of ear skin response following challenge of sensitized Th1-prone Dark Agouti (DA) rats, we have shown dose-dependent increase in the intensity of this response following application of 0.66 or 1.3% DNCB to rats previously sensitized with two consecutive applications of 2 or 4% DNCB, respectively [34]. At low DNCB dose (0.4% DNCB in sensitization and 0.13% DNCB in elicitation), weak or no ear swelling could be detected, with meager cell infiltration close to control, vehicle (acetone-olive oil)-treated ears. In keeping with data which showed the need for skin inflammation (irritancy of contact sensitizers) for the development of optimal CHS responses in mice [12], we have demonstrated a dose-dependent increase in TNF and nitric oxide (NO) production using challenged ears skin organ culture system [34, 35] what correlated highly and significantly with the infiltration of leukocytes into the challenged skin. Microarray study of challenged ears skin and draining lymph nodes in CrI: CD (SD) rats [36] demonstrated up-regulation of genes for numerous inflammatory mediators beside iNOS and inflammatory cytokines, including chemokines, as well as genes for innate immunity effectors such as metalloproteinases (MMP) and complement components. The level of TNF and NO production was much higher in DA as compared to Th-2 prone Albino Oxford (AO) rats [35], signifying the importance of genetic background in CHS expression in rats. In corroboration, strain-dependent differences in sensitivity to DNCB were shown between Th1-prone Wistar rats and Th2-prone BN rats, as sensitized BN rats developed less prominent macroscopic and

microscopic changes in ear skin after challenge, compared to sensitized Wistar rats [37]. Increase in IFN- γ (and IL-12) production by draining lymph nodes in the efferent phase of CHS to DNCB in BN rats with somewhat increased IL-10 production and unchanged IL-4 implied rat CHS to this happen as Th1 type reaction [38] what suggested a role for Th1 cells as effectors in rat CHS. Higher levels of both IFN- γ and IL-17 production by draining lymph nodes during expression of CHS in DA rats as compared to AO rats (unpublished data) imply involvement of both Th1 and Th17 cells as effectors in rat CHS.

Sensitization studies in rats

Development of LLNA in rats [39] suggested that this animal species might be considered as useful experimental model for sensitization studies as well. Both Th-2 prone BN and Th1-prone Wistar rats responded dose dependently to sensitization with increasing doses of DNCB, by a rise in draining lymph node weight and numbers of dividing (BrdU⁺) cells, although with variability in randomly bred/outbred Wistar rats [39]. Rat LLNA assay has proved useful in discriminating between sensitizing and non-sensitizing potential of chemicals [40] and in determination of dose-dependent responses and threshold level doses in skin allergy [41]. Our initial work with AO rats sensitized with 2% DNCB demonstrated changes in draining lymph node (DLN) cell activity including increase in total cell number and accumulation of cells with dendritic morphology, as well as proliferation (either non-specific/spontaneous, hapten-stimulated or in response to exogenous IL-2) 3 days following sensitization [35]. Sensitization was accompanied by changes in cell subset composition (increase in percentages of CD8⁺ cells, concomitantly with a decrease in CD4⁺ cells), what resulted in a decrease in CD4⁺/CD8⁺ cell ratio (compared to unsensitized animals). Shift in favor of CD8⁺ cells was more pronounced 5 days following skin sensitization and is observed at both low (0.4%) and high (4%) DNCB doses. No changes in subset composition were observed in DA rats (unpublished data), what suggested strain-dependent differences in the sensitization phase of CHS as well. By investigating time-dependent changes in DLNs during 5 day-sensitization period, we have shown increase in basic indices of DLN cell activity (cellularity and proliferation) in both rat strains, but with higher values in sensitized DA rats [42]. Development of CHS was associated with a progressive decrease in IL-6 production (more pronounced in AO rats). The production of inflammatory cytokines IFN- γ and IL-17, necessary for the development of effector cells in CHS, was increased in both strains, but with differential pattern and generally lower levels of production of both cytokines in AO rats. In

contrast to inflammatory cytokines, IL-4 was undetectable during the entire sensitization phase. Production of IFN- γ concomitantly with low IL-4 in both strains, classify rat CHS as Th1/type 1 reaction, and increase in IL-17 imply the involvement of Th₁₇/T_{IL-17} cells in this reaction. Strain-dependent differences in inflammatory cytokine production might rely on anti-inflammatory cytokine IL-10, as higher production was noted in AO rats [42]. In analogy with data in humans [43], which showed that the principle factor that determines responses to challenge is the strength of sensitization, DLN cell activity during sensitization might be responsible for the observed differences in CHS expression between DA and AO rats.

Systemic innate response in rat CHS to DNCB

Inflammatory/immunostimulatory potential of DNCB was employed as topical immunotherapy for some skin dermatoses, and the use of topical DNCB is proposed as a systemic immunotherapy for some immune-based diseases including HIV disease [44] and lupus erythematosus [45]. On the basis of the efficacy of repeated topical DNCB application shown in HIV patients [46, 47], chronic prurigo [48] and in refractory atopic dermatitis [49] systemic effects of skin sensitization (increase in peripheral blood T-cell counts and stimulation of proinflammatory Th1 responses) were proposed as an underlying mechanisms of beneficial effects of topical DNCB therapy in these patients. Data from animal CHS studies revealed systemic Th1 response as judged on serum hapten-specific IgG2a [50] and IgG antibodies [37] following DNCB sensitization of mice and rats, respectively. Increase in plasma levels of IL-6 and acute phase reactants (haptoglobin and serum amyloid A) in sera of mice sensitized with oxazolone [51, 52] pointed out that beside local (in skin) proinflammatory activity, there is also a systemic inflammation in contact sensitization. Our study showed the priming effect of repeated skin sensitization with DNCB on peripheral blood granulocyte respiratory burst and adhesion in both DA and AO rats 1 day following repeated application of high doses (2 and 4%) of DNCB [53], which indicated involvement of a cellular component of innate systemic inflammation in contact sensitization with this hapten. Changes in other granulocyte activities relevant for inflammatory/effector functions of these cells (myeloperoxidase content, NO and TNF production and expression of CD11b, a main molecule involved in the firm, irreversible granulocyte adhesion to various matrices) were observed following application of low (0.4%) DNCB dose as well, and some of the activities were evident during the entire (5 days) sensitization period [54, 55]. Increased responsiveness of granulocyte NO and TNF production to exogenous stimulation depict the impact of DNCB

application to signaling pathways in granulocytes of sensitized rats. Distinctive pattern of expression as well as differential dynamics of some of the granulocyte activities in sensitized animals of the same strain imply differential regulation of some of the aspects of their activity [54] and upregulation of all granulocyte activities first day following sensitization in both rat strains demonstrated acute changes in these cells. Increase in numbers of peripheral blood granulocytes in DA rats and a lack of quantitative changes in AO rats, concomitantly with upregulation of all examined granulocyte activities in DA rats, might be an indicator of higher responsiveness of these cells to DNCB stimulation in this strain. Distinctions between DLN cell activity in DA and AO rats during sensitization phase along with those reported for peripheral blood granulocytes might have collectively contributed to differences in the intensity of expression of CHS reaction in these two strains.

Conclusion

Although knowledge about CHS is mostly derived from murine model, rat as model for contact allergy investigations has been also used, in respect of inflammatory and immune mechanisms involved. Studies conducted in different rat strains, with significant number of data obtained in our model of rat CHS to DNCB, point to the importance of genetic background in the induction and expression of reaction. Beside the importance in understanding mechanisms involved in CHS reaction, characterization of rat CHS can be useful in the development of immune-based intervention strategies in contact allergy.

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References

1. Anderson SE, Siegel PD, Meade BJ: The LLNA: a brief review of recent advances and limitations. *J Allergy* 2011; doi:[10.1155/2011/424203](https://doi.org/10.1155/2011/424203).
2. Saint-Mezard P, Rosieres A, Krasteva M, Berard F, Dubois B, Kaiserlian D, Nicolas JF. Allergic contact dermatitis. *Eur J Dermatol*. 2004;14:284–95.
3. Divkovic M, Pease CK, Gerberick GF, Basketter DA. Hapten-protein binding: from theory to practical application in the in vitro prediction of skin sensitization. *Contact Dermat*. 2005; 53:189–200.
4. Lappin MB, Dearman RJ, Norval M, Kimber I. Lack of association between interleukin-6 production by contact allergen-activated draining lymph node cells and lymphoproliferative activity. *Am J Contact Dermat*. 1998;9:34–9.
5. Bour H, Peyron E, Gaucherand M, Garrigue JL, Desvignes C, Kaiserlian D, Revillard JP, Nicolas JF. Major histocompatibility complex class I-restricted CD8⁺ T cells and class II-restricted CD4⁺ T cells, respectively, mediate and regulate contact sensitivity to dinitrofluorobenzene. *Eur J Immunol*. 1995;25:3006–10.
6. Hope JC, Dearman RJ, Debicki RJ, Kimber I, Hopkins SJ. Interleukin-6 production by draining lymph node cells following primary contact sensitization: relationship to the proliferative response. *Int Arch Allergy Immunol*. 1994;103:378.
7. Hope JC, Dearman RJ, Kimber I, Hopkins SJ. The kinetics of cytokine production by draining lymph node cells following primary exposure of mice to chemical allergens. *Immunology*. 1994;83:250–5.
8. Hope JC, Campbell F, Hopkins SJ. Deficiency of IL-2 or IL-6 reduces lymphocyte proliferation, but only IL-6 deficiency decreases the contact hypersensitivity response. *Eur J Immunol*. 2000;30:197–203.
9. Kimber I, Dearman RJ. The local lymph node assay: developments and applications. *Toxicology*. 1994;93:13–31.
10. Larsen JM, Geisler C, Nielsen MW, Boding L, von Essen M, Hansen AK, Skov L, Bonefeld CM. Cellular dynamics in the draining lymph nodes during sensitization and elicitation phases of contact hypersensitivity. *Contact Dermat*. 2007;57:300–8.
11. Dilulio NA, Engeman T, Armstrong D, Tannenbaum C, Hamilton TA, Fairchild RL. Gα_o-mediated recruitment of neutrophils is required for elicitation of contact hypersensitivity. *Eur J Immunol*. 1999;29:3485–95.
12. Zhang L, Tinkle SS. Chemical activation of innate and specific immunity in contact dermatitis. *J Invest Dermatol*. 2000;115: 168–76.
13. Engeman T, Gorbachev AV, Kish DD, Fairchild RL. The intensity of neutrophil infiltration controls the number of antigen-primed CD8 T cells recruited into cutaneous antigen challenge sites. *J Leukoc Biol*. 2004;76:941–9.
14. Grabbe S, Schwarz T. Immunoregulatory mechanisms involved in elicitation of allergic contact hypersensitivity. *Immunol Today*. 1998;19:37–44.
15. Gocinski B, Tigelaar R. Roles of CD4⁺ and CD8⁺ T cells in murine contact sensitivity revealed by in vivo monoclonal antibody depletion. *J Immunol*. 1990;144:4121–8.
16. Xu H, Dilulio NA, Fairchild RL. T cell populations primed by hapten sensitization in contact sensitivity are distinguished by polarized patterns of cytokine production: interferon-γ-producing (Tc1) effector CD8⁺ T cells and interleukin (IL) 4/IL-10-producing (Th2) negative regulatory CD4⁺ T cells. *J Exp Med*. 1996;183:1001–12.
17. Wang B, Fujisawa H, Zhuang L, Freed I, Howell BG, Shahid S, et al. CD4⁺ Th1 and CD8⁺ type 1 cytotoxic T cells both play a crucial role in the full development of contact hypersensitivity. *J Immunol*. 2000;165:6783–90.
18. Saint-Mezard P, Berard F, Dubois B, Kaiserlian D, Nicolas J. The role of CD4⁺ and CD8⁺ T cells in contact hypersensitivity and allergic contact dermatitis. *Eur J Dermatol*. 2004;14:131–8.
19. Moussavi A, Dearman RJ, Kimber I, Kemeny DM. Cytokine production by CD4⁺ and CD8⁺ T cells in mice following primary exposure to chemical allergens: evidence for functional differentiation of T lymphocytes in vivo. *Int Arch Allergy Immunol*. 1998;116:116–23.
20. Gorbachev AV, Fairchild RL. Induction and regulation of T-cell priming for contact hypersensitivity. *Crit Rev Immunol*. 2001; 21:451–72.
21. Saulnier MS, Huang M, Aguet M, Ryffel B. Role of interferon-γ in contact hypersensitivity assessed in interferon-γ receptor deficient mice. *Toxicology*. 1995;102:301–12.
22. Reeve VE, Bosnic M, Boehm-Wicox C, Nishimura N, Ley RD. Ultraviolet A radiation (320–400 nm) protects hairless mice from immunosuppression induced by ultraviolet B radiation. *Int Arch Allergy Immunol*. 1999;115:316–22.

23. Gautman SC, Chikkala NF, Hamilton TA. Negative regulation of contact sensitivity to trinitrochlorobenzene. *J Immunol.* 1992; 148:1411–5.
24. Weigmann B, Schwing J, Huber H, Ross R, Mossmann H, Knop J, et al. Diminished contact hypersensitivity response in IL-4 deficient mice at a late phase of the elicitation reaction. *Scand J Immunol.* 1997;45:308–14.
25. Traidl C, Jugert F, Krieg T, Merk H, Hunzelmann N. Inhibition of allergic contact dermatitis to DNCB but not to oxazolone in interleukin-4-deficient mice. *J Invest Dermatol.* 1999;112: 476–82.
26. Watanabe H, Unger M, Tuvel B, Wang B, Sauder DN. Contact hypersensitivity: the mechanism of immune responses and T cell balance. *J Interferon Cytokine Res.* 2002;22:407–12.
27. Nakae S, Komiyama Y, Nambu A, Sudo K, Iwase M, Homma I, Sekikawa K, Asano M, Iwakura Y. Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity.* 2002;17:375–87.
28. He D, Wu L, Kim HK, Li H, Elmetts CA, Xu H. IL-17 and IFN- γ mediate the elicitation of contact hypersensitivity responses by different mechanisms and both are required for optimal responses. *J Immunol.* 2009;183:1463–70.
29. Kish DD, Li X, Fairchild RL. CD8 T cells producing IL-17 and IFN- γ initiate the innate immune response required for responses to antigen skin challenge. *J Immunol.* 2009;182:5949–59.
30. Scheynius A, Skoglund C, Johansson C, Holmdahl R, Van der Meide PH. The rat as an experimental model for studies on immune regulating mechanisms in contact allergic reactions. *Acta Derm Venereol Suppl (Stockh).* 1988;135:54–6.
31. Skoglund C, Scheynius A, Holmdahl R, Van der Meide PH. Enhancement of DTH reaction and inhibition of the expression of class II transplantation antigens by in vivo treatment with antibodies against γ -interferon. *Clin Exp Immunol.* 1988;71:428–32.
32. Skoglund C, Scheynius A. Effects of interferon- γ treatment of the cutaneous DTH reaction in rats. *Arch Dermatol Res.* 1990; 282:318–24.
33. Peszkowski MJ, Warfvinge G, Larsson Å. Allergic and irritant contact responses to DNFB in BN and LEW rat strains with different Th1/Th2 profiles. *Acta Derm Venereol.* 1994;74:371–4.
34. Kataranovski M, Kandolf-Sekulović L, Milosavljević I. Production of TNF- α by skin explants of dinitrochlorobenzene-challenged ears in rats: a model for the evaluation of contact hypersensitivity. *Vojnosanit Pregl.* 2002;59:581–6.
35. Kataranovski M, Milojević G, Kandolf L, Milošević V. Skin-organ culture as an approach in evaluation of nitric oxide (NO) involvement in contact hypersensitivity expression. *Acta Dermatoven APA.* 2002;11:3–10.
36. Hartmann B, Staedler F, Hartmann N, Meingassner J, Firat H. Gene expression profiling of skin and draining lymph nodes of rats affected with cutaneous contact hypersensitivity. *Inflamm Res.* 2006;55:322–34.
37. Kuper CF, Stierum RH, Boorsma A, Schijf MA, Prinsen M, Bruijntjes JP, Bloksma N, Arts JHE. The contact allergen dinitrochlorobenzene (DNCB) and respiratory allergy in the Th2-prone Brown Norway rat. *Toxicology.* 2008;246:213–21.
38. Dearman RJ, Warbrick EV, Skinner R, Kimber I. Cytokine fingerprinting of chemical allergens: species comparisons and statistical analyses. *Food Chem Toxicol.* 2002;40:1881–92.
39. Arts JH, Droge SC, Bloksma N, Kuper CF. Local lymph node activation in rats after dermal application of the sensitizers 2,4-dinitrochlorobenzene and trimellitic anhydride. *Food Chem Toxicol.* 1996;34:55–62.
40. Arts JH, Dröge SC, Spanhaak S, Bloksma N, Penninks AH, Kuper CF. Local lymph node activation and IgE responses in Brown Norway and Wistar rats after dermal application of sensitizing and non-sensitizing chemicals. *Toxicology.* 1997;117: 229–34.
41. Arts JH, Mommers C, Heer C. Dose-response relationships and threshold levels in skin and respiratory allergy. *Crit Rev Toxicol.* 2006;36:219–51.
42. Popov A, Mirkov I, Miljkovic DJ, Belij S, Zolotarevski L, Kataranovski D, Kataranovski M. Contact allergic response to dinitrochlorobenzene (DNCB) in rats: insight from sensitization phase. *Immunobiology.* 2011;216:763–70.
43. Friedmann PS. The relationships between exposure dose and response in induction and elicitation of contact hypersensitivity in humans. *Br J Dermatol.* 2007;157:1093–102.
44. Stricker RB, Zhu YS, Elswood BF, Dumlao C, Van Elk J, Berger TG, Tappero J, Epstein WL, Kiprov DD. Pilot study of topical dinitrochlorobenzene (DNCB) in human immunodeficiency virus infection. *Immunol Lett.* 1993;36:1–6.
45. Stricker RB, Goldberg B, Epstein WL. Immunological changes in patient with systemic lupus erythematosus treated with topical dinitrochlorobenzene. *Lancet.* 1995;345:1505–6.
46. Stricker RB, Goldberg B, Mills LB, Epstein WL. Decrease in viral load associated with topical dinitrochlorobenzene therapy in HIV disease. *Res Virol.* 1997;148:343–8.
47. Traub A, Margulis SB, Stricker RB. Topical immune modulation with dinitrochlorobenzene in HIV disease: a controlled trial from Brazil. *Dermatology.* 1997;195:369–73.
48. Yoshizawa Y, Kitamura K, Maibach HI. Successful immunotherapy of chronic nodular prurigo with topical dinitrochlorobenzene. *Br J Dermatol.* 1999;141:387–9.
49. Yoshizawa Y, Matsui H, Izaki S, Kitamura K, Maibach HI. Topical dinitrochlorobenzene therapy in the treatment of refractory atopic dermatitis: systemic immunotherapy. *J Am Acad Dermatol.* 2000;42:258–62.
50. Dearman RJ, Kimber I. Differential stimulation of immune function by respiratory and contact chemical allergens. *Immunology.* 1991;72:563–70.
51. Kimber I, Ward RK, Shepherd CJ, Smith MN, McAdam KPWJ, Raynes JG. Acute-phase proteins and the serological evaluation of experimental contact sensitivity in the mouse. *Int Arch Allergy Immunol.* 1989;89:149–55.
52. Kimber I, Cumberbatc M, Humphreys M, Hopkins SJ. Contact hypersensitivity induces plasma interleukin 6. *Int Arch Allergy Immunol.* 1990;92:97–9.
53. Kataranovski M, Draskovic-Pavlovic B, Jovcic G, Milojevic G, Todorovic V, Colic M, Popovic P. Peripheral blood granulocyte activity following contact sensitization of rats with dinitrochlorobenzene. *Toxicology.* 2001;162:121–36.
54. Belij S, Popov A, Zolotarevski L, Mirkov I, Djokic J, Kataranovski D, Kataranovski M. Systemic immunomodulatory effects of topical dinitrochlorobenzene (DNCB) in rats. Activity of peripheral blood polymorphonuclear cells. *Environ Toxicol Pharmacol.* 2012;33:168–80.
55. Belij S, Popov A, Mirkov I, Zolotarevski L, Kataranovski D, Kataranovski M. Percutaneous toxicity of dinitrochlorobenzene (DNCB) in rats. *Cutan Ocul Toxicol.* 2012;31:7–13.