IL-15 IMPAIRS REGULATORY RESPONSES TO DIETARY PROTEINS

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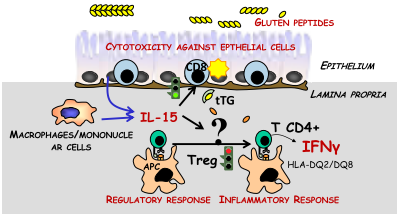
INTRODUCTION & OBJECTIVES

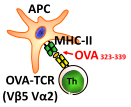
Previous studies have pointed to a possible contribution of IL-15, a cytokine massively up-regulated in coeliac disease (CD), in the loss of oral tolerance to gluten. *In vitro* studies in humans showed that IL-15 prevents effector T lymphocytes (L) and notably CD8+TL to respond to the immunosuppressive effect of Tregs via the activation of PI3-kinase in effector cells (Ben-Ahmed, 2009).



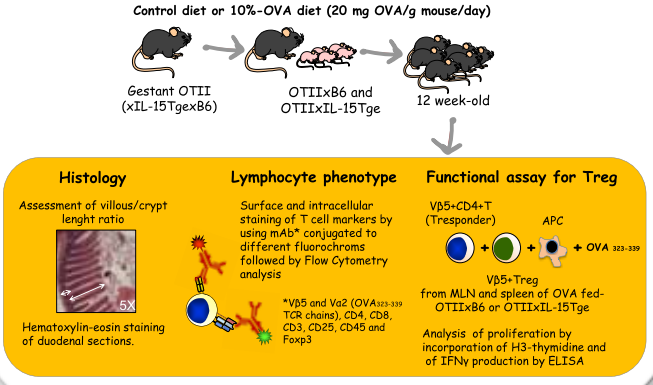
Herein, we have investigated whether and how IL-15 might jeopardize intestinal immune-regulation and promotes loss of tolerance to dietary antigens.

EXPERIMENTAL DESIGN & METHODS

OTII mice that possess CD4+ TL with a T cell receptor specific for ovalbumin peptide $OVA_{323-339}$ were crossed with transgenic mice over-expressing a secreted form of human IL-15 under an intestine-specific promoter (Ohta, 2002)

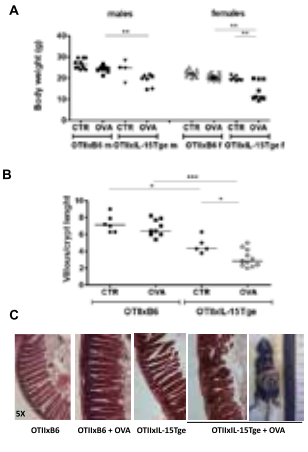


Homozygous OTII females were crossed with heterozygous IL-15TgexB6 males. Control or 10% OVA containing diets were introduced during the last week of gestation and pursued in OTIIxB6 and OTIIxIL-15Tge offspring after weaning. At 12 weeks, mice were sacrificed to sutdy duodenal histology, lymphocyte phenotype and Treg functions



RESULTS

CHRONIC ORAL EXPOSURE TO OVA HAS NO DELETERIOUS EFFECT IN OTIIXB6 MICE BUT EXACERBATES INTESTINAL LESIONS IN OTIIXIL-15TGE MICE



At 12 weeks, body weight and villous/crypt length ratio were comparable in OTIIxB6 mice on control or OVA diets (1A and 1B).

OTIIxIL-15Tge mice on control diet had a weight comparable to OTIIxB6 littermates, but a diminished duodenal villous/crypt length ratio (1B).

OVA-fed OTIIxIL-15Tge mice , notably females presented with growth retardation (1A), an enlarged duodenum and vsignificantly decreased illous/crypt ratiocompared to the same mice on control diet. Epithelial lesions remained patchy (Figure 1B).

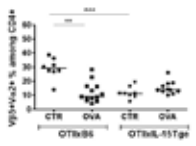
Figure 1. Control or 10% OVA containing diets were introduced during the last week of gestation and pursued in OTIIxB6 and OTIIxIL-15Tge offspring after weaning until 12 weeks.

A) Body weight of 12 week-old mice. Significant p values (ANOVA followed by Tukey's test) are indicated in the figure.

B) Villous/crypt length ratio in duodenum from 12 week-old mice. Each point corresponds to a median of 15-20 villous/crypt from a mouse. Medians are shown by horizontal lines and significant p values (Mann-Whitney U test) between groups are indicated in the figure (*p<0.05 and ***p<0.0001).

C) Representative hematein-eosin-stained sections of duodenum from 12 week-old females . A small OVA-fed OTIIxIL-15Tge mice with enlarged duodenum is shown

IL-15 PRESERVES OVA-SPECIFIC CD4+T CELLS IN LAMINA PROPRIA



Ancient observations in TCR-transgenic mice have shown that oral tolerance can be associated with deletion of T cells specific of the dietary antigen (Chen, 1995). Accordingly, proportions of OVA-CD4+ TL were markedly reduced in LP of OVA-fed OTIIxB6 mice. In contrast, they remained unchanged in LP from OVA-fed OTIIxIL-15Tge mice, suggesting that IL-15, known for its potent anti-apoptotic properties, may help to preserve the survival of activated OVA-specific CD4+ TL (Figure 2).

Figure 2. Percentage of Vβ5+Va2+ among CD4+TL (OVA-CD4T) in LP from OVA and control diet-fed OTIIxB6 and OTIIxIL-15Tge mice. Medians are shown by horizontal lines and significant p values (Mann-Whitney U test) between groups are indicated in the figure (*p<0.05, **p<0.01 and ***p<0.0001).

FUNCTIONAL OVA-SPECIFIC CD25+ FOXP3+ TREG CELLS EXPAND IN BOTH OTIIXB6 AND OTIIXIL-15TGE MICE.

One major mechanism for tolerance to dietary proteins is the induction of CD4+CD25+ FoxP3+ Treg that migrate into LP (Hadis, 2011). A strong expansion of OVA-Treg was observed in LP of both OVA-fed OTIIxB6 and IL-15TgexOTII mice, which was more particularly striking in OVA-fed IL-15TgexOTII mice (figure 3A).

The latter cells were functional Treg. Thus, Vβ5+CD25+TL from both OVA-fed OTIIxB6 and IL-15TgxB6 mice completely blocked proliferation and IFNγ secretion of Vβ5+CD25-T responders stimulated with $OVA_{323-339}$ (figure 3B)

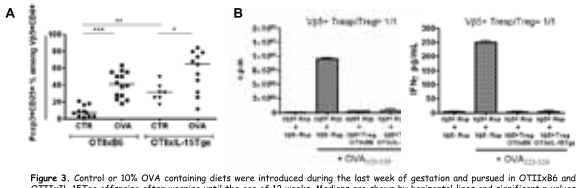


Figure 3. Control or 10% OVA containing diets were introduced during the last week of gestation and pursued in OTIIxB6 and OTIIxIL-15Tge offspring after weaning until the age of 12 weeks. Medians are shown by horizontal lines and significant p values (Mann-Whitney U test) between groups are indicated in the figure (*p<0.05, **p<0.01 and ***p<0.0001).

A) Percentage of Foxp3+CD25+ among Vβ5+CD4+TL.

B) Proliferation and IFNγ secretion of splenic Vβ5+CD4+CD25- stimulated with 0.5 µg/mL of $OVA_{323-339}$ for 3 days in the presence or absence of Vβ5+CD4+CD25+TL from OVA-fed OTIIxB6 and OTIIxIL-15Tge mice.

IFNγ-PRODUCING CD4+ AND CD8 T CELLS EXPAND IN OTIIXIL-15TGE MICE

In OTIIxIL-15Tge mice, a striking observation was a massive increase in proportions and/or absolute numbers of IFNγ+ CD8+ T cells in LP, MLN and spleen compared to OTIIxB6 littermates (Figure 4A and data not shown). In LP where most OVA-specific T cells had a T reg phenotype, the proportion of IFNγ+ OVA-CD4+ T cells did not change significantly except in two OTIIxIL15Tge mice which exhibited particularly severe intestinal lesions (Figure 4B).

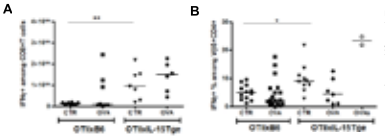


Figure 4. Control or 10% OVA containing diets were introduced during the last week of gestation and pursued in OTIIxB6 and OTIIxIL-15Tge offspring after weaning until the age of 12 week-old. OVAs group corresponds to OVA-fed OTIIxIL-15Tge mice with a villous/crypt lengthmedian of the latter group. Medians are shown by horizontal lines and significant p values (Mann-Whitney U test) between groups are indicated in the figure (*p<0.05, **p<0.01 and ***p<0.0001).

A) IFNγ-producing CD8+T cells in LP

B) IFNγ-producing cells among OVA-CD4+TL in LP

OVA DIET PROMOTES THE EXPANSION OF CD8+T EXPRESSING GRANZYME B AND NK RECEPTORS IN OTIIXIL-15TGE

In CD, IL-15 is thought to promote a cytolytic attack of epithelial cells by stimulating granzyme B/perforin dependent cytotoxity of IEL expressing NK receptors. In OTIIxIL-15Tge mice, OVA-feeding induced a significant increase of NKG2D+NK1.1+CD8+TL, a population particularly associated to the development of enteropathy in old IL-15Tge mice (Figure 5A). Strikingly, granzyme B synthesis in LP CD8+ T cells increased massively after OVA feeding (Figure 5B).

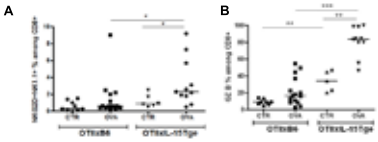


Figure 5. Control or 10% OVA containing diets were introduced during the last week of gestation and pursued in OTIIxB6 and OTIIxIL-15Tge offspring after weaning until the age of 12 week-old. OVAs group corresponds to OVA-fed OTIIxIL-15Tge mice with a villous/crypt lengthmedian of the latter group. Medians are shown by horizontal lines and significant p values (Mann-Whitney U test) between groups are indicated in the figure (*p<0.05, **p<0.01 and ***p<0.0001).

A) NKG2D+NK1.1+ cells among CD8+T L in LP

B) Granzyme B-producing cells among CD8+TL in LP

IL-15 RENDERS T CELLS AND NOTABLY CD8+ T CELLS LESS SENSITIVE TO IMMUNO-SUPPRESSION BY CD4+CD25+TREG

IL-15 partially reversed the inhibitory effect of Treg on the production of IFNγ by CD45+CD25- spleen cells stimulated with anti-CD3 and anti-CD28 antibodies (Figure 6A). Consistent with the preferential response of CD8+ TL to IL-15, reversion of the inhibitory effect of T regs by IL-15 was more efficient in CD8+ than in CD4+ T cells as indicated by the proliferative responses of Cell Trace labeled CD45+ CD25- spleen cells stimulated with anti-CD3 Ab (Figure 6B).

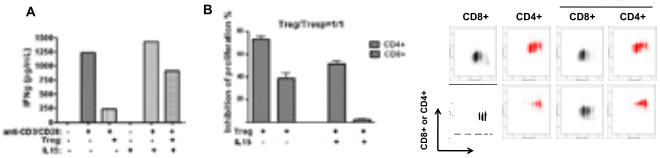


Figure 6. A) IFNγ secretion of splenic CD45+CD25- stimulated with 0.2 µg/mL of soluble anti-CD3 and anti-CD28 for 2 days in the presence or absence of CD4+CD25+TL (Treg) and 20 ng/mL of IL-15.

B) Inhibition of proliferation (%) of Cell Trace™ Violet-labeled CD4+ and CD8+ TL from spleen of B6 mice stimulated with 0.1 µg/mL of soluble anti-CD3 for 2 days in the presence or absence of CD4+CD25+TL (Treg) and 20 ng/mL of IL-15.