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Modulation of allergic airway inflammation by the histamine H_4 receptor: analysis by flow cytometry

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Introduction
The incidence of allergic airway diseases, such as asthma, is increasing throughout the world¹. Although histamine (HA) is found at high concentrations in asthmatic lungs², a role for HA in bronchial asthma is still a neglected topic in clinical research. In particular, the capacity of HA to modulate the underlying immune reaction, i.e., allergic sensitization, is far from being understood. The most recently identified histamine H₄ receptor (H₄R) is involved in acute inflammation and Th2 cytokine production³,⁴. Consequently, we intended to analyze the role of H₄R in a murine model of experimental asthma⁵ on the cellular level and specifically examined the ability of H₄R expressed on dendritic cells (DCs) to modulate T cell function.

Materials and methods
DCs and CD4+ T cell isolation
Spleens from wild-type or H₄R⁻/⁻ BALB/c mice were minced into small fragments using the gentleMACS™ Dissociator (Miltenyi Biotec) and digested with collagenase and DNase I. DCs were enriched according to their density by centrifugation with Nycodenz® (Axis- Shield, Heidelberg, Germany) and thereafter purified by using CD11c MicroBeads in combination with the autoMACS Separator (both Miltenyi Biotec)⁶. The population of CD11c⁺ cells among splenocytes amounted to about 15–20% and could be enriched to about 87–91% (fig. 1A).

In order to isolate CD4⁺ T lymphocytes, single-cell suspensions from lymph nodes of ovalbumine (OVA)-transgenic DO11.10 mice were processed with the CD4⁺ T Cell Isolation Kit II (Miltenyi Biotec) and enriched using the autoMACS Separator. In the single-cell preparations, we observed about 12–15% CD4⁺ T cells, which were enriched to 92–95% by MACS³ Technology (fig. 1B).

Polarization, transfer of T lymphocytes, and allergen challenge in mice
OVA-specific polarized T cells were generated by coculture of purified DCs and DO11.10 CD4⁺ T cells in medium containing OVA-peptide, IL-4, IL-2, and anti-IFN-γ. Five days later, 4×10⁶ polarized cells were adoptively transferred into BALB/c recipients. On the following three consecutive days, 30 µg OVA (dissolved in 30 µL PBS) were applied intranasally. Twenty-four hours after the last application, mice were dissected and asthma-specific parameters were analyzed.

Cell differentiation of the BAL fluid
Total cell counts in bronchoalveolar lavage (BAL) fluid were determined using a Neubauer chamber. Leukocyte subsets (eosinophils, neutrophils, macrophages, and lymphocytes) were analyzed in BAL fluid using the MACSQuant³ Analyzer (Miltenyi Biotec) after staining with Siglec-F, F4/80, CD11c, CD45, and Ly-6G antibodies.

![Flow cytometric analysis of DCs and T cells.](image)
Results and discussion

In order to analyze the effect of the H₄R on DC function in the polarization of spleen T cells towards allergen-specific Th2 cells specifically, we employed in vitro T cell polarization followed by adoptive transfer. OVA-specific CD4⁺ T cells were stimulated in vitro in a Th2-favoring medium with OVA peptide–pulsed DCs, obtained either from wild-type or H₄R⁻/⁻ mice. Analysis of the polarized T cells after restimulation revealed a marked decrease of IL-4 production in T cells polarized in the presence of H₄R⁻/⁻ DCs compared to those polarized in the presence of wild-type DCs (not shown). Thus, on DCs, the H₄R is essential for proper stimulation of spleen T cells and for directing their polarization towards a Th2 phenotype, a phenomenon already observed⁷,⁴.

Through the transfer of in vitro polarized T cells into recipient mice and subsequent provocation, an asthma-like disease can be induced. A parameter indicating allergic inflammation is the enhanced influx of cellular infiltrates into bronchoalveolar spaces, mostly driven by eosinophils, which are virtually absent in non-asthmatics. The number of eosinophils and other leukocytes in BAL fluids can be determined in an objective manner by flow cytometry (fig. 3 and ref. 8). As expected, BAL fluids from wild-type mice, which were provoked without having previously received T cells, contain only few cells. Cell numbers increase dramatically in mice, which have received the in vitro polarized T cells, regardless of whether T cells were polarized in the presence of wild-type or H₄R⁻/⁻ DCs. However, when analyzing the number of eosinophils specifically, a dramatic difference due to the polarizing conditions of T cells occurs. In BAL fluids of mice that received T cells polarized in the presence of wild-type DCs, about 45–65% eosinophils were detected. In contrast, the transfer of T cells polarized in the presence of H₄R⁻/⁻ DCs yielded only about 10–20% eosinophils in BAL fluids. Thus, the H₄R on DCs not only affects in vitro polarization of T cells, but also the in vivo function of the obtained polarized T cells.

Conclusions

We demonstrated that the H₄R on DCs plays an important role for T cell polarization and consequently affects the allergic reaction during sensitization. Since the lack of the H₄R on DCs reduced their ability to stimulate proper Th2 polarization of CD4⁺ T cells, we conclude that HA via the H₄R significantly affects the manifestation of asthmatic inflammation, suggesting therapeutic H₄R antagonism as a potential target for novel therapies.

References


MACS Product | Order no.
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MACSQuant Analyzer | 130-092-197
gentleMACS Starting Kit | 130-093-235
autoMACS Pro Starting Kit | 130-092-545
CD4⁺ T Cell Isolation Kit II, mouse | 130-095-248
CD11c MicroBeads, mouse | 130-052-001