CD301b+ Dermal Dendritic Cell-Mediated Type 2 Immunity Plays a Critical Role in Oxazolone-induced Contact Hypersensitivity

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INTRODUCTION

- Contact hypersensitivity (CHS)** response induced by single epicutaneous sensitization/elicitation with hapten is widely used for a mouse model of human allergic contact dermatitis (ACD)
- It is generally accepted that types of hapten and solvent would determine the dominant immunological responses of CHS
 - Th1 IFN- γ model:** DNFB, DNCB, TNCB, Oxazolone
 - Th2 IL-4 model:** FITC/DBP
- HOWEVER**, human ACD lesions frequently demonstrate a mixed gene expression pattern of IFN- γ and IL-4
 - Nickel: Th1 > Th2
 - Fragrance, rubber: Th2 > Th1
- FURTHERMORE**, it has been poorly understood yet which type of immune response would play more critical role for a mixed immune types of CHS
- Oxazolone (Ox)-induced acute CHS response**
 - Ox has generally been regarded as Th1 hapten in acute CHS for a long-time
 - HOWEVER**, Ox-induced acute colitis is a well-defined Th2-deviated inflammation model

HYPOTHESIS

We hypothesized that Ox-induced CHS responses would involve Th2 immunity which plays a pathogenic role in this DTH responses of the skin

METHODS

- Mice:** Wild-type (WT), Mgl2-DTR mice, Langerin-DTR (BL/6 background), IL-4-GFP(BALB/c background)

Ox-induced CHS model protocol

- The abdomens of mice were shaved and sensitized with 50 μ l of vehicle or 3% Ox in a 4:1 acetone:olive oil on day 0. On day 5, the mice were challenged on both sides of ear with 20 μ l of Ox or vehicle.
- The ear swelling response was measured 24hr after elicitation using dial thickness gauge.
- Ox-sensitized skin areas and skin draining lymph nodes were analyzed by FACS and quantitative PCR.

Skin single cell suspensions and FACS analyses

- The skins were harvested from the base and minced into small pieces. Total skins were incubated in collagenase A and DNase 1 digestion media for 1.5hr under 37°C.
- The resultant skin cells were further incubated for the ex vivo T cell stimulation with α CD3 ϵ and α CD28 supplemented with PMA, Ionomycin, and golgi blockers for 6hrs.

RESULTS

Increased level of IL4 expression in Ox-sensitized/challenged skins

Figure 1. Groups of C57BL/6 mice were sensitized and challenged with an indicated vehicle and haptens. Gene expression levels of an indicated cytokines from the lesional skins were analyzed 24hr after the challenge. Results are from at least two independent experiments with four to five mice per group. Error bars show mean $\pm$ standard error of the mean. *P<0.05.

Ox-induced CHS response was attenuated by IL-4 neutralization

Figure2. Groups of C57BL/6 mice were intraperitoneally injected with 250 μ g of isotype, α IFN- γ , and α IL-4 neutralizing antibodies at two days interval starting from 24hr before Ox sensitization. (a) Ear swelling was measured using dial thickness gauge(PEACOCK, Ozaki MFG Co. Ltd., Tokyo, Japan) 24hr after elicitation. (b) The mRNA expression of the indicated genes was analyzed from the Ox-challenged ears. (c) Histology (hematoxylin and eosin staining) of the ear skin 24hr after elicitation. Error bars show mean $\pm$ standard error of the mean. *P<0.05, **P<0.01.

Epicutaneous sensitization with Ox drives Th2 priming in the draining lymph nodes

Figure 3. (a) Groups of C57BL/6 mice were epicutaneously sensitized with Ox and gene expression levels from the draining lymph nodes were sequentially analyzed by quantitative PCR. (b) IL-4 protein expression from skin-draining lymph nodes of 4get mice 5 days after epicutaneous Ox sensitization was analyzed by FACS. IL-4 expression was evaluated by the level of GFP+ cells. Total lymph nodes cells were stimulated with α CD3 ϵ and α CD28 antibodies in the presence of PMA, Ionomycin, and golgi blockers for 6 hours. Error bars show mean $\pm$ standard error of the mean. ***P<0.001

Increased infiltration of IL-4+ Th2 cells in Ox-challenged skins

Figure 4. (a) Gating strategies for skin α BT cells. (b) Groups of 4get mice were sensitized and challenged with Ox. 24hr after Ox challenge, lesional skins of mice were harvested and single cell suspensions were prepared by collagenase A and DNase I digestion, and cells were stimulated with α CD3 ϵ and α CD28 antibodies in the presence of PMA, Ionomycin, and golgi blockers for 6 hours. IL-4 expression was evaluated by the level of GFP+ cells by FACS.

CD4+ T cells are a major producer of IL-4 during Ox sensitization

Figure 5. Groups of C57BL/6 mice were treated with 250ug of isotype or α CD4 Ab 24hr before Ox sensitization. (a) CD4+ T cells depletion was assessed by FACS. (b) Gene expression of IL-4 were performed from from skin-draining lymph nodes 5 days after Ox sensitization. (c) Ear swelling was measured using dial thickness gauge 24h after elicitation. (d) mRNA expression of IL-4 was analyzed from the Ox-challenged ears. (e) Histological analysis of the Ox-challenged ear skin. Error bars show mean $\pm$ standard error of the mean. **P<0.01, ***P<0.001

Ox-induced Th2 type CHS response was attenuated by depletion of CD301b+ cDC2(Mgl2+ cDC2) not by cDC1

Figure 6. Groups of C57BL/6, Langerin-DTR and Mgl2-DTR mice were intraperitoneally injected with 1 μ g of diphtheria toxin (DT) 24hr prior to Ox sensitization. (a,d) The mRNA expression of IL-4 was analyzed from skin-draining lymph nodes 5 days after epicutaneous Ox sensitization. (b,e) Ear swelling was measured using dial thickness gauge 24h after elicitation. (c,f) 24hr after Ox challenge, mRNA expression IL-4 was analyzed from the Ox-challenged ears. Error bars show mean $\pm$ standard error of the mean. **P<0.01, ***P<0.001

Ox drives TSLP expression in the skin of the abdomens

Figure 7. (a) Groups of C57BL/6 mice were sensitized with Ox and gene expression levels of TSLP from the abdomens were sequentially analyzed by quantitative PCR. (b) TSLP receptor(TSLPR) expression in cDC2(blue) and Fluorescence Minus One control (FMO, gray) from the skin of the abdomen in C57BL/6 mice was analyzed by FACS. Error bars show mean $\pm$ standard error of the mean. *P<0.05

Ox-induced CHS response was attenuated by IL-7R blocking

Figure 8. (a) Groups of C57BL/6 mice were treated with 500ug of isotype or α IL-7R α Ab 24hr and 2hr before Ox sensitization and Ear swelling was measured using dial thickness gauge 24h after challenge. (b) The mRNA expression of IL-4 was analyzed from the Ox-challenged ears. (c) Histology of the ear skin 24hr after elicitation. Error bars show mean $\pm$ standard error of the mean. *P<0.05, ***P<0.001.

SUMMARY OF RESULTS AND CONCLUSIONS

- Ox-induced acute CHS was characterized by the mixed type1 and type2 immune responses.
- Neutralization of IL-4 more significantly attenuated Ox-induced CHS response compared to IFN- γ blockade.
- CD4+ T cells were major cellular sources or driving forces for IL-4 production during Ox sensitization, indicating that Th2 immunity was efficiently generated in this model.
- Ox-induced Th2 CHS response was principally mediated by CD301b+ dermal cDC2.
- Gene expression of TSLP rapidly increased in the Ox-sensitized abdominal skins and blocking IL-7R α effectively ameliorated the level of IL-4 expression, indicating that TSLP receptor signaling is involved in type 2 CHS response.
- Our data suggest Th2 CHS response, critical in Ox-induced CHS response, is mediated by TSLP-CD301b+ dermal DCs-IL-4 producing CD4+ T cell cascade.

OVERALL, our data indicate that blocking IL-4 with biologics would be a promising therapeutic approach for alleviating highly recurrent ACD patients with Th2-deviated immune environment.

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