

Comparison of two DTH models for T cell-mediated immunity in preclinical screen

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Background

Delayed-type hypersensitivity (DTH) involves the recruitment of T cells into tissues and activation by antigen-presenting cells to produce cytokines, which further activates local endothelial cells and recruits macrophages. It results in tissue erythema, swelling and inflammation, being a classical model of in vivo T cell-mediated immunity screening model for atopic dermatitis, asthma and autoimmune diseases. Different antigens and haptens are used to establish DTH model with different Th1/Th2 balance. To reveal the immunity characterization of the most widely-used DTH models, we built DNFB and oxazolone-induced DTH model in both BALB/c and C57BL/6 mice with robust ear thickness as the readout.

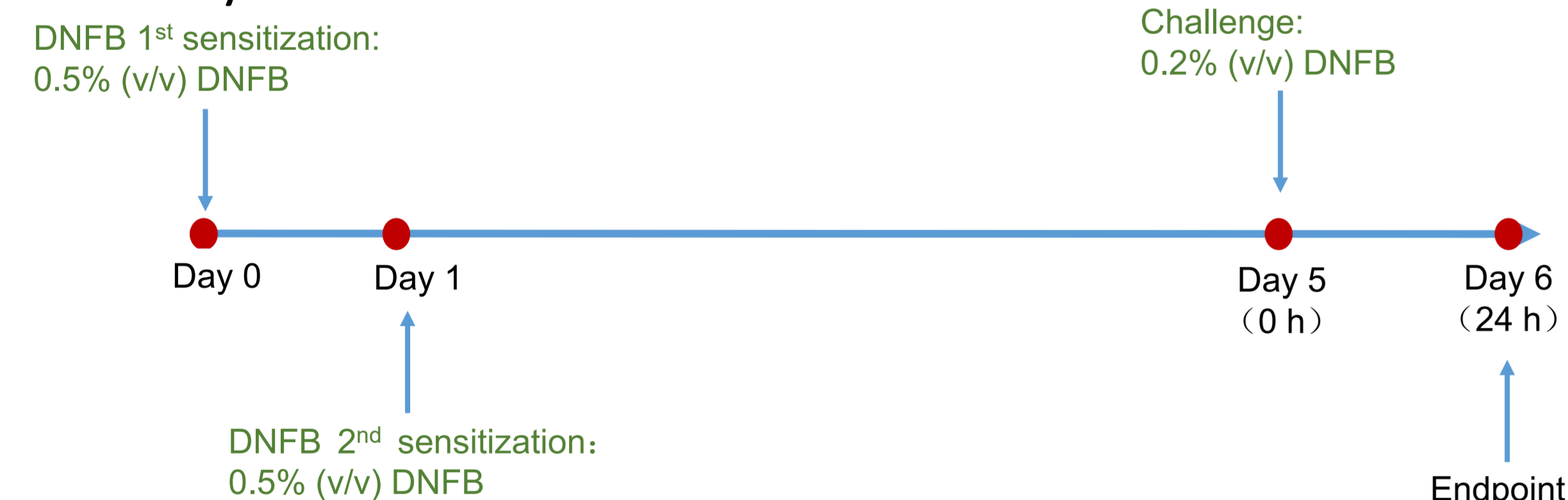
Histological examination was characterized with an influx of immune cells. At the endpoint, we collected the ear tissues for Th1 (ie, IFN- γ) / Th2 (ie, IL-4) cytokines and Myeloperoxidase (MPO) activity evaluation. We also explored extra parameters in spleen to characterize the disease progression and established a more systemically preclinical DTH model with a clearer Th1/Th2 profile for drug efficacy evaluation in T cell-mediated immune response. The systemic comparison helps to choose a suitable model for T cell-mediated immunity assays with different combinations of hapten and mouse strains.

Methods

Building DNFB-induced DTH model

1. Sensitization: The mice were given 0.5% DNFB solution on the shaved abdomen on day 1 and day 2.

2. Challenge: The mice were given 0.2% DNFB solution on the left ear on day 6.



Building oxazolone-induced DTH model

1. Sensitization: The mice were given 3% oxazolone solution on the shaved abdomen on day 1.

2. Challenge: the mice were given 1% oxazolone solution on the left ear on day 6.



Ear thickness and weight

Ear thickness was recorded by micrometer on day 0, day 5, day 6. Stimulated the left ear by DNFB/oxazolone could increase the ear thickness. The Δ (left)ear thickness(mm) was calculated by ear thickness(day 6)- ear thickness(day 5). As shown in Figure 1, dexamethasone exhibited inhibition on ear thickness increased by DNFB/oxazolone, and the weight of ear also followed the same trend.

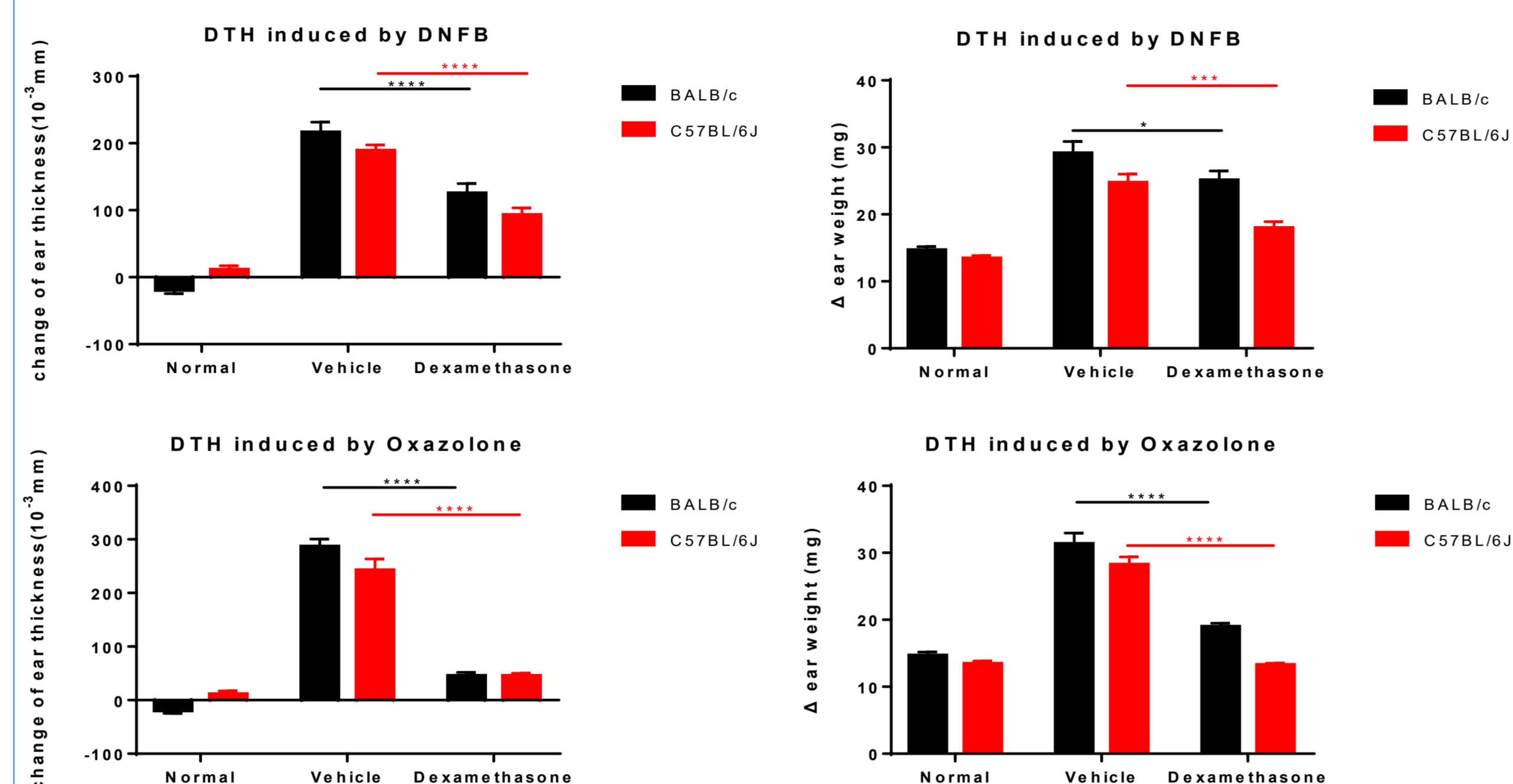


Figure 1: Ear thickness and ear weight in DNFB, oxazolone-induced DTH model in both BALB/c and C57BL/6 mice.

Cytokine

In Figure 2&3, oxazolone induced more potent Th1/Th2 type cytokines in the ear tissue of BALB/c mice and the inhibition of dexamethasone was in line with the in-life readouts. Cytokines in blood towards the same trend.

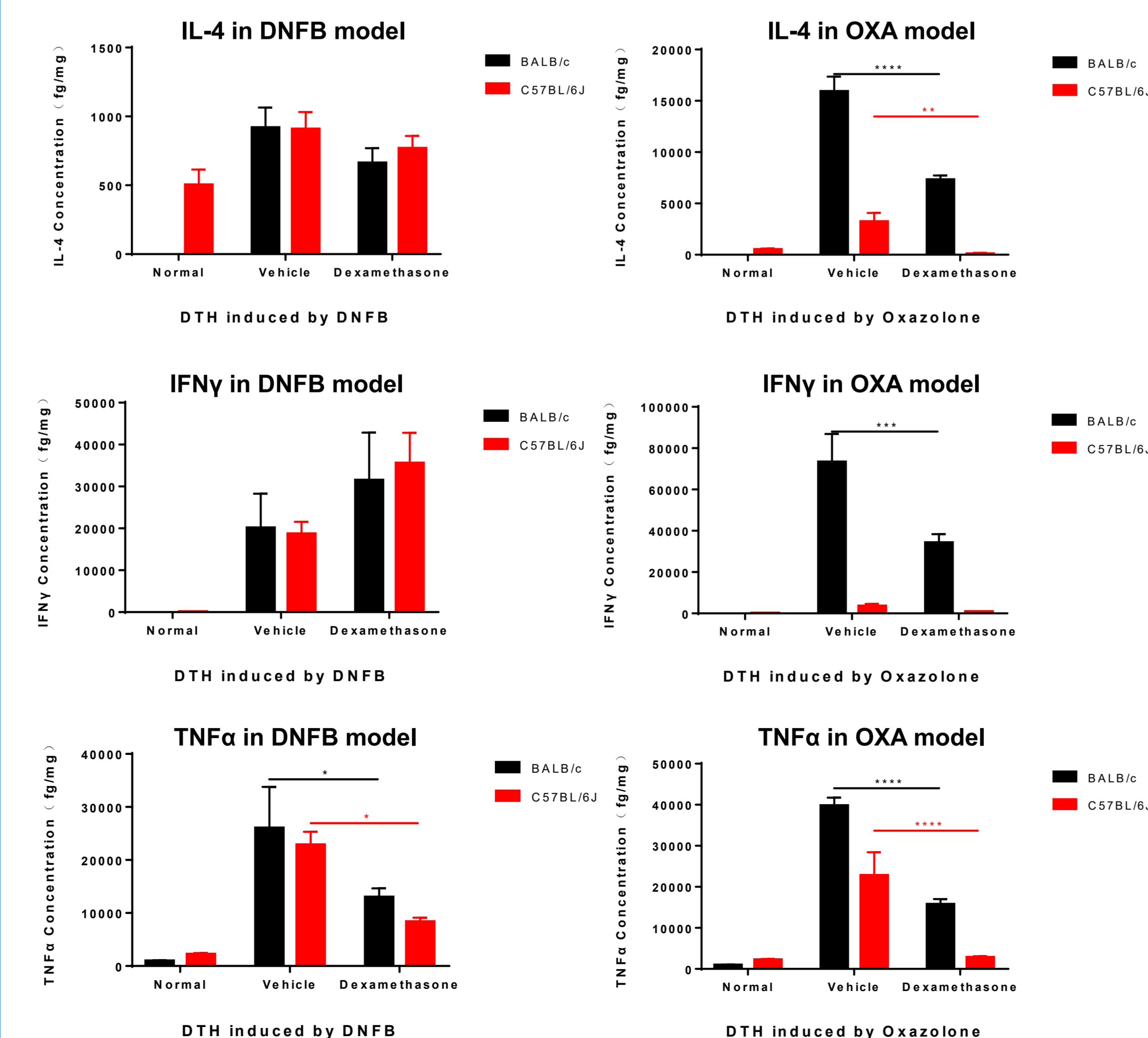


Figure 2: Cytokines level(IL-4, IFN γ , TNF α) in the ear tissue of DTH model.

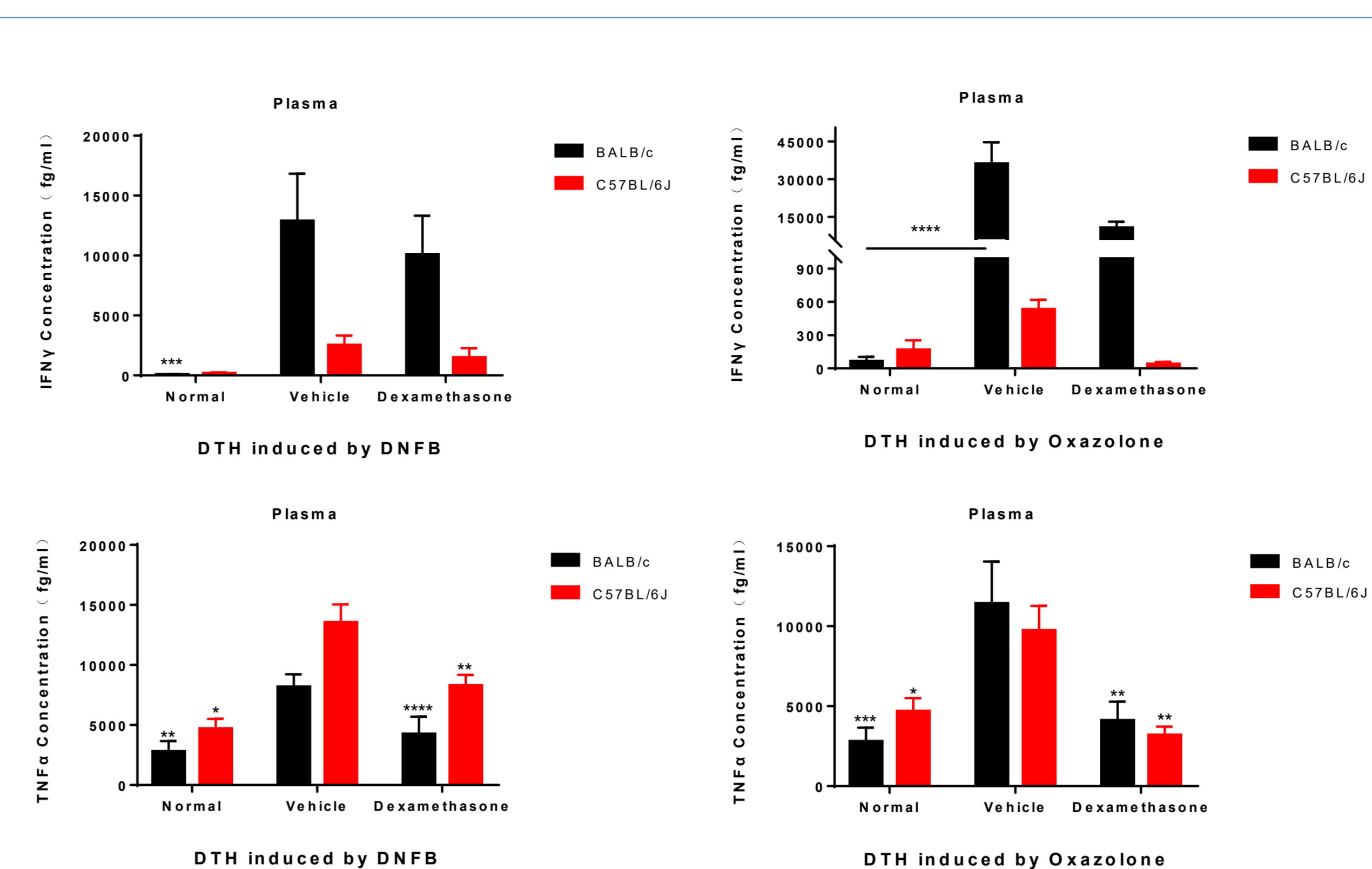


Figure 3: Cytokines level (IFN γ , TNF α) in the Plasma of DTH model.

H&E staining

Histopathological examination with H&E stain revealed a diffuse mononuclear infiltration in the dermis and necrosis of the intradermal connective tissue in the DNFB-induced/oxazolone-induced DTH model.

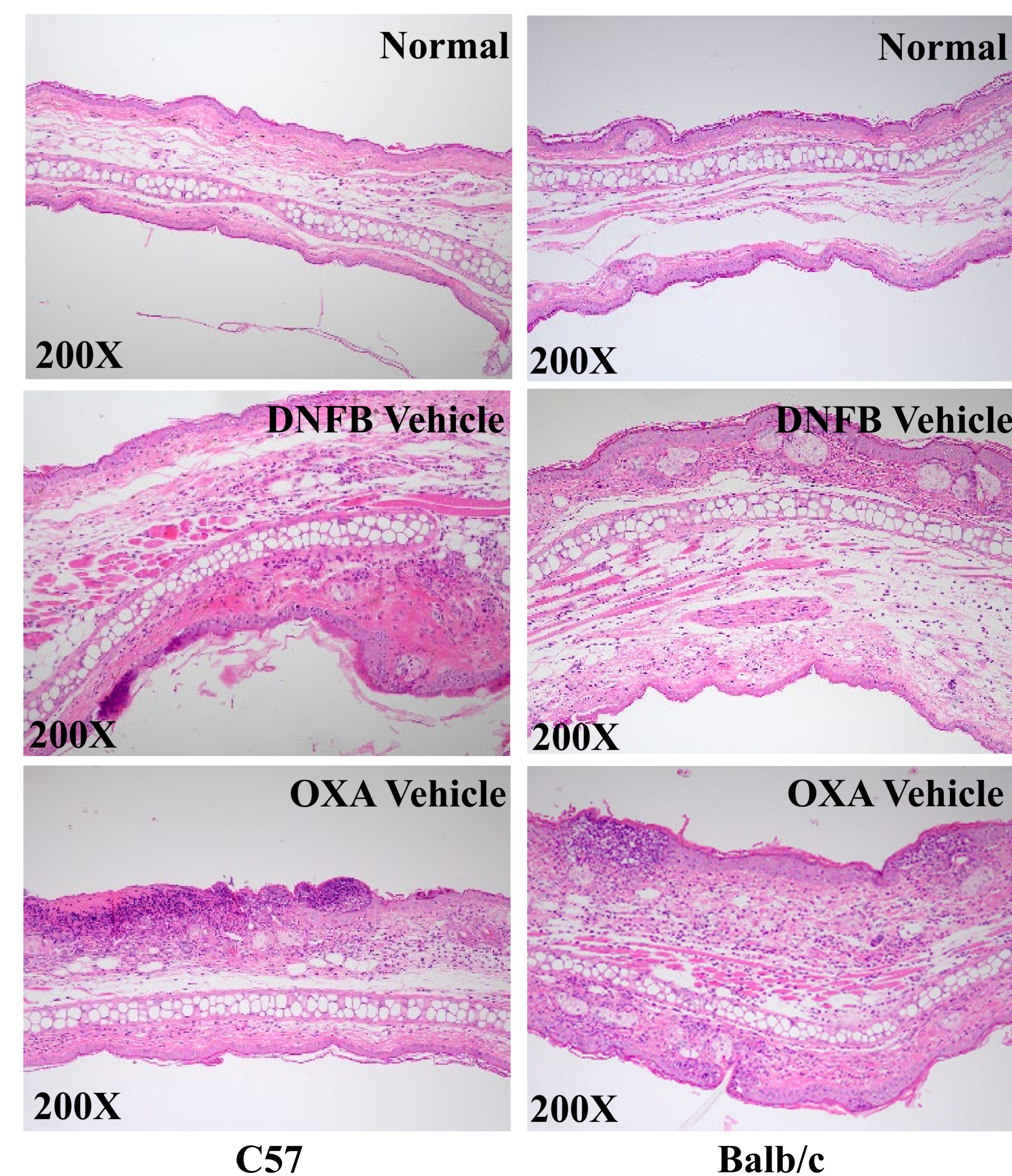


Figure 4: Representative pictures of H&E staining in the ears of DTH model.

Cell count in lymph node

In DTH model, the cell counts in lymph node were increased significantly.

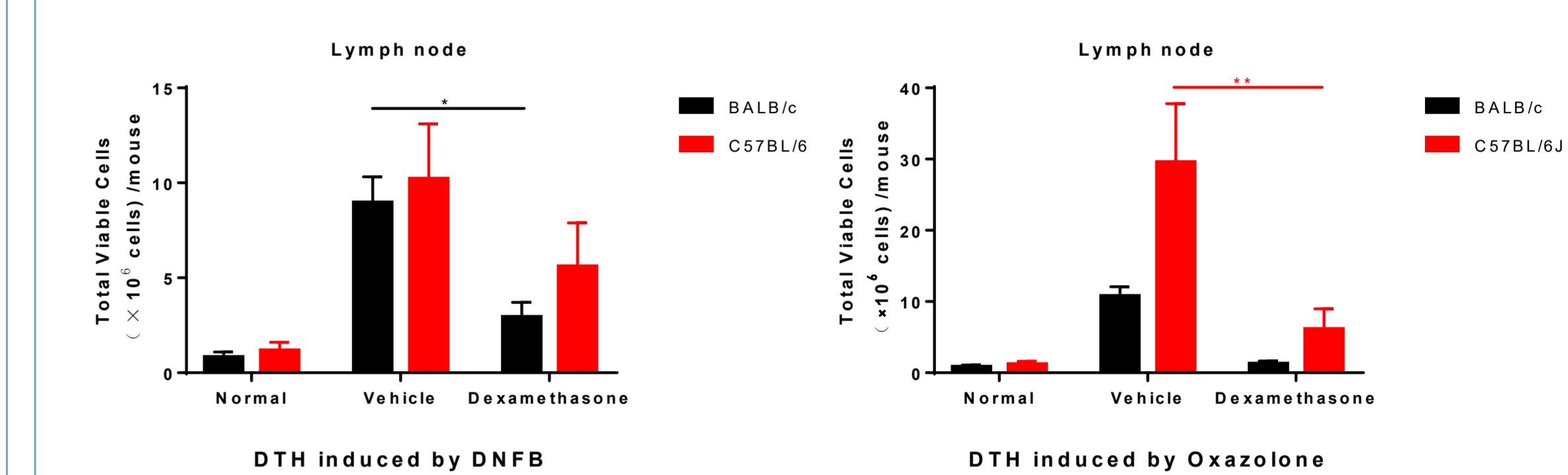


Figure 5: Cell count of lymph node in DTH model.

MPO level

In DTH model, the levels of MPO were increased in plasma and ear tissue.

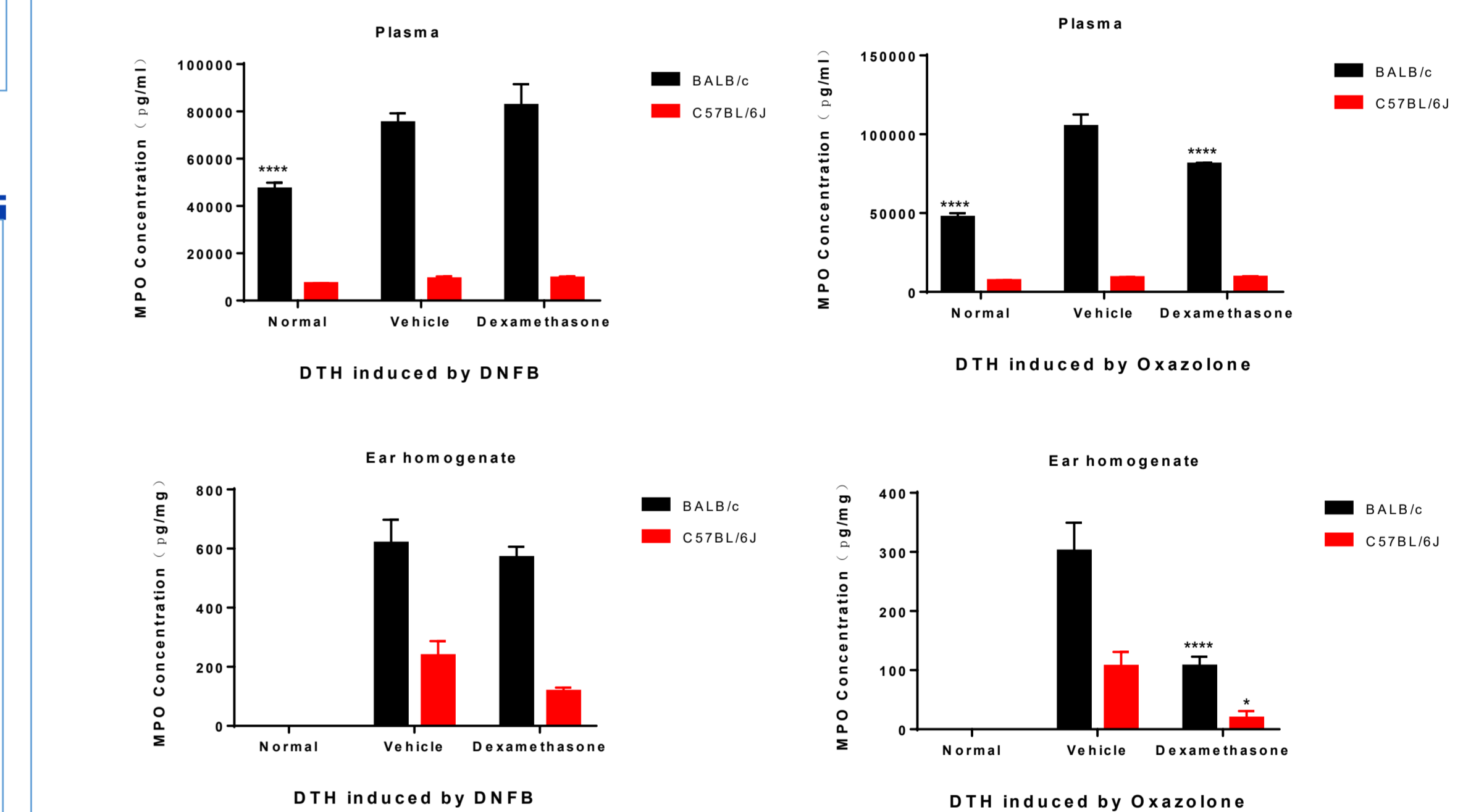


Figure 6: MPO level in plasma and ear tissue of DTH model.

Summary

Stimulating the left ear by DNFB/oxazolone increased the ear thickness and weight, leading to inflammatory cell infiltration, elevation of inflammatory cytokine and MPO in plasma and ear tissue.

Oxazolone induced more potent Th1/Th2 immune response in BALB/c mice and the inhibition of dexamethasone was more consistent with the in-life readouts.

Reference

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- [2] Bas, E., Recio, M. C., Giner, R. M., Máñez, S., López-Ginés, C., Gil-Benso, R., & Ríos, J. L. (2007). Demethylethylethylamine inhibits delayed-type hypersensitivity reactions, human lymphocyte proliferation and cytokine production. British journal of pharmacology, 152(8), 1272-1282.