

Role of regulatory T cells in the antigen specific induction of tolerance in murine asthma

Caroline Boudousquie, Céline Pellaton, Nathalie Barbier and François Spertini, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland.

Introduction

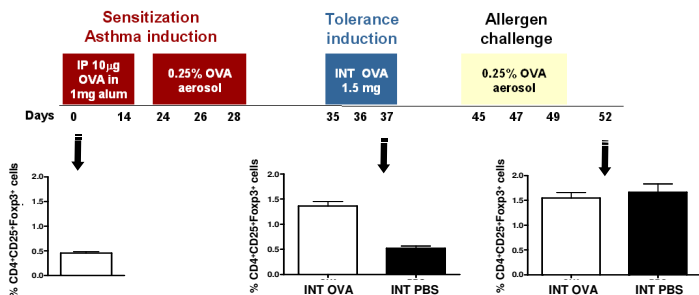
Allergic asthma is a complex inflammatory syndrome. Its severity correlates with the presence of activated T lymphocytes and eosinophils in the bronchoalveolar lavage fluid (BALF).

Induction of tolerance via the nasal route results in reduced recruitment of eosinophils into bronchial fluid (BALF) upon challenge, inhibition of T_H2 pro-inflammatory cytokine secretion and T cell hyporesponsiveness.

Regulatory T cells (Tregs) are key players in controlling the development of asthmatic inflammation.

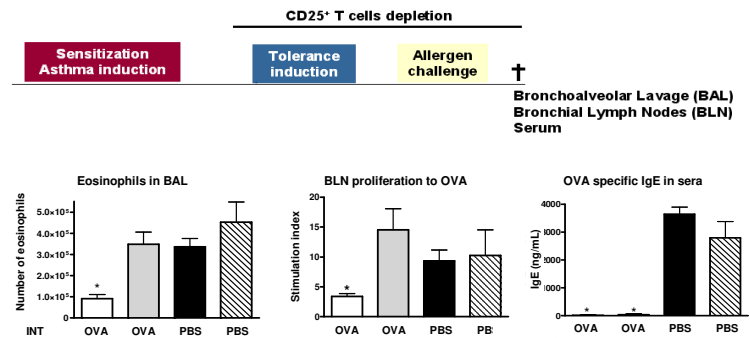
Here we showed that, in a mouse model of asthma, $CD4^+CD25^+Foxp3^+$ natural regulatory T cells were generated early after induction of tolerance. We also demonstrated that $CD25^+$ T cells depletion severely hampered tolerance induction. Transfer of $CD4^+CD25^+$ T cells in asthmatic mice was sufficient to induce tolerance whereas transfer of $CD4^+CD25^-$ T cells was not able to do so. However when $CD4^+CD25^-$ were purified from donor mice depleted of $CD25^+$ T cells, they were no longer able to transfer tolerance. Taken together, our data suggest that both $CD4^+CD25^+$ and $CD4^+CD25^-$ T cells are implicated in tolerance induction.

I- Tolerance induction leads to $CD4^+CD25^+Foxp3^+$ natural regulatory T cells generation in lungs



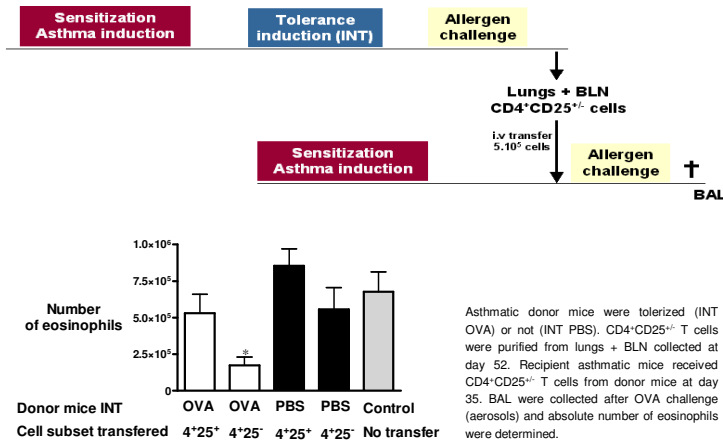
BALB/c mice were sensitized to OVA by two intraperitoneal (i.p.) injection of 10mg alum-adsorbed OVA on days 0 and 14. One week later, mice were exposed for 20 min to OVA 0.25% aerosol every other day for 3 days. At day 35 mice were intranasally treated (INT) with 1.5mg OVA every day for 3 days. One week later, mice were challenged with OVA 0.25% aerosol. Lungs were collected at day 0, 37 and 52. Lung tissue cells were stained with CD4, CD25 and intracellular Foxp3. They were then analysed by flow cytometry.

II- $CD25^+$ T cells depletion severely hampers tolerance induction



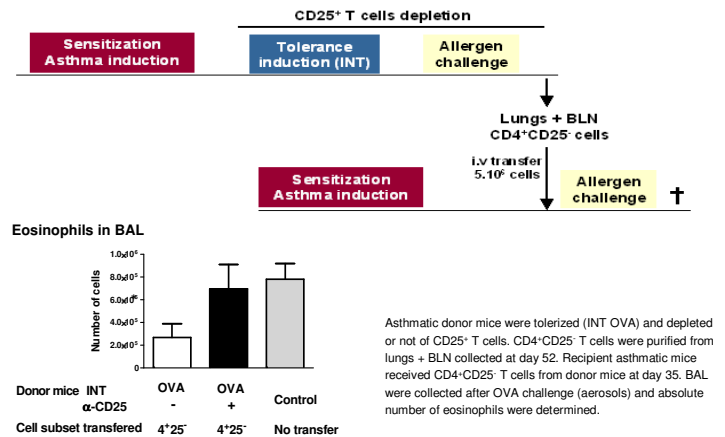
Asthmatic BALB/c mice were treated with anti-CD25 (PC61) (three i.p. injection of 1mg at day 31, 38 and 45). Mice were challenged with OVA 0.25% aerosol and sacrificed at day 52. Number of eosinophils in BAL, proliferation of BLN cells to OVA and IgE levels in sera were analysed.

III- Transfer of $CD4^+CD25^+$ T cells attenuates eosinophilic inflammation



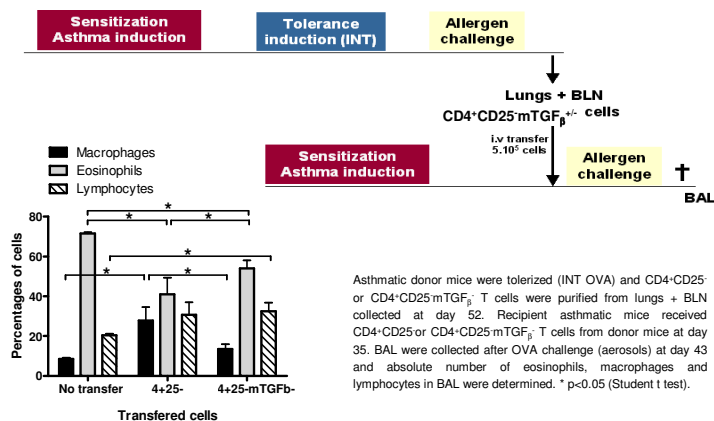
Asthmatic donor mice were tolerized (INT OVA) or not (INT PBS). $CD4^+CD25^{+/+}$ T cells were purified from lungs + BLN collected at day 52. Recipient asthmatic mice received $CD4^+CD25^{+/+}$ T cells from donor mice at day 35. BAL were collected after OVA challenge (aerosols) and absolute number of eosinophils were determined.

IV- Both $CD4^+CD25^-$ and $CD4^+CD25^+$ T cells are implicated in tolerance induction



Asthmatic donor mice were tolerized (INT OVA) and depleted or not of $CD25^+$ T cells. $CD4^+CD25^-$ T cells were purified from lungs + BLN collected at day 52. Recipient asthmatic mice received $CD4^+CD25^-$ T cells from donor mice at day 35. BAL were collected after OVA challenge (aerosols) and absolute number of eosinophils were determined.

V- $CD4^+CD25^+mTGF\beta_1$ T cells play a role in tolerance induction



Asthmatic donor mice were tolerized (INT OVA) and $CD4^+CD25^+$ or $CD4^+CD25^+mTGF\beta_1$ T cells were purified from lungs + BLN collected at day 52. Recipient asthmatic mice received $CD4^+CD25^+$ or $CD4^+CD25^+mTGF\beta_1$ T cells from donor mice at day 35. BAL were collected after OVA challenge (aerosols) at day 43 and absolute number of eosinophils, macrophages and lymphocytes in BAL were determined. * p<0.05 (Student t test).

Conclusion

In our murine model of asthma intranasal treatment led to the generation of natural Tregs ($CD4^+CD25^+Foxp3^+$ T cells) that were crucial for tolerance induction. However cell transfer experiments revealed that only $CD4^+CD25^+$ T cells were able to tolerize recipient asthmatic mice. These $CD4^+CD25^+$ T cells were however not suppressive if purified from a donor mice depleted of $CD25^+$ T cells.

In conclusion, both $CD4^+CD25^+$ T cells and $CD4^+CD25^-$ T cells appear to be essential in tolerance induction. The relationship both subsets will have to be investigated.