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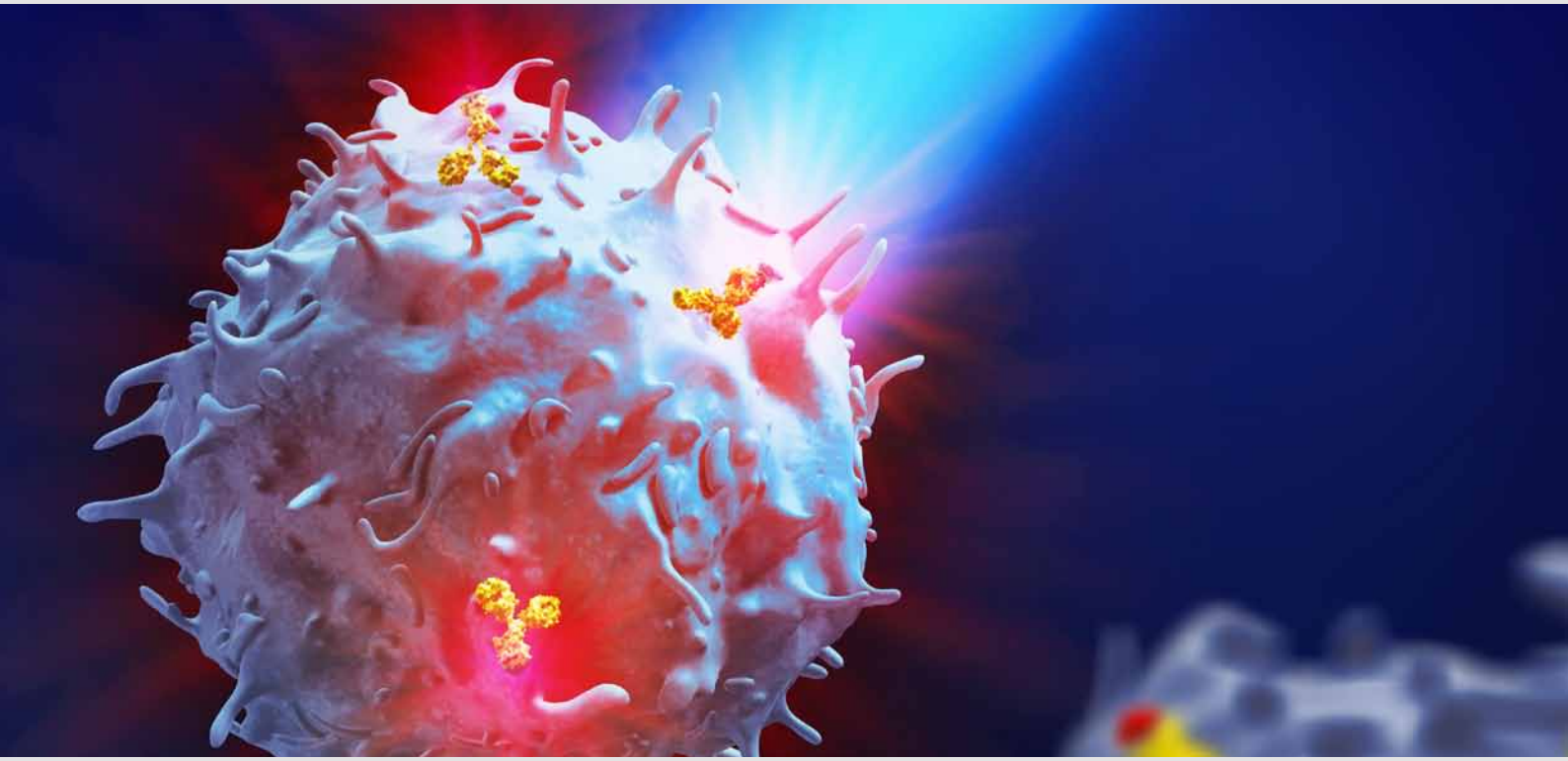
Christina Hartwig, Roland Seifert, and Detlef Neumann

Pharmacology and Toxicology, Hannover Medical School, Hannover, Germany



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 world[1]. Although histamine (HA) is found at high concentrations in asthmatic lungs[2], 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 H_4 receptor (H_4R) is involved in acute inflammation and TH2 cytokine production[3,4]. Consequently, we intended to analyze the role of H_4R in a murine model of experimental asthma[5] on the cellular level and specifically examined the ability of H_4R expressed on dendritic cells (DCs) to modulate T cell function.

Materials and methods

DCs and CD4$^+$ T cell isolation

Spleens from wild-type or $H_4R^{-/-}$ 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 MACS® 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, 4×10^6 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 MACSQuant® Analyzer (Miltenyi Biotec) after staining with Siglec-F, F4/80, CD11c, CD45, and Ly-6G antibodies.

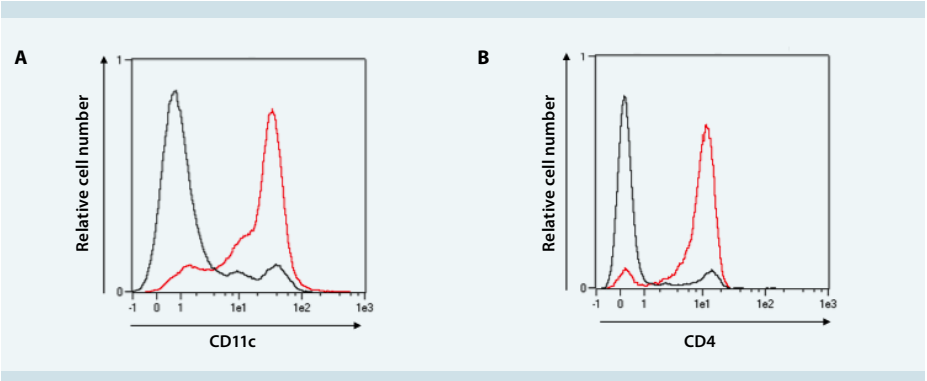

Figure 1 **Flow cytometric analysis of DCs and T cells.** A, Splenocytes were analyzed for CD11c expression before (black) and after (red) purification of DCs. B, Lymph node cells were analyzed for CD4 expression before (black) and after (red) purification of CD4$^+$ T cells.

Results and discussion

In order to analyze the effect of the H_4R 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 $H_4R^{-/-}$ 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 $H_4R^{-/-}$ DCs compared to those polarized in the presence of wild-type DCs (not shown). Thus, on DCs, the H_4R is essential for proper stimulation of spleen T cells and for directing their polarization towards a TH2 phenotype, a phenomenon already observed[7,4].

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 $H_4R^{-/-}$ 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 $H_4R^{-/-}$ DCs yielded only about 10–20% eosinophils in BAL fluids. Thus, the H_4R on DCs not only affects *in vitro* polarization of T cells, but also the *in vivo* function of the obtained polarized T cells.

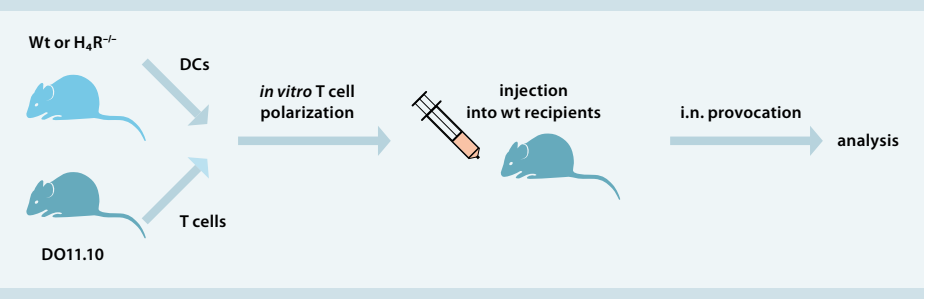

Figure 2 **Experimental asthma in mice, induced by adoptive T cell transfer.**

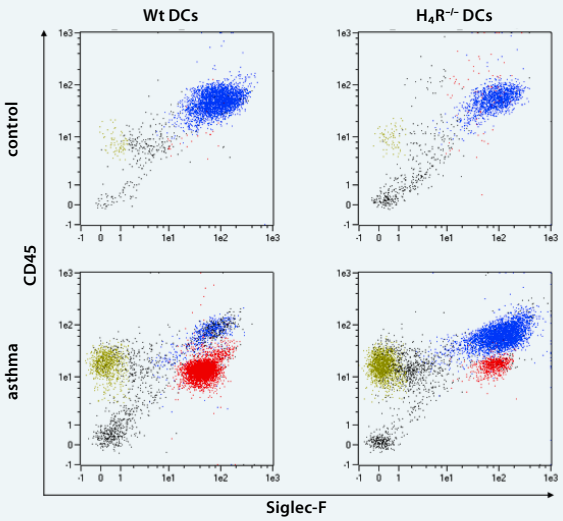

Figure 3 **Characterization of cells in BAL fluid.** Eosinophil (red), macrophage (blue), and lymphocyte (green) counts of BAL fluids obtained from control mice (w/o application of T cells) or asthma mice (application of polarized T cells) after provocation. Applied T cells were polarized in the presence of either wild-type (wt) or $H_4R^{-/-}$ DCs, as indicated above.

Conclusions

We demonstrated that the H_4R on DCs plays an important role for T cell polarization and consequently affects the allergic reaction during sensitization. Since the lack of the H_4R on DCs reduced their ability to stimulate proper TH2 polarization of $CD4^+$ T cells, we conclude that HA via the H_4R significantly affects the manifestation of asthmatic inflammation, suggesting therapeutic H_4R antagonism as a potential target for novel therapies.

References

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