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Maria
Exogenous Stimuli Maintain Intraepithelial Lymphocytes via Aryl Hydrocarbon Receptor Activation

Ying Li,1 Silvia Innocentin,4 David R. Withers,5 Natalie A. Roberts,5,6 Alec R. Gallagher,2 Elena F. Grigorieva,3 Christoph Wilhelm,1 and Marc Veldhoen1,4,*

1Division of Molecular Immunology
2Division of Biological Services
3Division of Developmental Neurobiology
MRC National Institute for Medical Research, Mill Hill, London NW7 1AA, UK
4Laboratory of Lymphocyte Signalling and Development, Babraham Institute, Cambridge CB22 3AT, UK
5MRC Centre for Immune Regulation, Institute for Biomedical Research, Medical School, University of Birmingham, Birmingham B15 2TT, UK
6Present address: Department of Molecular, Cell and Developmental Biology, Yale University, New Haven, CT 06520, USA

*Correspondence: marc.veldhoen@babraham.ac.uk
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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.

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 its 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?

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

- Green vegetables
- AhR ligand
- Immune surveillance
- Growth factors
- Epithelial and mucus layer

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

Without AhR activity

- microbes
- IFNγ
Natural Aryl Hydrocarbon Receptor Ligands Control Organogenesis of Intestinal Lymphoid Follicles

Elina A. Kiss,1,2,3 Cedric Vonarbourg,1 Stefanie Kopffmann,1 Elias Hobeika,4 Daniela Finke,5 Charlotte Esser,6 Andreas Diefenbach1,2,3*

1IMMH, Institute of Medical Microbiology and Hygiene, University of Freiburg Medical Center, Hermann-Herder-Strasse 11, D-79104 Freiburg, Germany. 2Spemann Graduate School of Biology and Medicine, Albertstrasse 19A, D-79104 Freiburg, Germany. 3Research Training Group of Organogenesis (GRK1104), Hauptstrasse 1, D-79104 Freiburg, Germany. 4Max-Planck-Institute of Immunobiology & Epigenetics and Department of Molecular Immunology, Faculty of Biology III, University of Freiburg, Stübeweg 51, D-79108 Freiburg, Germany. 5Developmental Immunology, Department of Biomedicine, University of Basel, Mattenstrasse 28, CH-4058 Basel, Switzerland. 6Leibniz Research Institute for Environmental Medicine at the Heinrich-Heine-University of Düsseldorf, Auf’m Hennekamp 50, D-40225 Düsseldorf, Germany.
The formation of isolated lymphoid follicles

RORγt+ innate lymphoid cell
Innate lymphoid cells (ILCs)

- CD16^{++}
- CD16^{-} or CD16^{+}

- IFN-γ
- cNK

- IL-15
- IL-15
- γc cytokine

- ILCP

- Extracellular bacteria
  - Autoimmune disease

- ILCP
- IL-7
- IL-17

- RORγt
- ILC22
  - (NK22, NCR22, NKR^{+} LTi cells)

- Extracellular bacteria
  - Autoimmune disease (IBD)

- ILCP
- IL-7
- IL-2
- IL-22

- RORγt
- ILC17

- Extracellular parasites
  - Allergy (asthma)

- ILC1
  - (Thymic NK cells)

- Functions
  - Intracellular pathogens, virus
  - Inflammation

- LT-α–LT-β
  - LN formation
  - Isolated lymphoid follicle formation
  - T cells–independent B cell help
Is AHR-signaling important in ILCs?

RORyt+ ILCs express the AHR

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

Induce Cyp1a1 and Cyp1b1 in response to AHR-stimulation

-> receptor is functional
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

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

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.
How does AHR signaling maintain RORyt+ ILCs?

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.

Impaired proliferation in AHR-deficient cells

Reduced c-kit expression in AHR-deficient cells

RORyt+ ILCs upregulate Kit in response to AHR stimulation.
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?

<table>
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<tr>
<th>Defined diet</th>
<th>AHR agonist -&gt; broccoli</th>
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<tr>
<td>-</td>
<td>+ 2 g/kg I3C</td>
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<td>Control diet</td>
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Mice 4 weeks 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 !!!!!!
An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor

Christiane A. Opitz1,2*, Ulrike M. Litzenburger1,2*, Felix Sahm3, Martina Ott1,2, Isabel Tritschler4, Saskia Trump5, Theresa Schumacher1,2, Leonie Jestaedt6, Dieter Schrenk2, Michael Weller7, Manfred Jugold8, Gilles J. Guillemin9, Christine L. Miller10, Christian Lutz11, Bernhard Radlwimmer12, Irina Lehmann7, Andreas von Deimling6, Wolfgang Wick1,13 & Michael Platten1,2