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*Short communication*

# Distribution of CD4<sup>+</sup>CD8<sup>+</sup> double positive T cells in a mouse model of allergic asthma

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## Abstract

The present study describes the distribution of CD4<sup>+</sup>CD8<sup>+</sup> double-positive (DP) T cells in various immune compartments of mice with ovalbumin (OVA)-induced allergic asthma. It was found that the absolute number of DP T cells was considerably increased in the mediastinal lymph nodes and lungs of asthmatic mice as compared with that determined in the healthy subjects. On the contrary, the absolute counts of DP T cells was significantly decreased in the head and neck lymph nodes, and in peripheral blood of OVA-immunized mice. These results suggest that DP T cells may be involved in the pathogenesis of allergic asthma.

**Key words:** CD4<sup>+</sup>CD8<sup>+</sup> double-positive (DP) T cells, asthma, MLNs, HNLNs, lungs

## Introduction

Asthma is a chronic inflammatory lung disease characterized by infiltration of CD4 lymphocytes, eosinophils, neutrophils and by accompanying secretion of pro-inflammatory cytokines. During our current studies with the use a mouse model of ovalbumin (OVA)-induced asthma, we observed that (apart from changes in the number of CD4<sup>+</sup> single positive T cells, which are typical of allergic asthma) the disease caused alterations in the absolute counts of CD4<sup>+</sup>CD8<sup>+</sup> double-positive (DP) T cells in the lungs, mediastinal lymph nodes (MLNs), head and neck lymph nodes (HNLNs) and in peripheral blood. Extrathymic DP T cells have been described in several pathological conditions as well as in healthy individuals (Parel and Chizzolini 2004). Although it has been established that DP T cells can show both helper and

cytotoxic activities (Nam et al. 2000, Quandt et al. 2014), the roles performed by these cells are unclear, and it is unknown how they are distributed in the body. The aim of the present paper is to describe changes in the amount of DP T cells occurring in the course of OVA-induced allergic asthma in mice.

## Materials and Methods

All the procedures were approved by the Local Ethics Commission (35/2014/N). The experiments were carried out on 6-week-old Balb/c mice. Mice were divided into the control group (PBS-treated mice) and experimental group (OVA-immunized mice). The animals from the experimental group were sensitized on days 0 and 14 *via* i.p. injection with 20 µg of OVA emulsified in 2 mg aluminum hydroxide

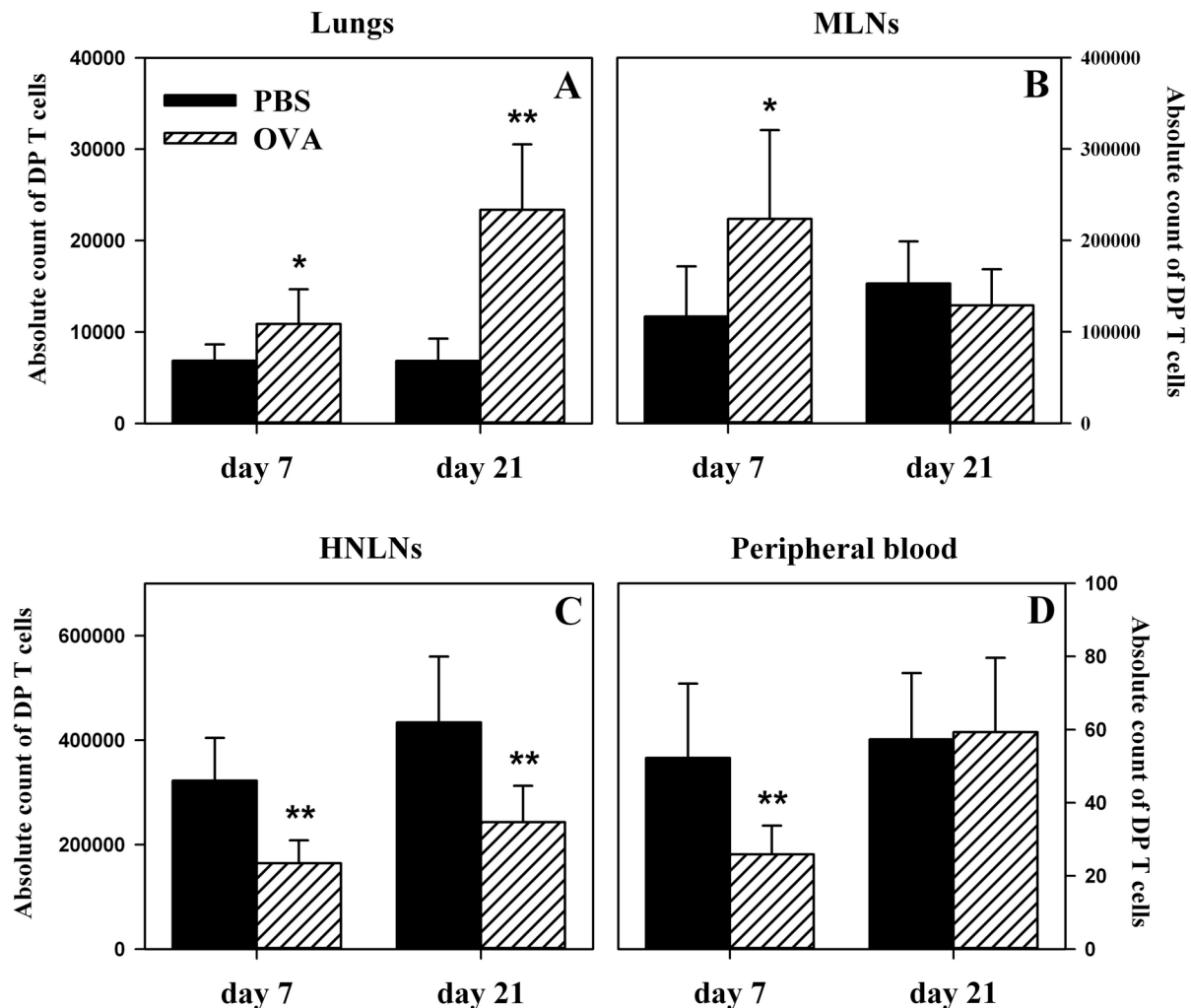


Fig. 1. Absolute counts of CD4<sup>+</sup>CD8<sup>+</sup> double-positive (DP) T cells in the lungs (A), mediastinal lymph nodes (MLNs) (B), head and neck lymph nodes (HNLNs) (C) and peripheral blood (D) of healthy mice (PBS) and mice with ovalbumin (OVA)-induced allergic asthma (OVA). Results are expressed as the mean ( $\pm$ SD) of two independent experiments with  $n=5$ /group/time point/experiment (overall  $n=10$ , unpaired Student's t-test, \* $p<0.01$ , \*\* $p<0.001$ ). Peripheral blood samples and whole MLNs and HNLNs were collected from individual mice, whereas each lung sample consisted of cells pooled from two animals.

(both from Sigma-Aldrich, Germany) and challenged intranasally with 100  $\mu$ g of OVA from day 21 to 42. The control mice were sensitized/challenged with PBS instead of OVA. The animals were euthanized (by CO<sub>2</sub> asphyxiation) on days 7 and 21 after the first challenge.

Peripheral blood samples (100  $\mu$ l) and whole MLNs and HNLNs were collected from individual mice. Because the cell yield from lung samples of a single mouse is relatively poor, whole lungs obtained from two animals were pooled into one sample. Hence, in each experiment the number of mice was doubled ( $n=20$ /group/time point): lungs were dissected from all the animals, whereas MLNs, HNLNs and peripheral blood were collected from ten mice per group per time point. MLNs and HNLNs were removed and subjected to homogenization with Dounce

tissue grinder. Lung lymphocytes were isolated by enzymatic digest (collagenase type IV, 50 U/ml; Sigma-Aldrich) and enriched by density gradient centrifugation (Percoll, Sigma-Aldrich). The resulting cell suspensions were filtered, washed with FACS buffer [FB; Dulbecco's PBS with 2% (v/v) heat-inactivated FBS (Sigma-Aldrich)], re-suspended in FB and counted. For flow cytometry analysis, 100  $\mu$ l of peripheral blood was subjected to red cell lysis and subsequently washed twice with FB. Cells were stained for surface antigens with fluorochrome conjugated monoclonal antibodies: FITC rat anti-mouse CD4 (clone H129.19) and APC-Cy7 rat anti-mouse CD8a (clone 53-6.7) (both from BD Biosciences, USA). Flow cytometry analysis was performed using a FACSCanto II cytometer (BD Biosciences). Routine complete blood count (CBC) was performed using

a hematology analyzer Advia 2120i (Siemens Healthcare Diagnostics, Germany). The absolute counts of DP T cells (i.e. number of DP T cells per  $\mu$ l of blood or per tissue sample) were calculated using the dual platform method, i.e. the absolute cell count was determined by calculating the data obtained from CBC (peripheral blood) or cell counting chamber (the total yield from the lungs, MLNs and HNLNs) by the percentage of CD4<sup>+</sup>CD8<sup>+</sup> double-positive T cells (the data from flow cytometric immunophenotyping).

## Results and Discussion

The research demonstrated that immunization with OVA induced a significant increase in the absolute number of DP T cells in the lungs and MLNs (Fig. 1A and B). An increase in the number of lymphocytes in the lymph nodes is interpreted as a result of their proliferation in response to the antigen, therefore the increased amount of DP T cells in MLNs could be due to their clonal expansion in response to OVA peptide. In turn, an increase in the absolute counts of DP T cells in the lungs of asthmatic mice indicates that these cells infiltrated the lungs in response to OVA challenge.

Following antigen presentation by dendritic cells to recirculating naïve T cells in the MLNs, specific CD4<sup>+</sup> T cells are activated and differentiated into T helper 2 type cells, which migrate to the lungs and orchestrate pulmonary immune responses (Lambrecht and Hammad 2003). Taking into consideration the above and the fact that DP T cells show helper activities (Nam et al. 2000, Quandt et al. 2014), it

could be hypothesized that infiltration of the lungs with these cells is a result of their recruitment from the MLNs to sites of allergen-induced inflammation. This hypothesis is supported by recently published results of Quandt et al. (2014) who demonstrated that DP T cells were recruited into synovial tissues of rheumatoid arthritis patients and that these cells showed features of T helper cells. But it cannot be excluded that the lung infiltration with DP T cells could be additionally caused by redistribution of these cells from the HNLNs and peripheral blood. Such a possibility is indicated by the significant depletion of DP T cells detected in both of these compartments (Fig. 1C and D). The results suggest that DP T cells may be involved in the development of allergic asthma, but the elucidation of their role in the pathogenesis of this disease requires further investigations.

## References

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