

Allergic asthma is a major complication of atopy. Its severity correlates with the presence of activated T lymphocytes and eosinophils in the bronchoalveolar lavage fluid (BALF). Mechanisms that protect against asthma are poorly understood.

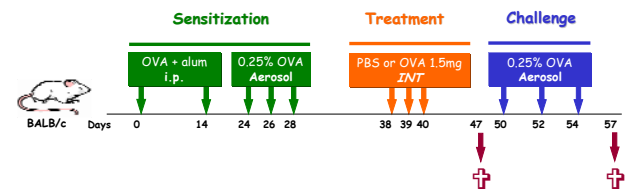
Based on oral models of mucosal tolerance induction, models using the nasal route showed that uptake of important amount of antigen can induce tolerance and reverse the allergic phenotype.

Here we showed that intranasal treatment (INT) with ovalbumin (OVA) protects sensitized mice against an OVA aerosol challenge by inhibiting inflammatory cell recruitment for an extended period of time.

OVA INT was also able to markedly downregulate Th2 cytokine production, including IL-10, upon allergen challenge.

Respective role of inhibitory cytokines IL-10 and TGFβ has been investigated.

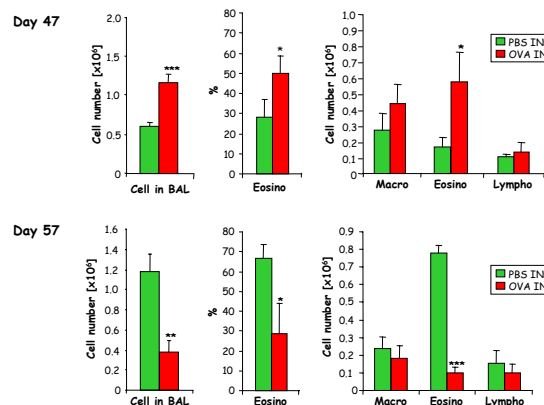
Protocol: Induction of a nasal tolerance



BALB/c mice were sensitized to OVA by two intraperitoneal (i.p.) injection of 10µg alum-adsorbed OVA on days 0 and 14. Ten days later, mice were exposed for 20 min to 0.25% OVA aerosol every other day for 3 days. At day 38, mice were intranasally treated (INT) with 1.5 mg OVA every day for 3 days. Ten days later, mice were challenged with 0.25% OVA aerosol.

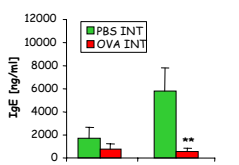
Tolerance induction in a mouse model of asthma

Cell recruitment in BALF



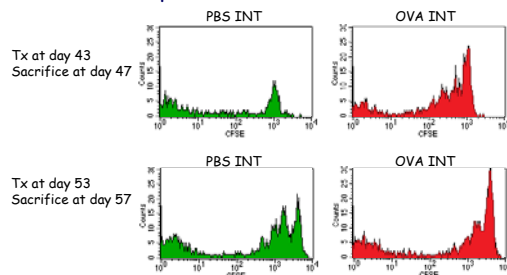
BALF were harvested 7 days after INT (day 47, upper panels) and 3 days after OVA challenge (day 57, lower panels). Total cell count and cellular repartition were performed on cytospins.

OVA-specific IgE



OVA-specific IgE measured in serum by ELISA at indicated time points.

Transfer of OVA-specific T cells



Splenocytes of OVA₁₁₃₋₃₃₅-specific DO11.10 TCR transgenic mice were transferred (Tx) to sensitized BALB/c mice either after INT (upper panels) or at the time of the challenge (lower panels).

Asthmatic mice intranasally treated with OVA or PBS as a control are subsequently challenged with OVA aerosol. INT OVA very efficiently inhibits immune reaction occurring upon aerosol re-exposure:

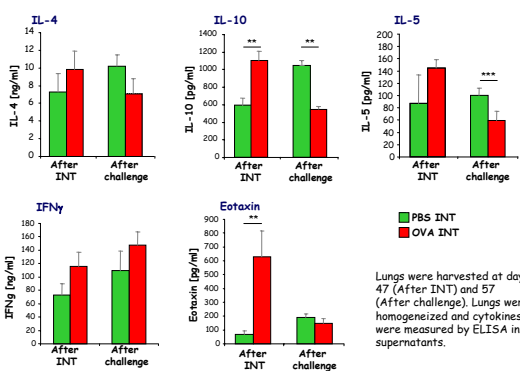
- ↓ Inflammatory cell recruitment into the BALF
- ↓ IgE production
- ↓ Cell division in draining lymph nodes

After OVA INT, a transient activation phase occurred, probably necessary for tolerance induction.

The OVA INT is efficient for an extended period of time and resists to a second set of OVA aerosol re-exposure (data not shown).

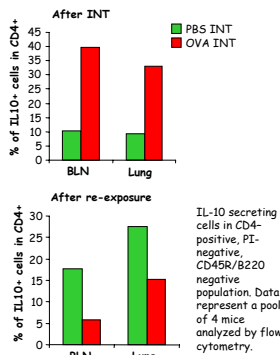
Cytokines production and role of IL-10

OVA INT prevents cytokines production in lungs upon allergen challenge



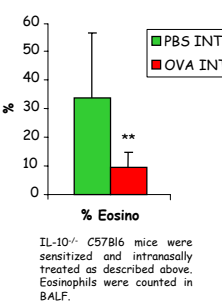
Lungs were harvested at day 47 (After INT) and 57 (After challenge). Lungs were homogenized and cytokines were measured by ELISA in supernatants.

IL-10 production by CD4⁺ cells



IL-10 secreting cells in CD4⁺ positive, PI-negative, CD45R/B220 negative population. Data represent a pool of 4 mice analyzed by flow cytometry.

Tolerance induction in IL-10^{-/-} mice



IL-10^{-/-} C57Bl6 mice were sensitized and intranasally treated as described above. Eosinophils were counted in BALF.

Upon challenge, INT OVA drastically reduced T_H2 cytokines, including IL-10, production by lungs.

Seven days after INT, expression of all measured cytokines was exacerbated.

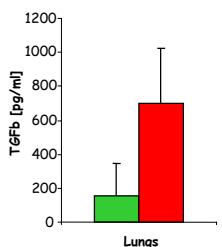
The same pattern was found when measuring IL-10 production by CD4⁺ T cells in bronchial lymph nodes (BLN) and lungs.

Transgenic mice deficient in IL-10 were also protected by INT OVA.

→ Taken together, these results suggest that IL-10 is not necessary for tolerance induction in OVA-sensitized mice.

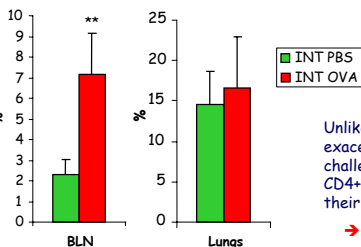
Role of TGFβ

TGFβ in lungs supernatants



Lungs were harvested at day 57 (After challenge) and homogenized. TGFβ was measured by ELISA in supernatants.

Membrane-bound TGFβ in CD4⁺CD25⁺



Bronchial lymph nodes (BLN) and lungs were harvested at day 57 (After challenge). Single-cell suspensions were analysed by flow cytometry after cell surface labelling with antibodies against CD4, CD25 and TGFβ.

Unlike other cytokines, TGFβ is exacerbated in lungs after challenge. In draining lymph nodes, CD4⁺CD25⁺ cells bearing TGFβ on their surface are upregulated.

→ Taken together, these results suggest that TGFβ play a role in tolerance induction.

Conclusion

INT OVA, in a therapeutic model, is able to inhibit several inflammatory markers of an asthma model of allergy to OVA. Inflammatory cell recruitment, particularly eosinophils, OVA-specific IgE induction upon challenge and Th2 cytokines recruitment in the bronchial lymph nodes and lungs are inhibited by the treatment.

The protection against inflammatory effects of an OVA challenge is efficient for an extended period of time.

Cellular recruitment, IgE production and increase of T_H2 cytokines level measured one week after INT indicate that a transient inflammatory phase occurs upon treatment.

The cytokine IL-10 has been described as an important regulator of allergy. We showed here that its production is downregulated in lungs of OVA-treated mice after challenge with OVA. CD4⁺ cells also produce less IL-10 after challenge. It was possible to induce tolerance in a mouse lacking IL-10 indicating that IL-10 is probably not implicated in the induction of the immunomodulation observed.

On the other hand, TGFβ and CD4⁺CD25⁺ cells bearing TGFβ on their surface are upregulated upon challenge in OVA-treated mice indicating a role for TGFβ in tolerance induction.