Journal Club

11.11.2011

Maria

Exogenous Stimuli Maintain Intraepithelial Lymphocytes via Aryl Hydrocarbon Receptor Activation

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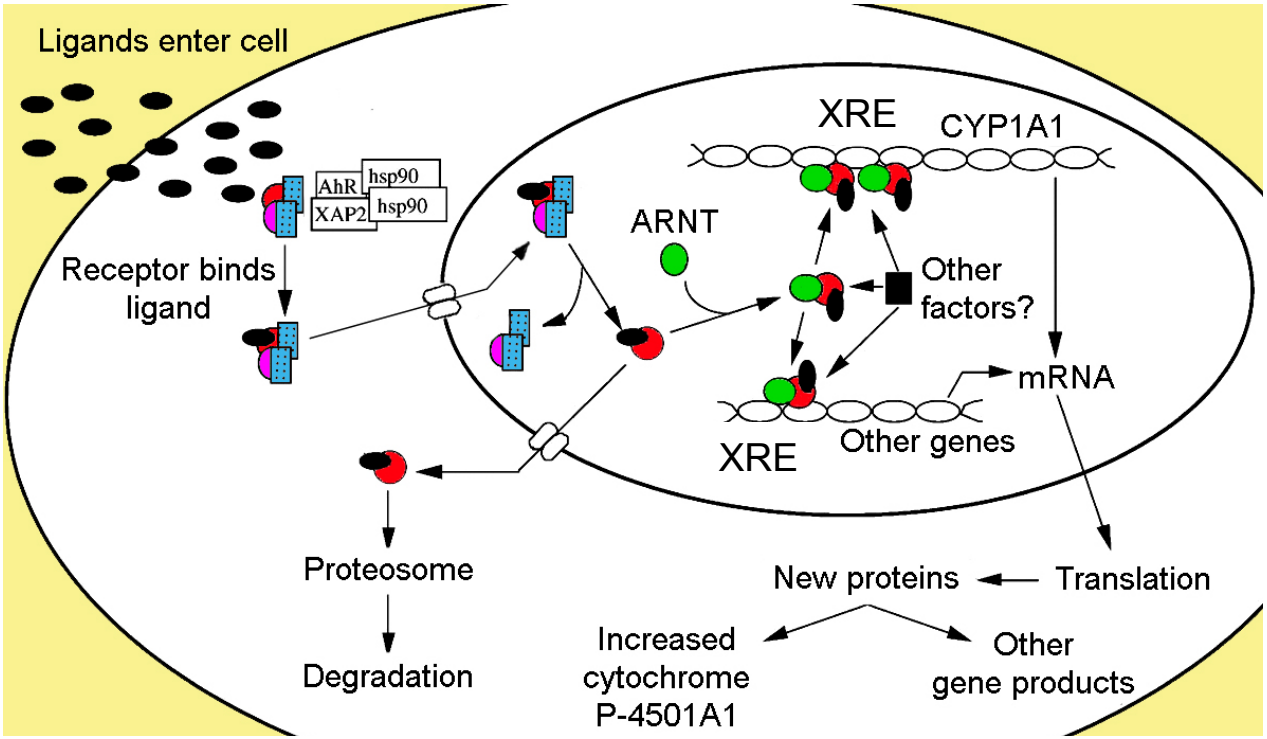
Aryl Hydrocarbon Receptor (AHR)

- Member of the family of basic-helix-loop-helix transcription factors
- Two classes of ligands: synthetic (dioxin), natural (Tryptophan derivatives, arachidonic acid metabolites, bilirubin, carotinoids…).
- Identified in drosophila to be involved in T-cell-, neuron-, hepatocyte- development.



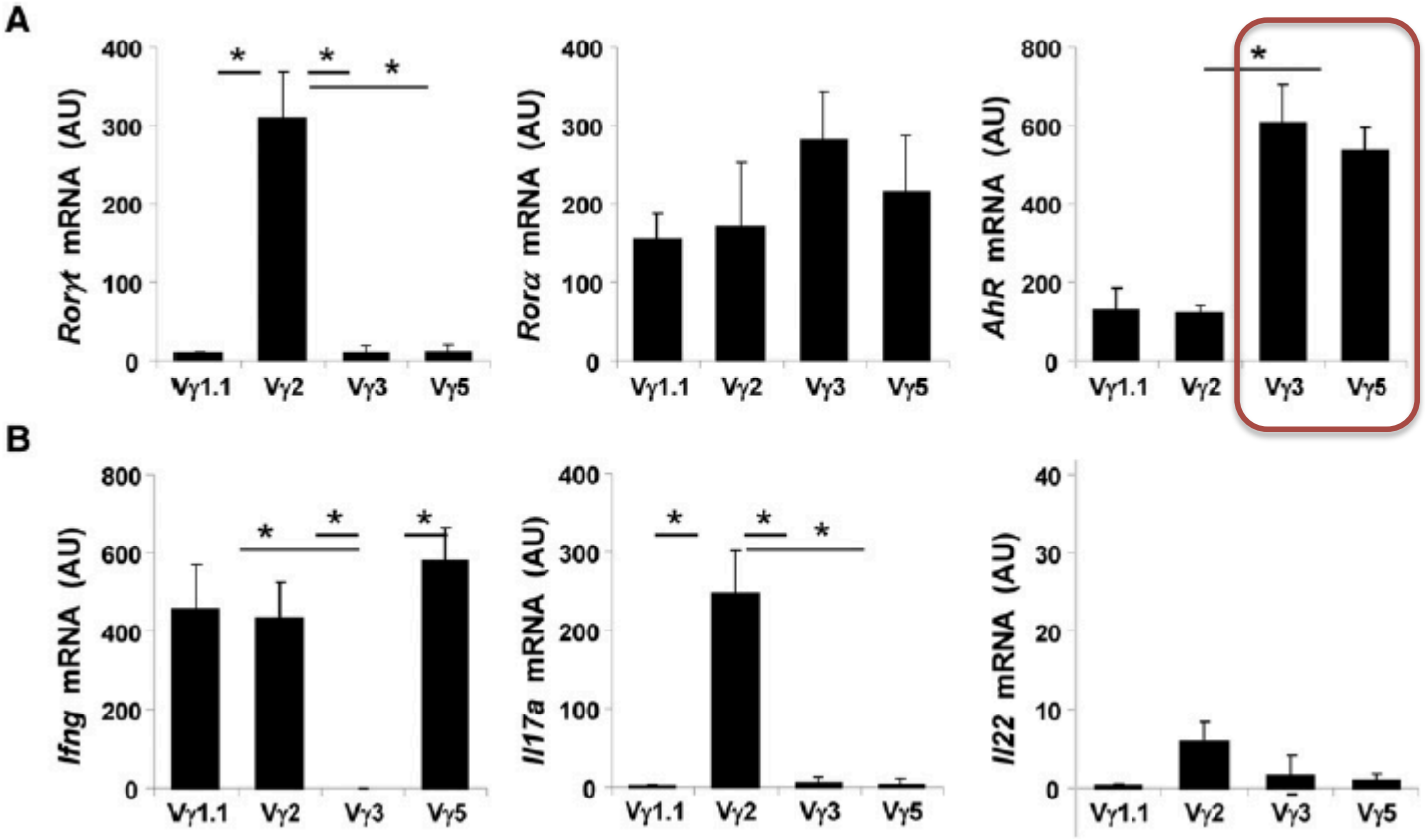
Denison *et al.* 2003 Annu. Rev. Pharmacol. Toxicol.

What is known about AHR-function in immunology?

- AHR expression is up-regulated in **Th17** cells (Veldhoen et al. 2008)
- AhR expression in γδ T cells in secondary lymphoid organs is essential for the production of **IL-22** (Martin et al. 2009)
- Innate lymphoid cells do express AHR, but it's role is unknown (Cella *et al.* 2009)
- Mice injected with AHR-agonists are protected in the DSS model (Monteleone *et al* 2011)
- AHR is important for Treg induction by bone marrow derived DCs after LPS or CpG stimulation (Nguyen *et al.* 2010)

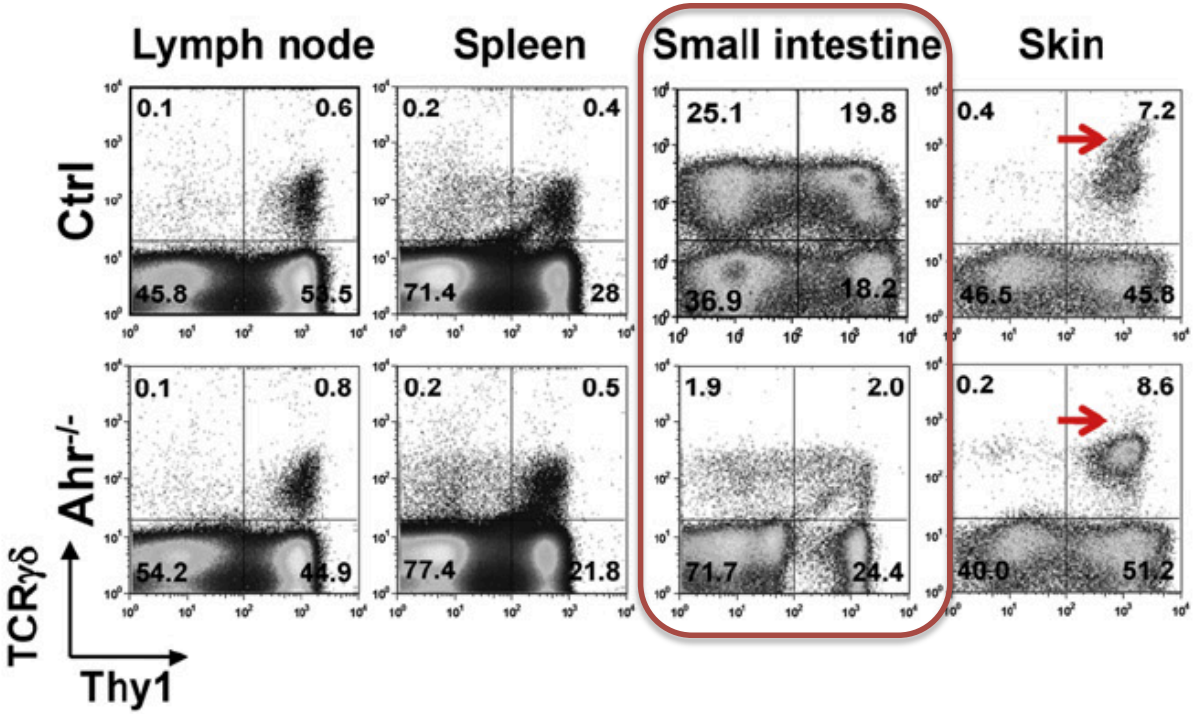
Is AHR expressed in Intra-epithelial lymphocytes?

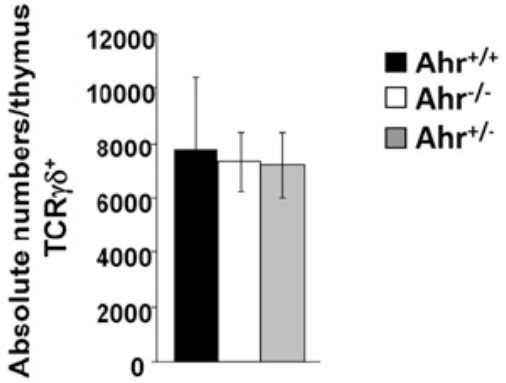
IELs express an invariant TCR and can be classified based on the γ-chain they express. Intestine: TCRVγ5; Skin: TCRVγ3, LN: TCRVγ1.1 and TCRVγ2

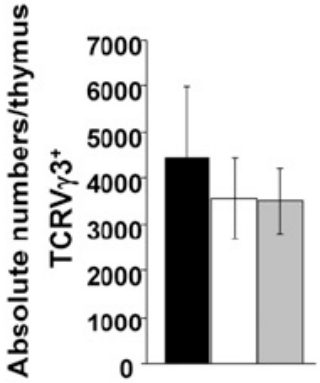


RT-PCR from FACS sorted CD8α+, TCRβ- IELs, according to their TCRVγ usage

What happens in the absence of AHR?



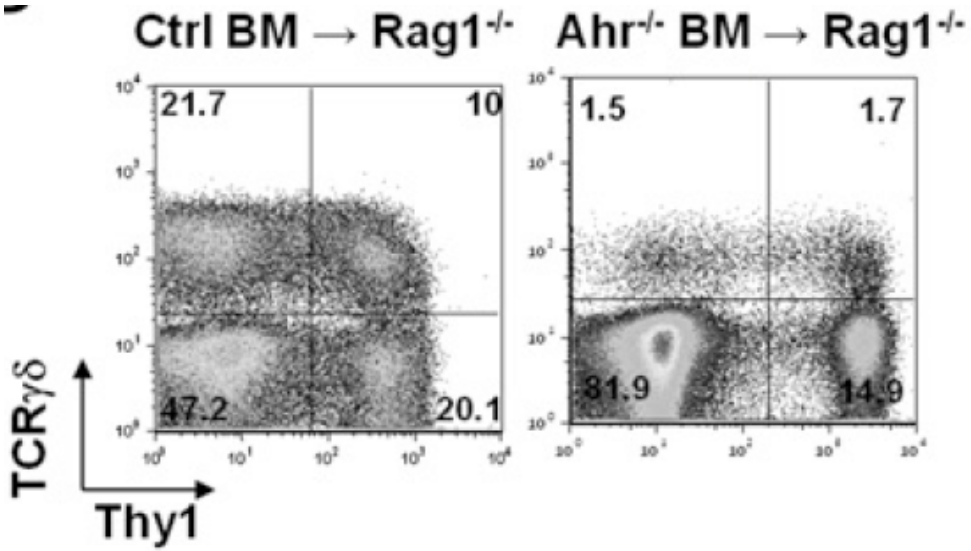




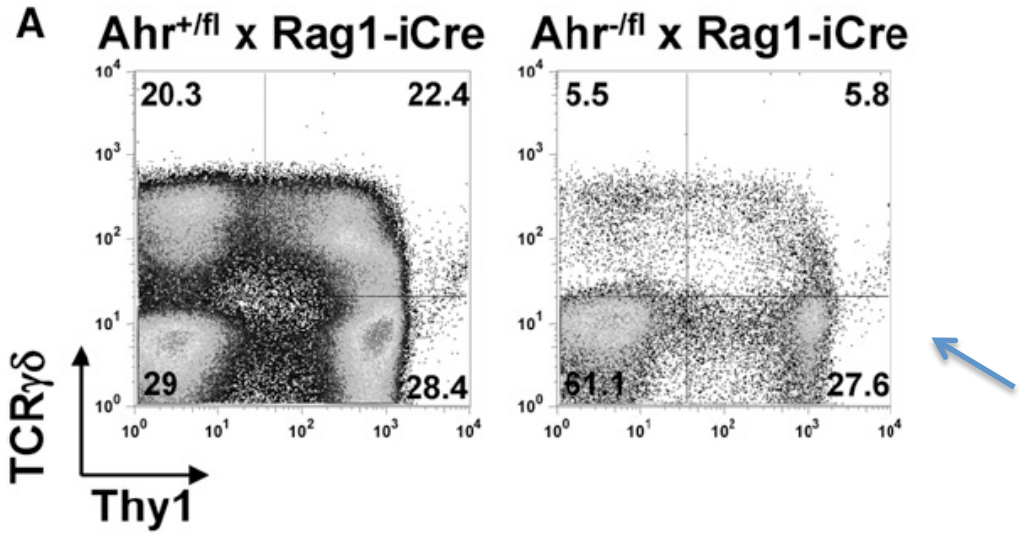
Development of these cells in the thymus is normal and they are able to migrate to the intestine

-> problem of maintenance at mucosal sites!

In what cell type is AHR-signaling important?



AHR expression on BM derived cells is important for IEL generation

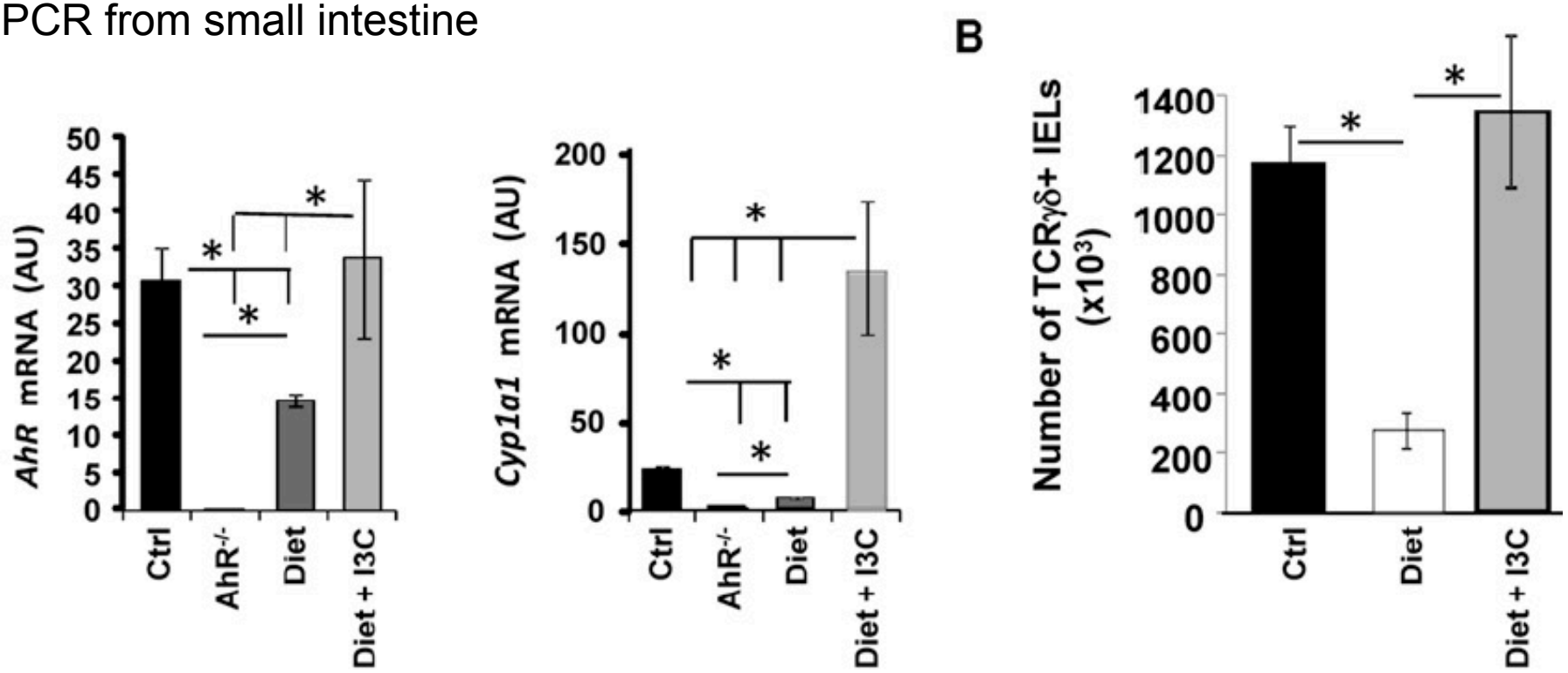


Maintenance of IELs depends on T-cell intrinsic AHR-activity

(These mice lack AHR expression in all cells that express Rag)

What is the ligand for AHR in IELs?

RT-PCR from small intestine

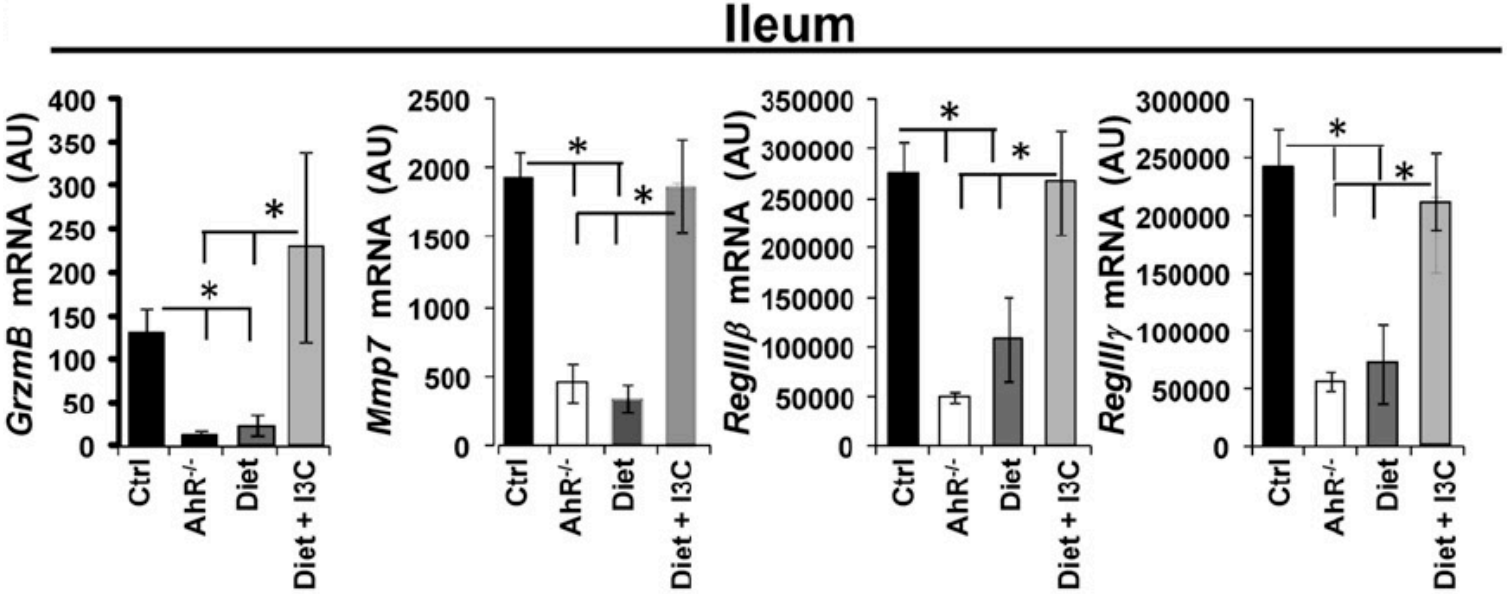


Cyp1a1: target gene of AHR

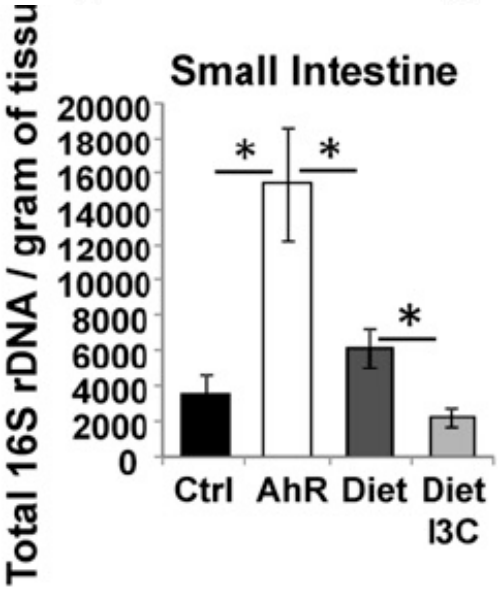
I3C: phytochemical found in cruciferous vegetables that activates AHR

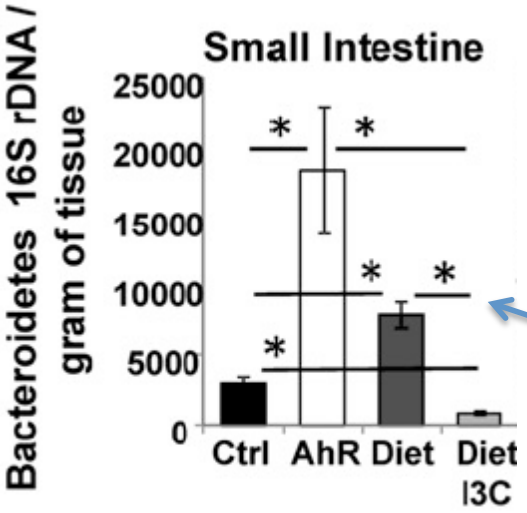
Diet: synthetic diet given for 3 weeks

Is there any consequence of AHR-mediated IEL-loss?



Bactericidal and anti-microbial genes are downregulated in absence of AHR



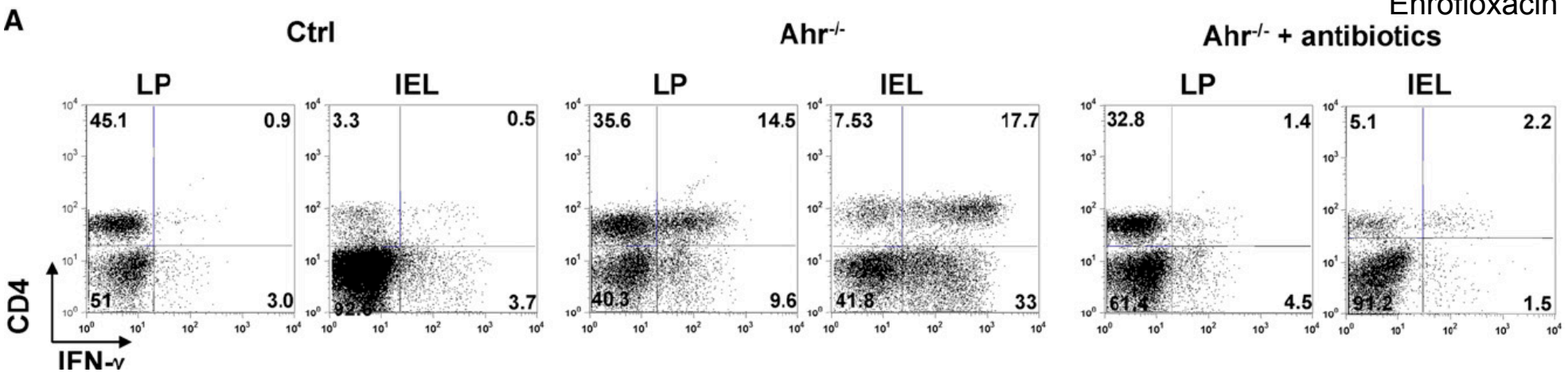


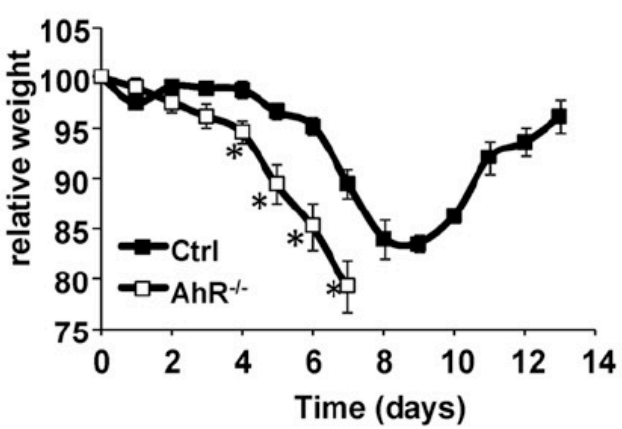
Increased bacterial load and high abundance of *Bacteroidetes*

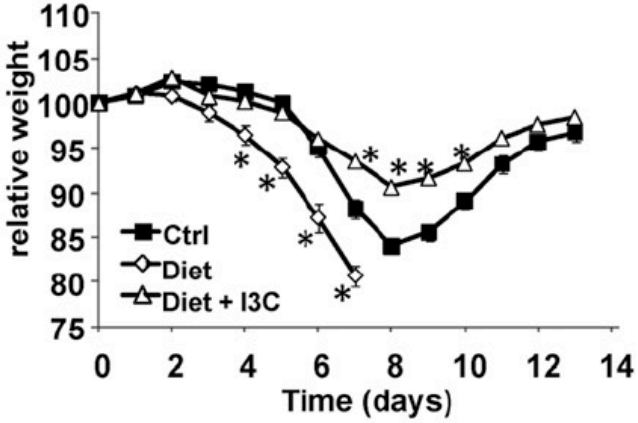
Funniest significance lines I've ever seen

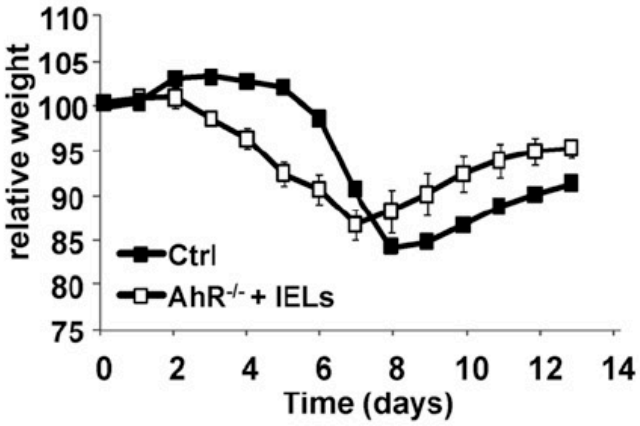
Is there any consequence of AHR-mediated IEL-loss?

Enrofloxacin







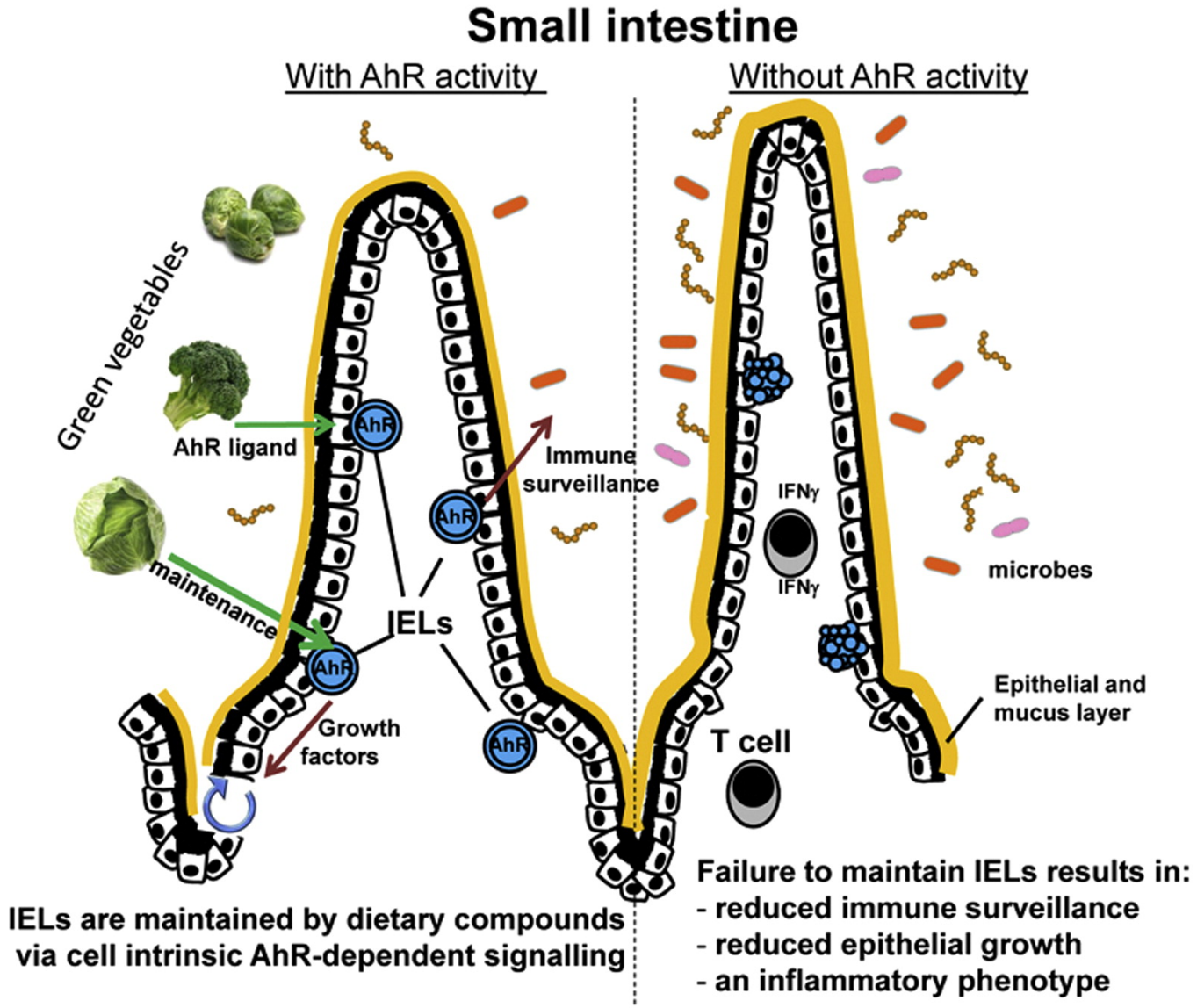


AHR-mediated signaling in IELs protects in the the DSS model (3% DSS for 6 days)

Summary

- IELs have a cell-intrinsic requirement for the ligand activation of the AHR
- Its activation affects maintenance but not development, homing or proliferation of IELs
- Reduction in AHR-activity in mice reduces their intestinal cytotoxic capacity leading to changes in microbiota composition -> *Bacteroidetes*
- AHR deficient mice accumulate more IFNy secreting CD4+ T-cells in the intestine.
- AHR-deficiency or its ligands results in increased susceptibility to DSS colitis

Summary


Small intestine
With AhR activity
Without AhR activity
Green vegetables
AhR ligand
maintenance
AhR
IELs
Growth factors
Immune surveillance
IFNγ
IFNγ
microbes
Epithelial and mucus layer
T cell
IELs are maintained by dietary compounds via cell intrinsic AhR-dependent signalling
Failure to maintain IELs results in:
- reduced immune surveillance
- reduced epithelial growth
- an inflammatory phenotype

Sciencexpress Report

Natural Aryl Hydrocarbon Receptor Ligands Control Organogenesis of Intestinal Lymphoid Follicles

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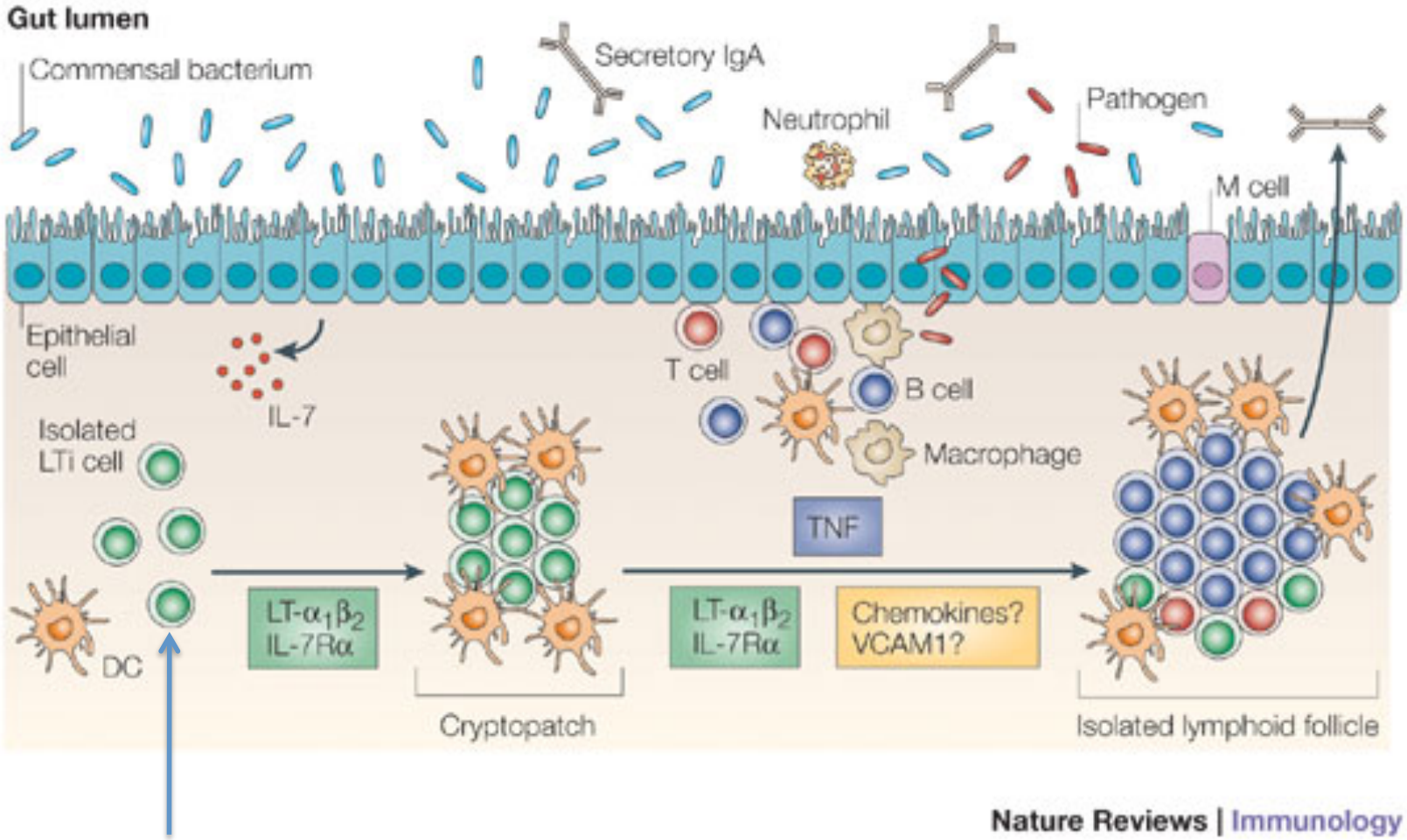
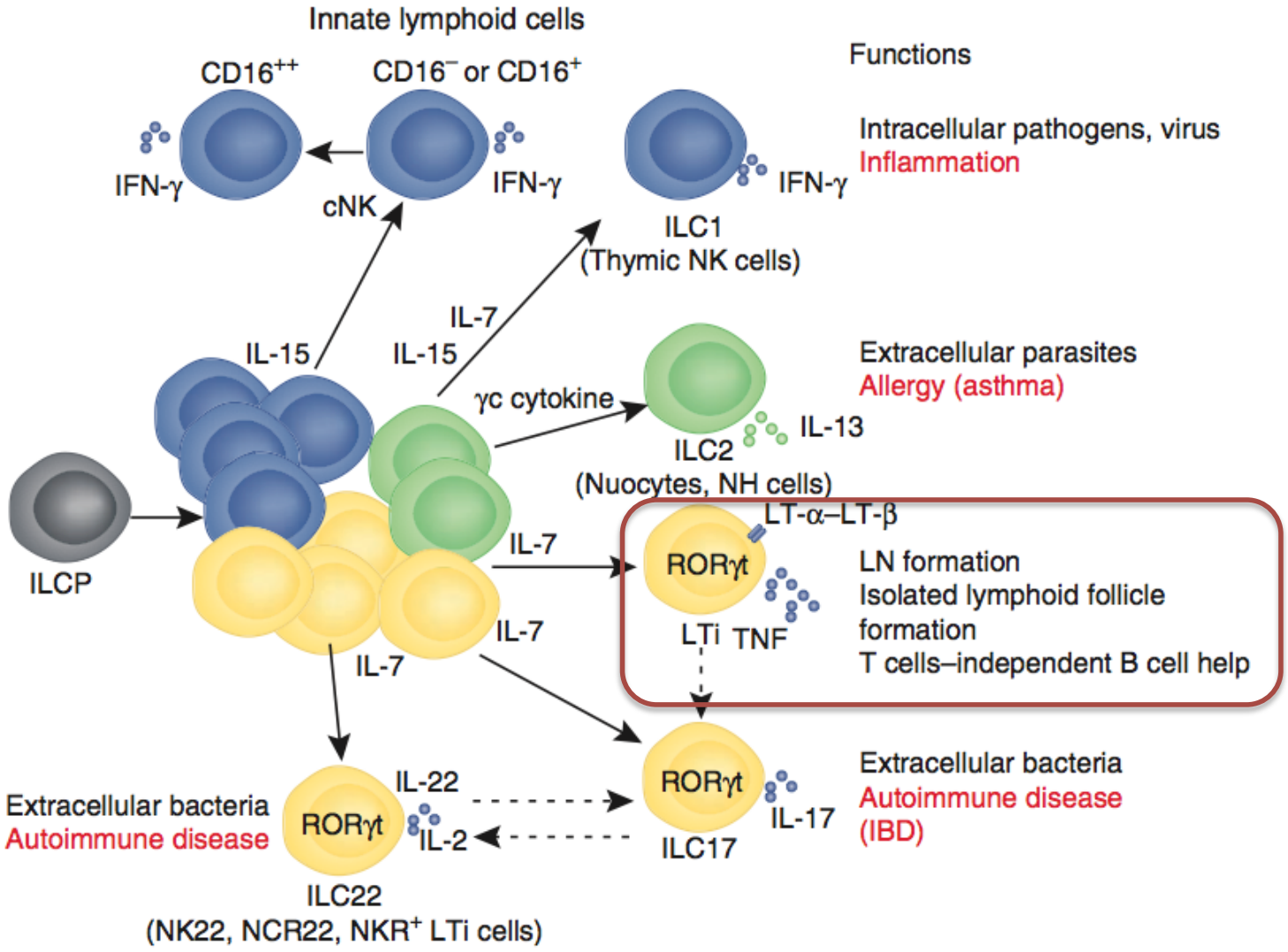
The formation of isolated lymphoid follicles



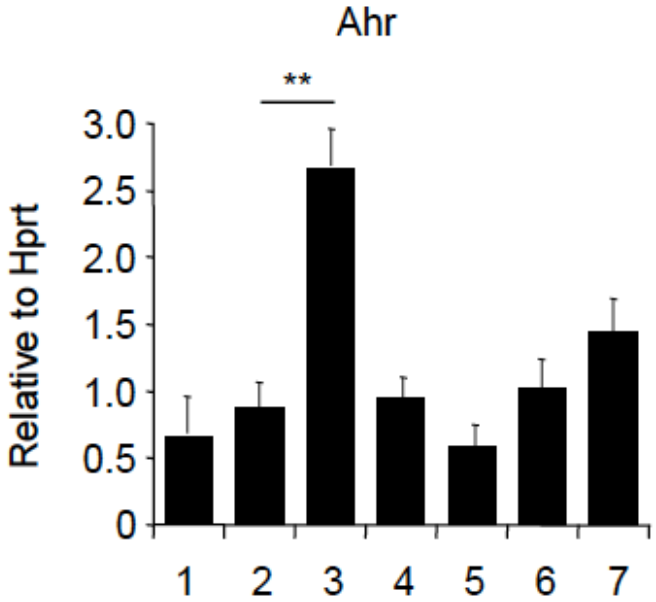
RORγt+ innate lymphoid cell

Innate lymhoid cells (ILCs)



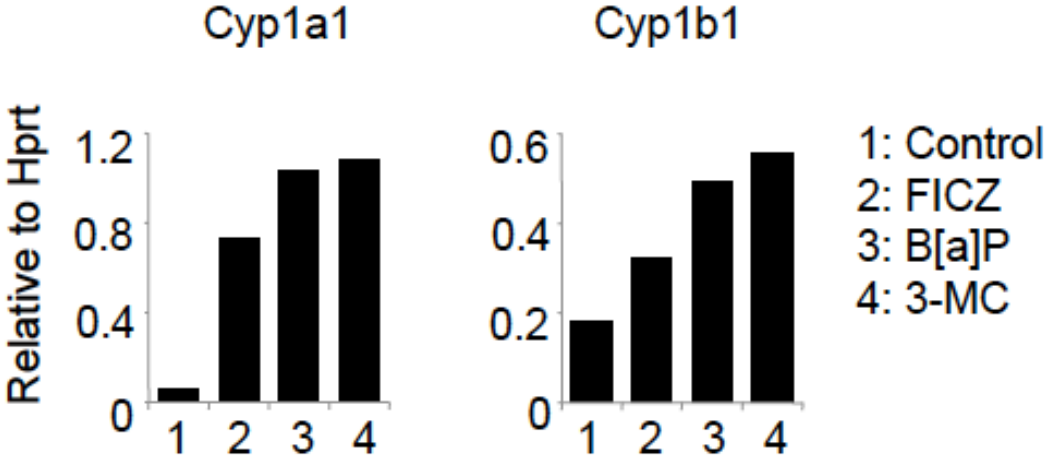
Hergen Spits and James Di Santo Nature Immunology Volume: 12, Pages: 21–27 (2011)

Is AHR-signaling important in ILCs?



1: NK cells
2: NKR$^+$ RORγt$^+$ ILC
3: NKR$^-$ CD4$^-$ RORγt$^+$ ILC
4: NKR$^-$ CD4$^+$ RORγt$^+$ ILC
5: CD4$^+$ RORγt$^-$ T cells
6: CD4$^+$ RORγt$^+$ T cells
7: Hepa1.6

RORyt+ ILCs express the AHR

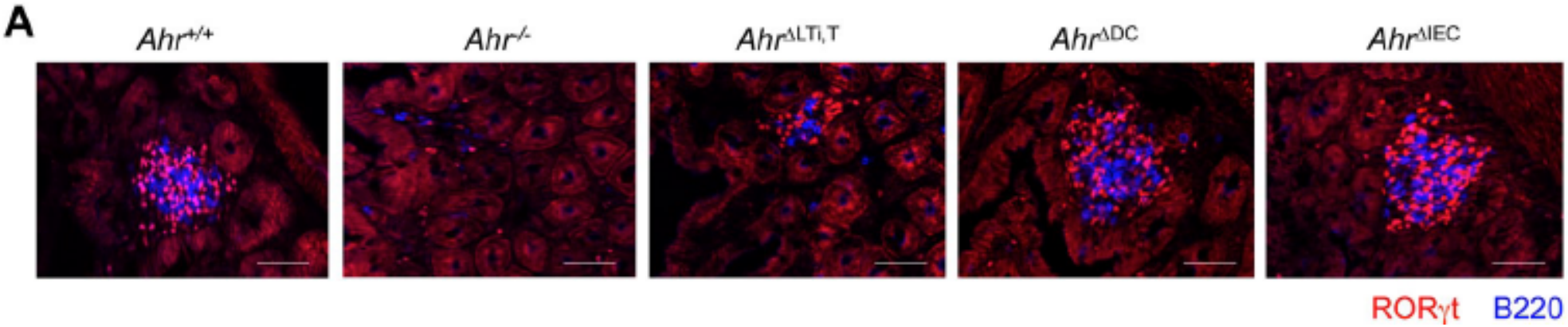


1: Control
2: FICZ
3: B[a]P
4: 3-MC

Induce Cyp1a1 and Cyp1b1 in response to AHR-stimulation
-> receptor is functional

Purified RORyt+ ILCs stimulated with AHR-ligands for 4 hours.

In what cell type is AHR-signaling important?

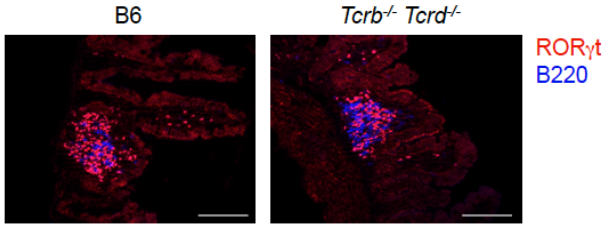


Ahr deleted in RORyt expressing cells-> ILCs and αβT-cells, and some γδ T-cells

Ahr deleted in Itgax expressing cells -> DCs

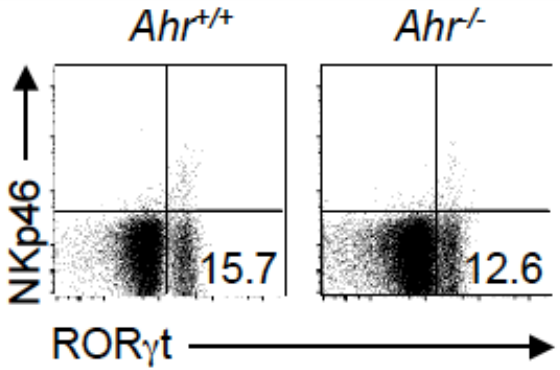
Ahr deleted in Villin1 expressing cells -> intestinal epithelium

Small intestine from 8 weeks old mice



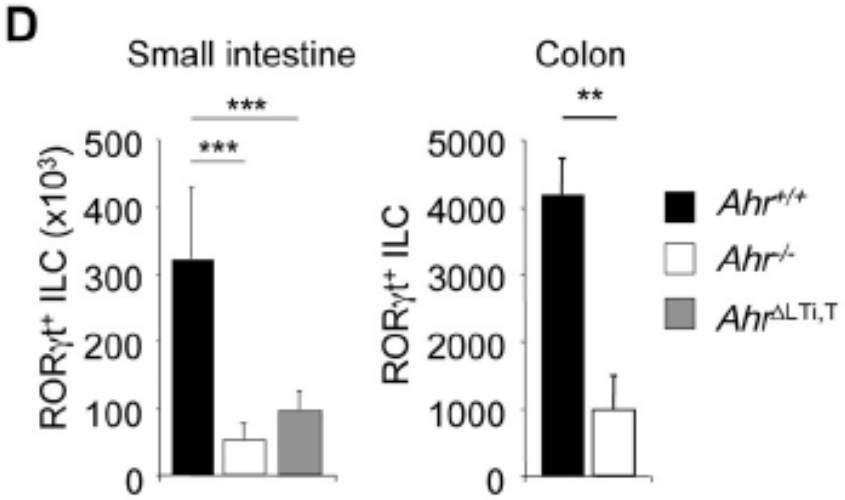
The postnatal initiation of CP development in the small intestine requires AHR expression by RORyt+ ILCs

In what cell type is AHR-signaling important?



NKp46 and RORyt expression by CD3-CD19- lamina propria lymphocytes from the small intestine of newborn mice.

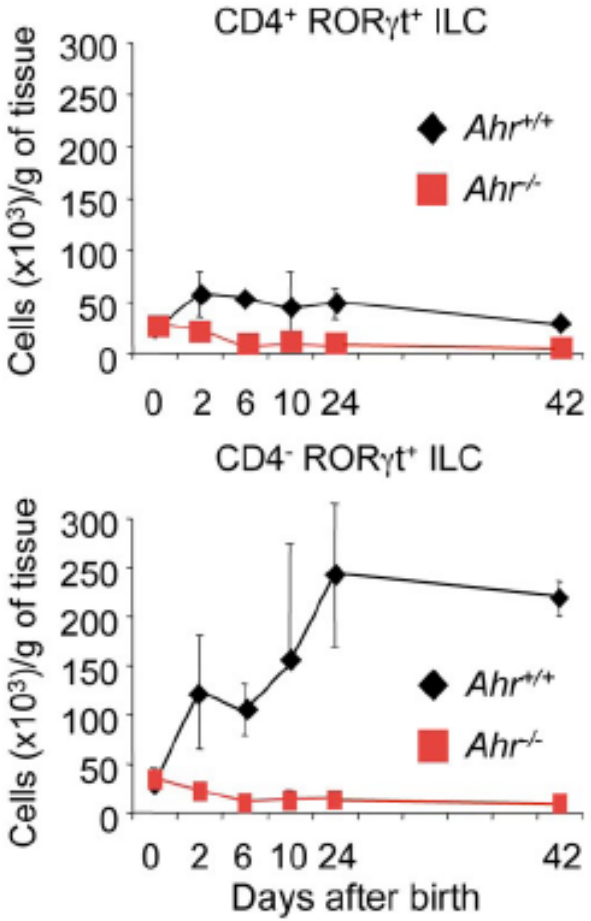
Normal development of RORyt+ ILCs in absence of AHR…



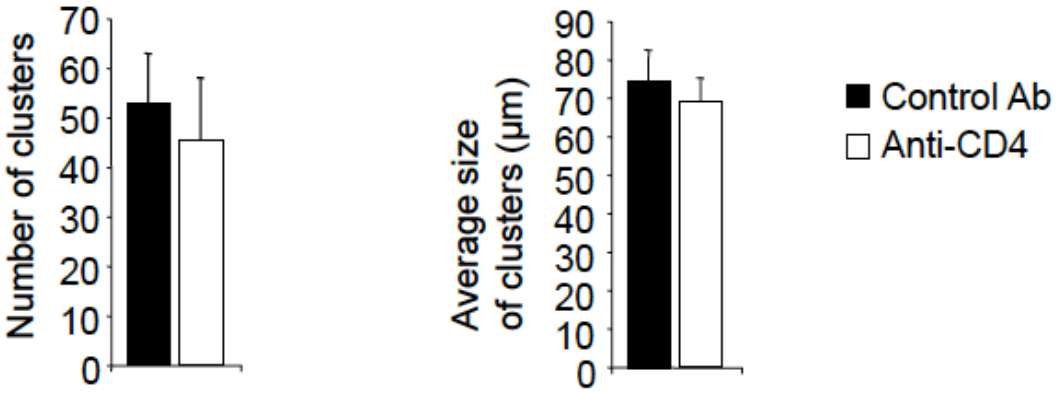
… but strongly diminished pool of RORyt+ ILCs in adult AHR-deficient mice

AHR-mediated signals in RORyt+ ILCs are not required for their development but rather for postnatal maintenance or expansion.

Two subsets of ILC according to CD4 expression



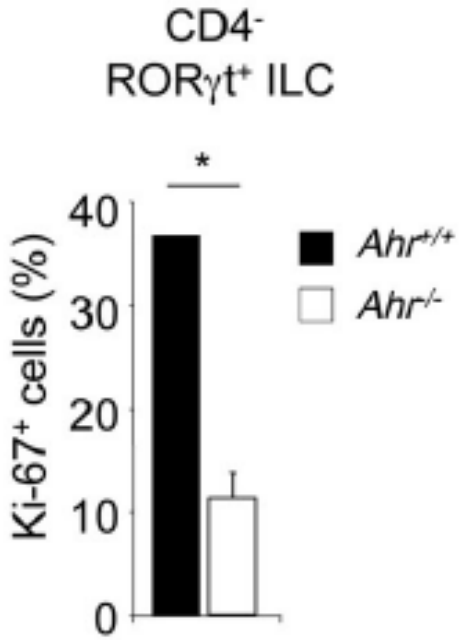
Absolute numbers per gramme of intestinal tissue

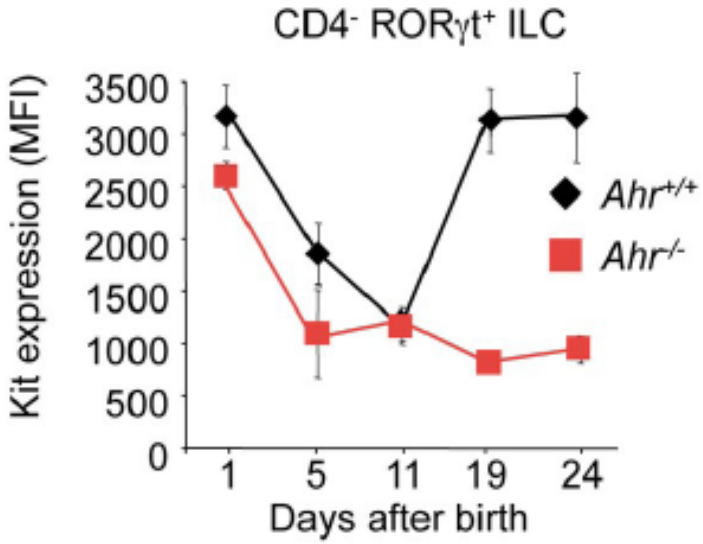


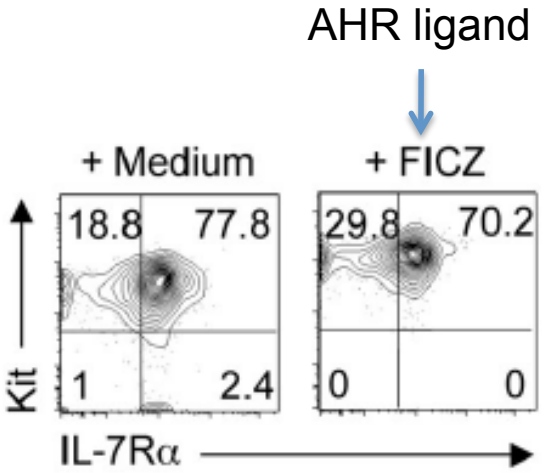
Mice were injected with a depleting CD4 antibody (GK1.5) or isotype control antibody every 3d and the formation of intestinal lymphoid clusters was analyzed at 3w of age.

AHR signals are predominantly important in CD4-RORyt+ ILCs for the genesis of CP/ILF

How does AHR signaling maintain RORyt+ ILCs?







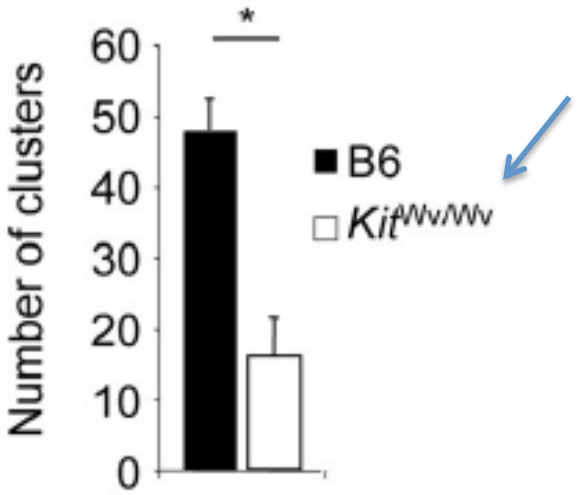
Impaired proliferation in AHR-deficient cells

Reduced c-kit expression in AHR-deficient cells

RORyt+ ILCs upregulate Kit in response to AHR stimulation.

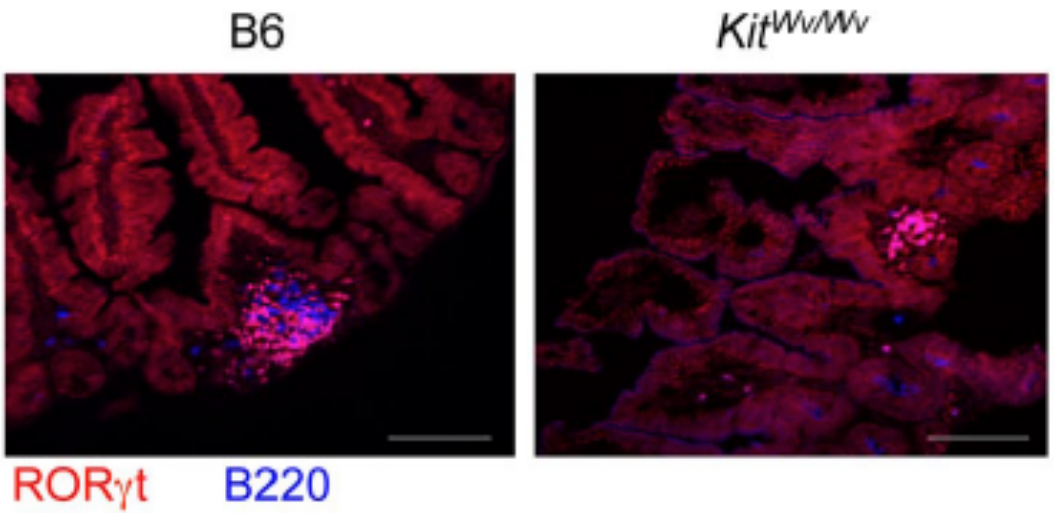
AHR ligands mediate Kit expression in RORyt+ ILCs at the timepoint where ILF induction occurs (11d). AHR ligands directly bind to XRE elements within Kit promoter.

Does Kit expression mediate ILF generation?

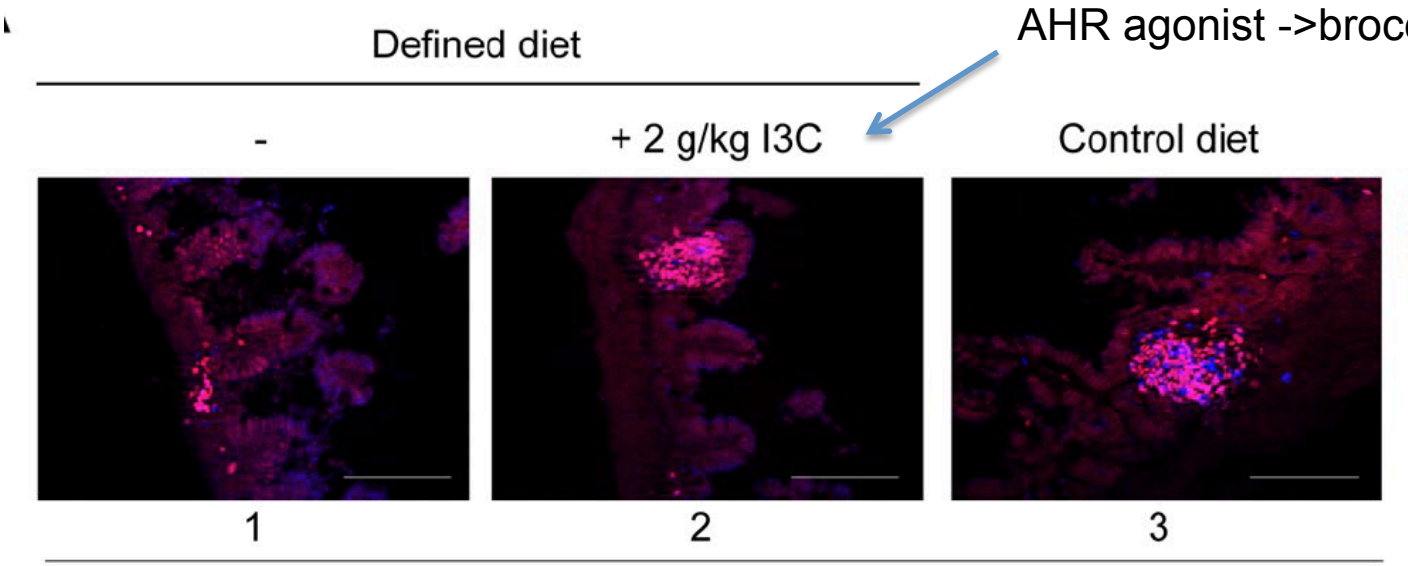


Mice with dysfunctional Kit

Number of ILF clusters is reduced in mice with defective Kit signaling



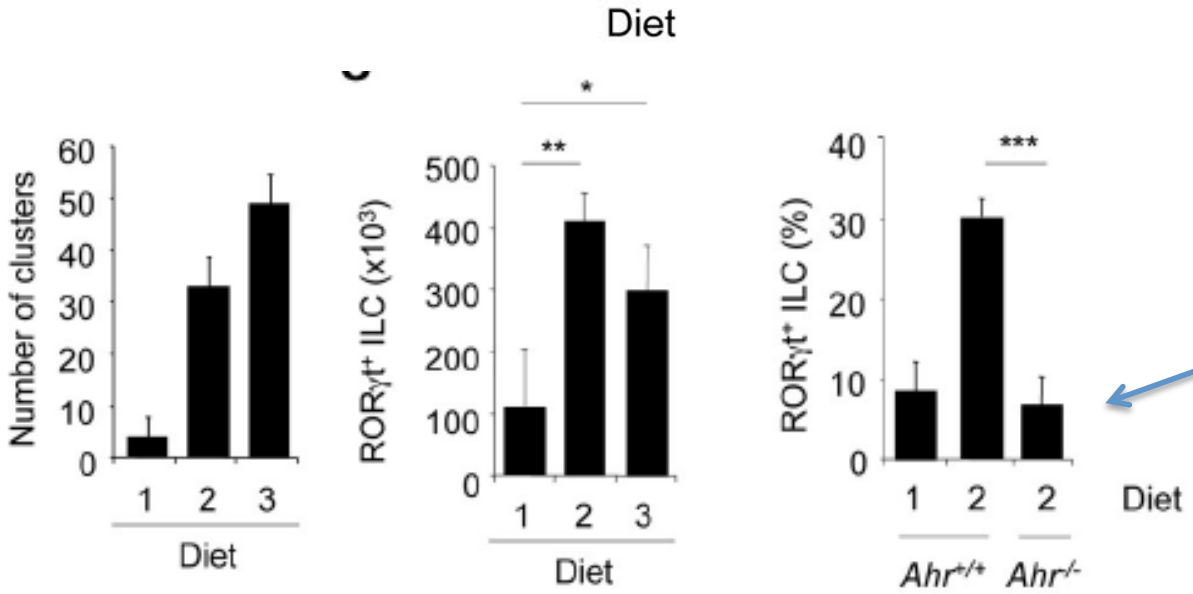
What is the ligand for AHR-mediated ILF generation?



AHR agonist ->broccoli

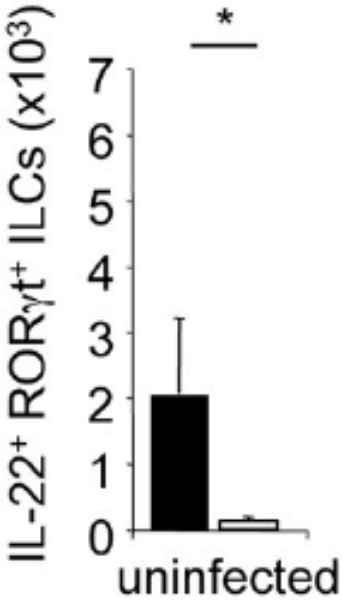
RORγt
B220

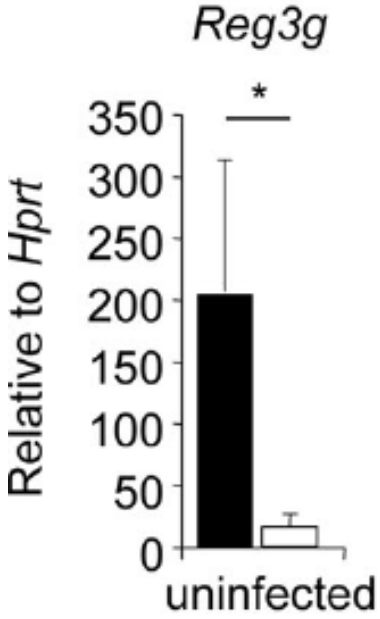
Mice 4weeks of age

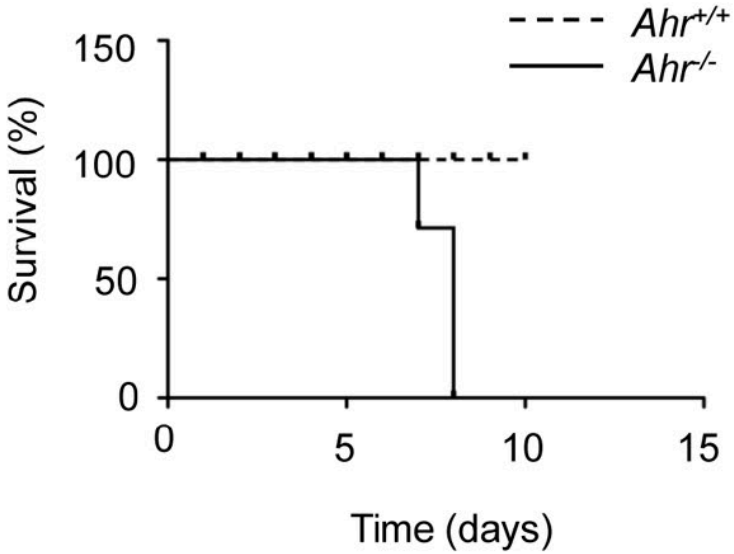


Mice fed a phytochemical-free diet have reduced CP/ ILF formation. Effect is AHR-dependent

Why is this important?







Reduced IL-22 production in absence of AHR

Reduced Reg3g production by colonic epithelial cells

Decreased survival after *C. rodentium* infection

AHR-dependent postnatal expansion of RORyt+ ILC is required for the protection against intestinal infections.

Summary

• AHR controls the pool size of RORyt+ ILC and consequently postnatal formation of CP/ILF

• AHR mediates c-kit expression and proliferation in these cells

• AHR activation by phytochemicals is sufficient to promote ILF formation

• AHR-mediated generation of ILFs is crucial for protection against intestinal infections. -> Dietary AHR-ligand sources may also be infested with intestinal pathogens, therefore this may be a preventive event

Conclusion: eat broccoli !!!!!

BUT….

ARTICLE

doi:10.1038/nature10491

An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor

Christiane A. Opitz[1,2]*, Ulrike M. Litzenburger[1,2]*, Felix Sahm[3], Martina Ott[1,2], Isabel Tritschler[4], Saskia Trump[5], Theresa Schumacher[1,2], Leonie Jestaedt[6], Dieter Schrenk[7], Michael Weller[4], Manfred Jugold[8], Gilles J. Guillemin[9], Christine L. Miller[10], Christian Lutz[11], Bernhard Radlwimmer[12], Irina Lehmann[5], Andreas von Deimling[3], Wolfgang Wick[1,13] & Michael Platten[1,2]