The impact of environmental stimuli on innate and adaptive immune responses


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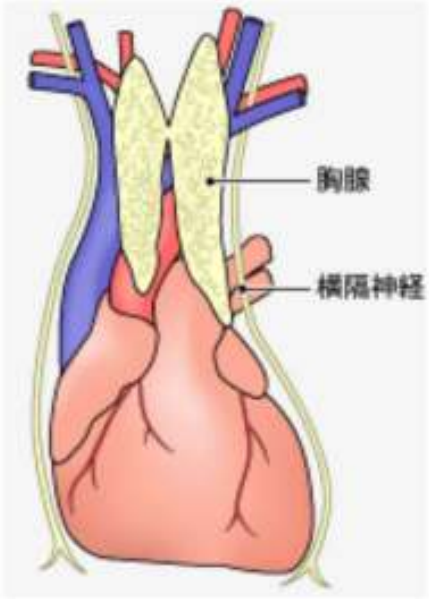


Treg

CD4

T helper cells

CD4

Killer cells

CD8

experience

iTreg

Th1

Th2

The other players in the immune system….

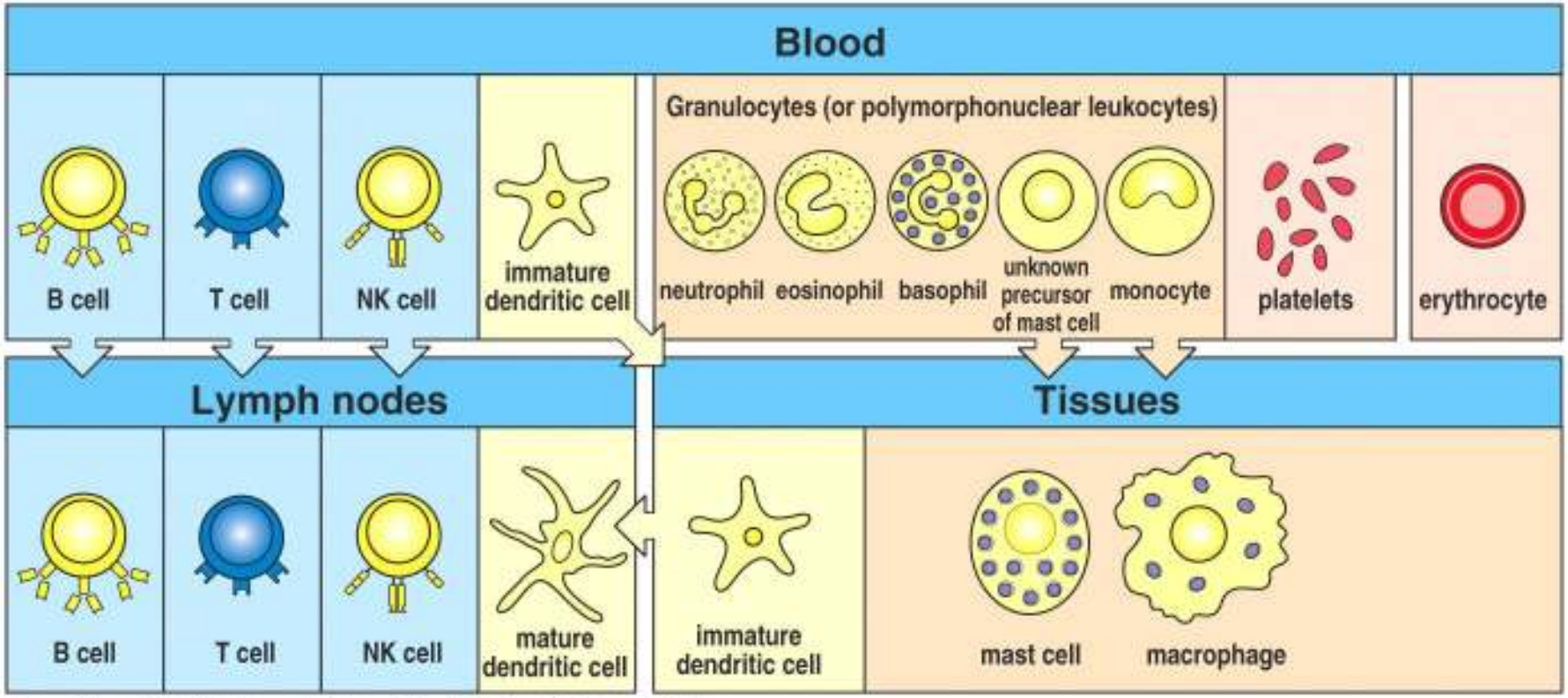
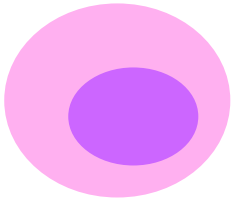
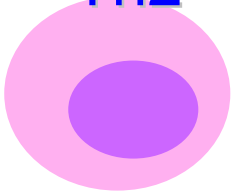
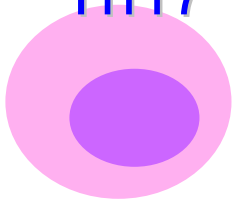
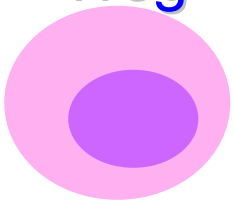
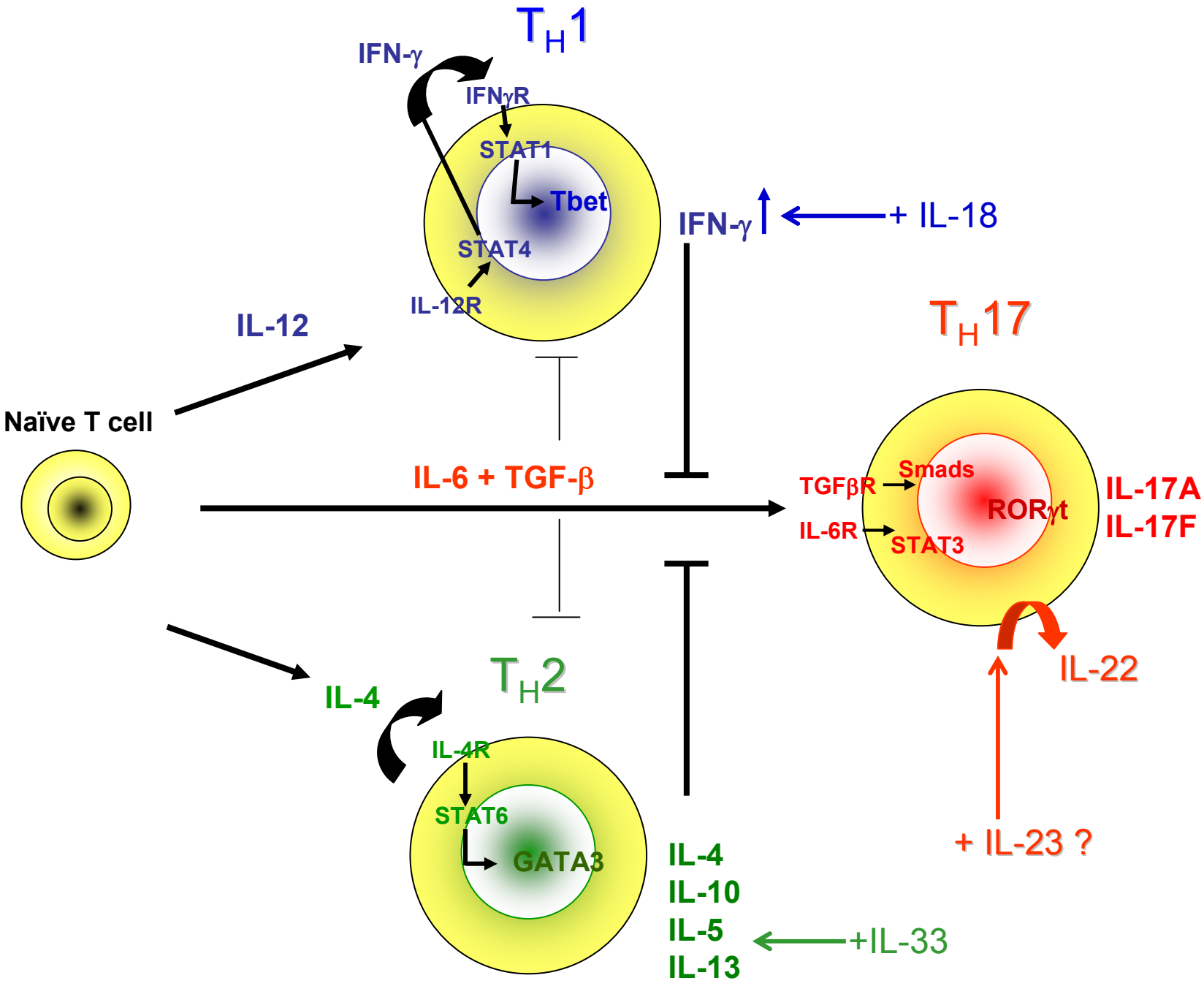


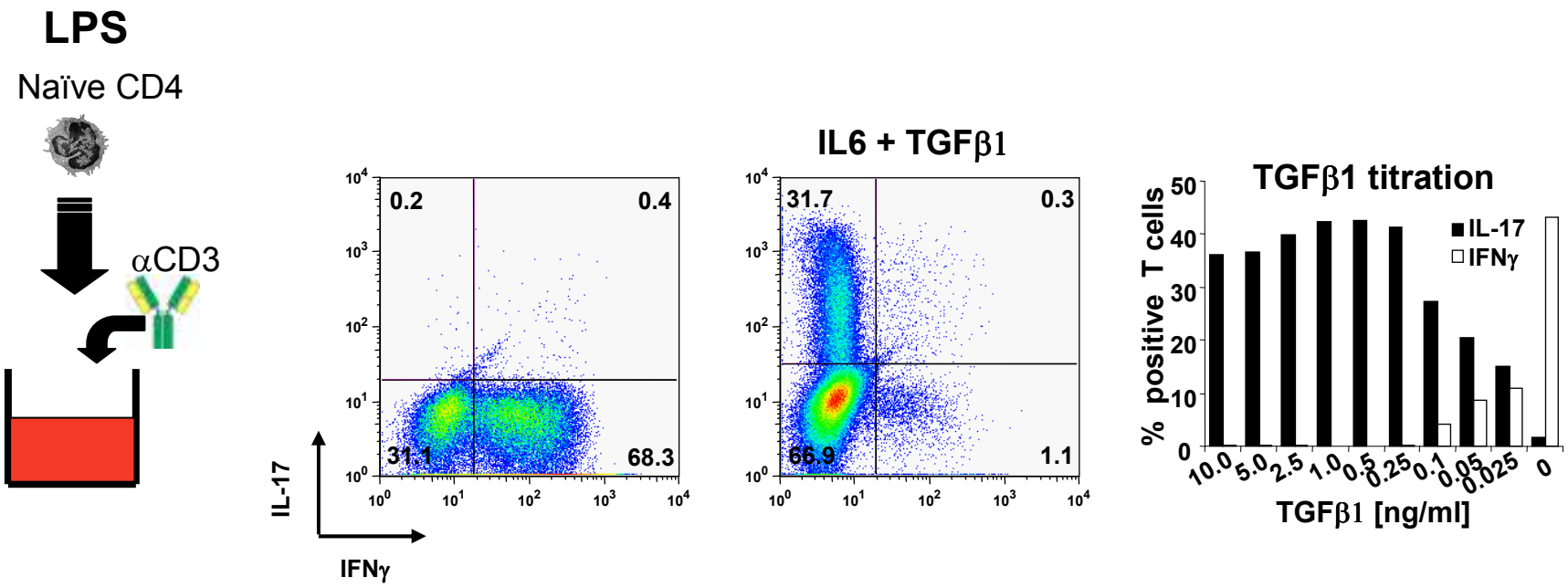
Figure 1-3 part 3 of 4 Immunobiology, 6/e. (© Garland Science 2005)

CD4 T cell subsets in the periphery

	Job description	Side effects
Th1 	Activate macrophages to fight intracellular pathogens (IFN-γ)	autoimmunity
Th2 	Help B cells to make antibodies against extracellular pathogens	allergic reactions
Th17 	Coordinate innate and adaptive immune responses against pathogens Fungi, bacteria	autoimmunity
Treg 	Limit immune pathology by exuberant Th1 or Th2, Th17	block anti-tumour responses

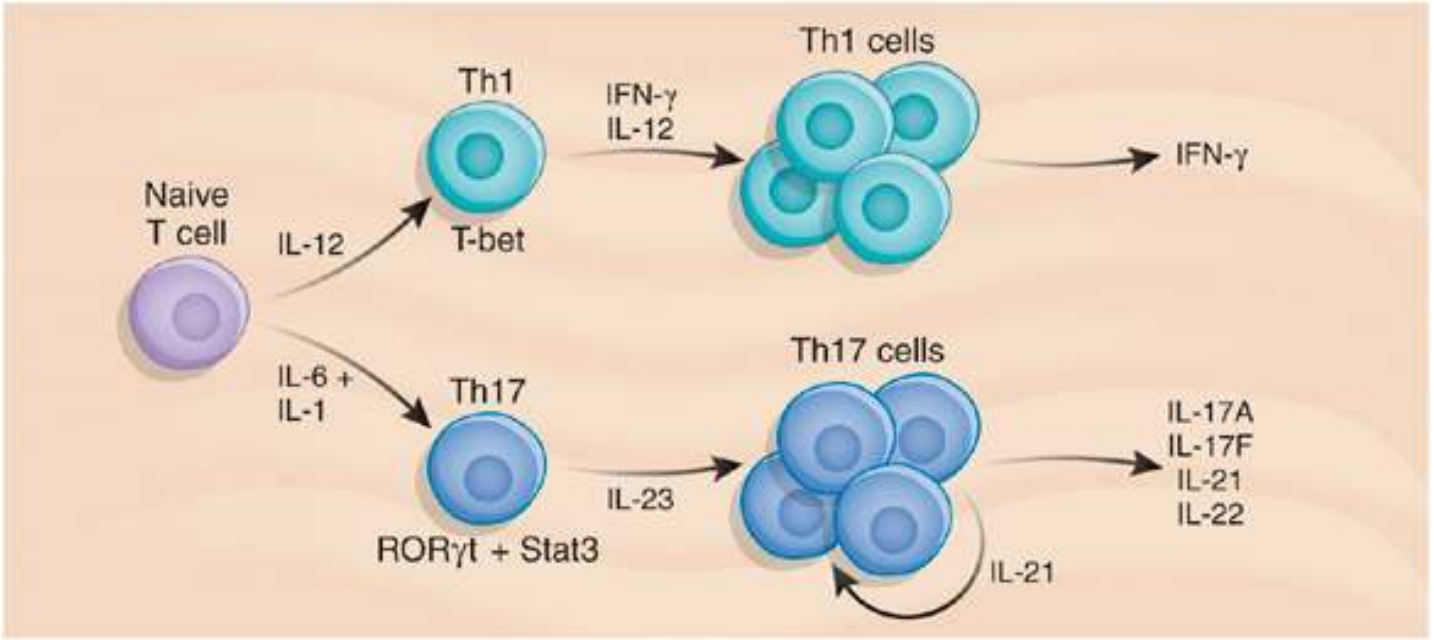

T_H1
IFN-γ
IFNγR
STAT1
Tbet
STAT4
IL-12R
IL-12
IFN-γ
+ IL-18
T_H17
Naïve T cell
IL-6 + TGF-β
TGFβR
Smads
RORγt
IL-6R
STAT3
IL-17A
IL-17F
IL-22
+ IL-23 ?
T_H2
IL-4
IL-4R
STAT6
GATA3
IL-4
IL-10
IL-5
IL-13
+IL-33

TGFβand IL-6 are essential and sufficient for differentiation of Th17 cells
(IL-1β and TNFα enhance differentiation)



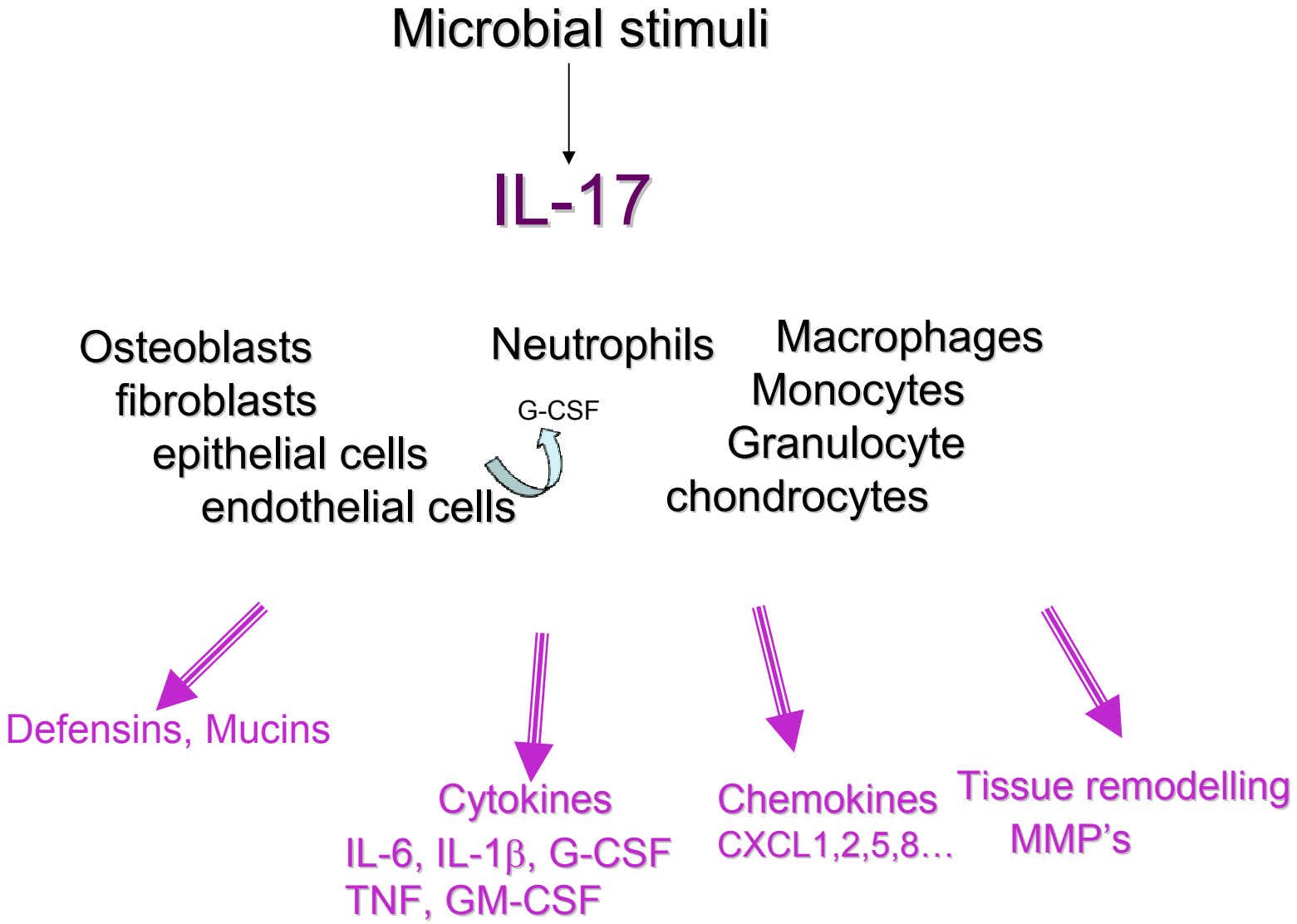
RORγt and RORα are the lineage defining transcription factors for Th17

IL-23 is not needed for Th17 differentiation, but is essential for their survival and function in vivo

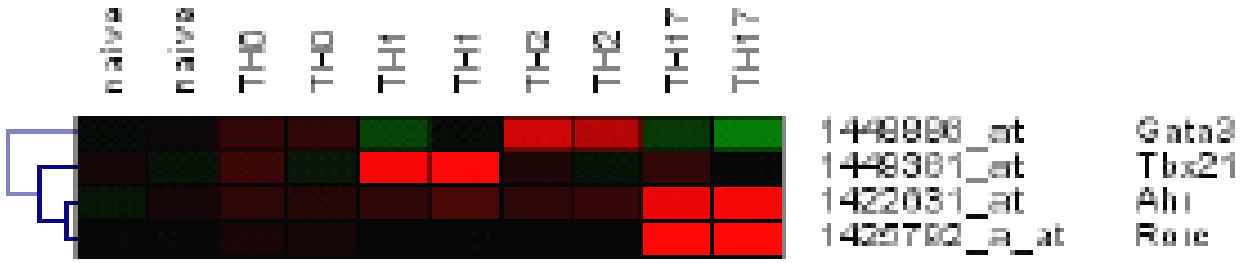


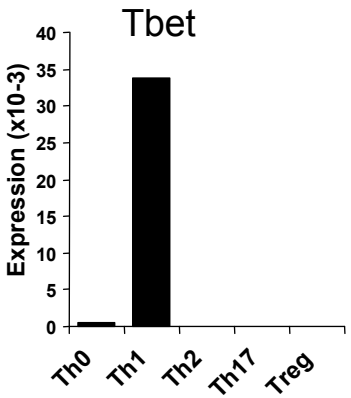
Th17 cells are distinct from Th1 cells, differentiate in the presence of IL-1 and IL-6, proliferate in response to IL-23, and secrete a characteristic set of cytokines. (Modified from Fitch et al., 2007.)

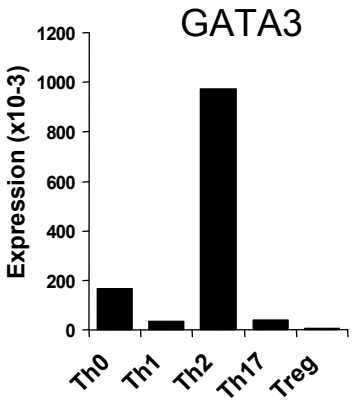
IL-17 stimulates the innate immune system and amplifies the innate response against pathogens

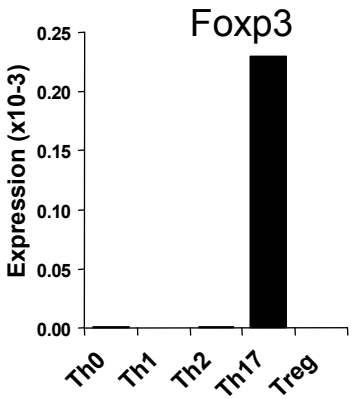


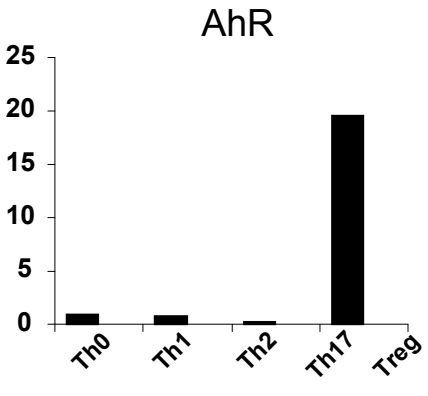
Transcription factors in CD4 T cell subsets



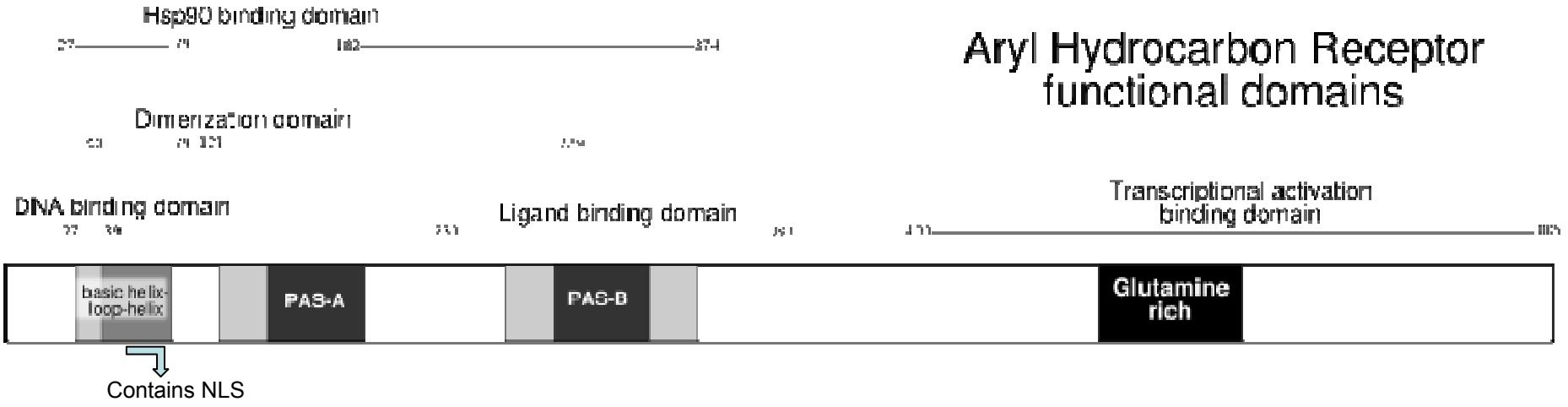








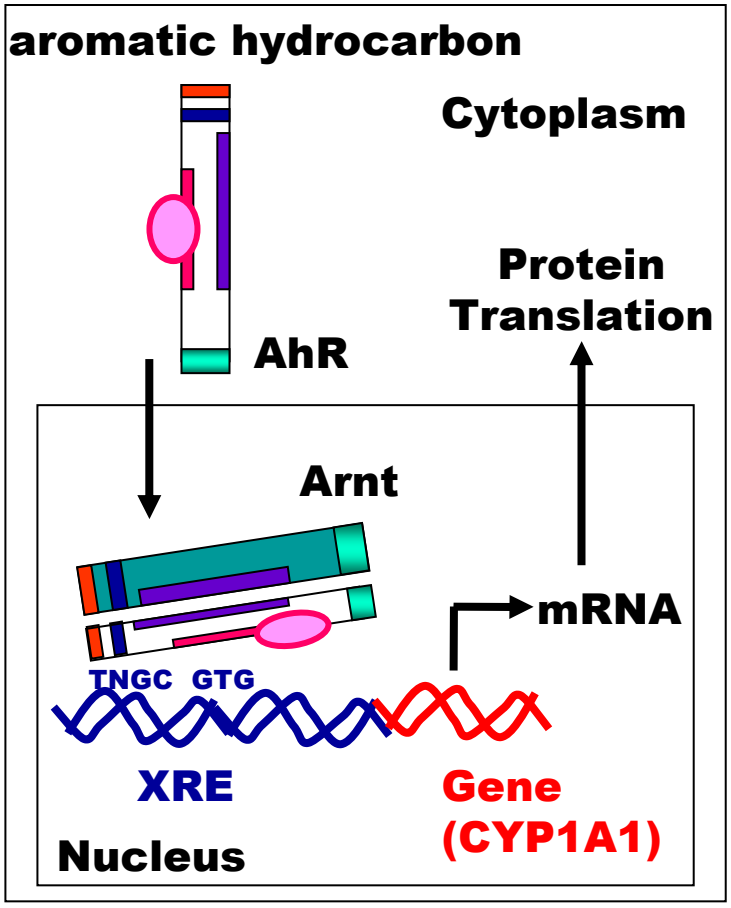
AhR: Chromosome 7:17.3 in humans
Chromosome 12 in mice



Aryl Hydrocarbon Receptor functional domains

Mediates the toxicity of dioxin and other environmental pollutants

Aryl hydrocarbon receptor (AhR)



(Gu et al., 2000)

Phase I enzymes:
CYP1A1,CYP1A2,CYP1B1

CYP1A1 encodes a member of cytochrome P450 superfamily of enzymes

Primarily involved in xenobiotic and drug metabolism

Phase II enzymes:

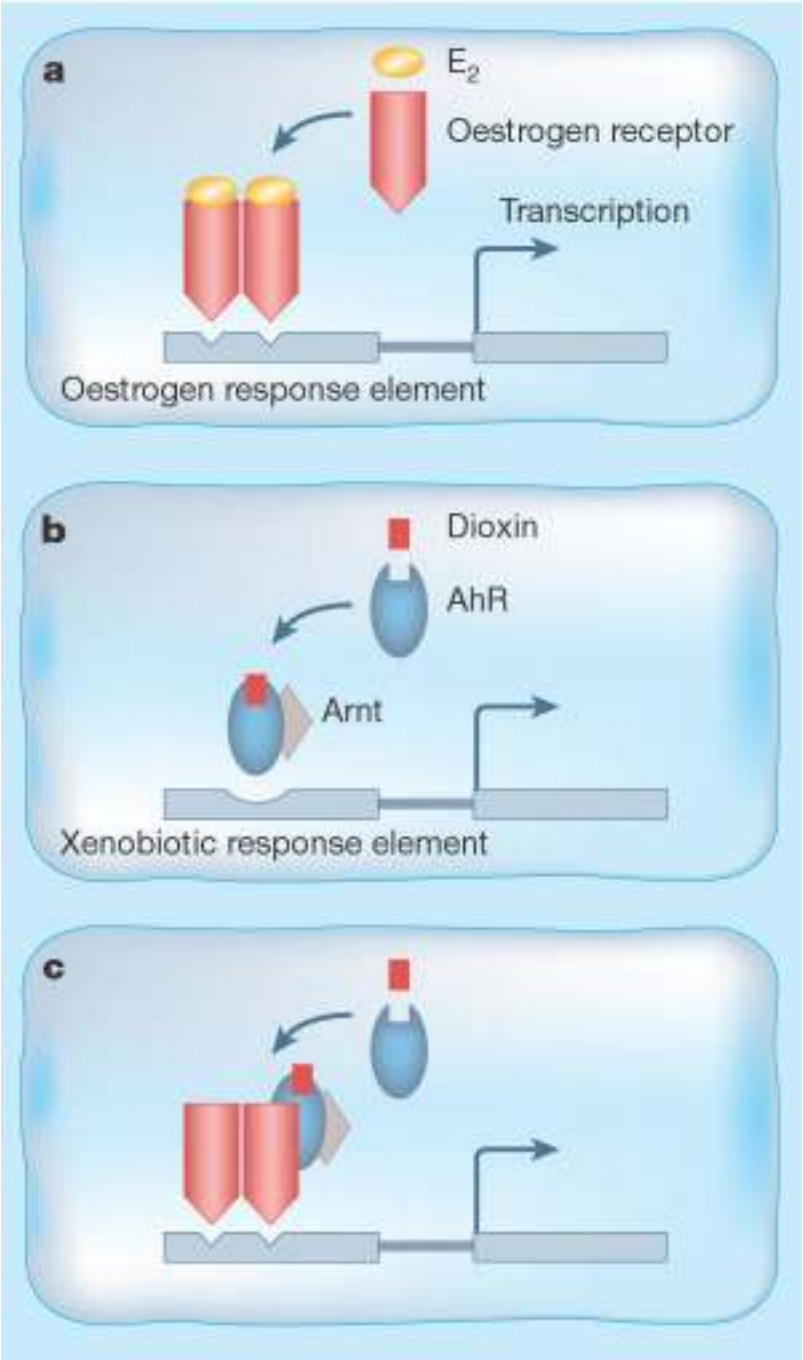
NQO1, ALHD3A1, UGT1A2 and GSTA1

Substrates quinones, aldehydes, p-nitrophenol, 1-chloro-2,4-dinitrobenzen

AhR pathway downregulation

1. AhR repressor (Mimura et al., 1999)
    1) Competes with AhR for ARNT heterodimerization
    2) Transcriptional repressor via AhRR/Arnt binding to XRE

2. Proteolysis of AhR following ligand activation
    - Leads to decreased receptor number
    - Ligand degradation via CYP enzymes- apart from dioxin…..



Cross talk between AhR and ER

Ligand-activated AhR-Arnt complex can activate *unliganded* ER to mimic estrogens

Ohtake et al. (2003)

Brosens & Parker (2003). *Nature* 423: 487.

Physiological roles of the AhR

Observations in mice lacking WT AhR:

- **Schmidt et al. (1996)**
  - **Hepatic growth and development stunted**
- **Mimura et al. (1997)**
  - **Lack of teratogenic response to TCDD**
- **Lund et al. (2003)**
  - **Cardiac hypertrophy in adult AhR null mice**

Additional physiological functions highly likely….

Aryl hydrocarbon receptor in C.elegans AHR-1 regulates neuronal development

AhR homologue in Drosophila is called the *spineless* gene (sensory bristles reduced, transformation of distal antenna into distal leg

Stochastic *spineless* expression creates the retinal mosaic for colour Vision

Wernet et al, Nature 440, 174 (2006)

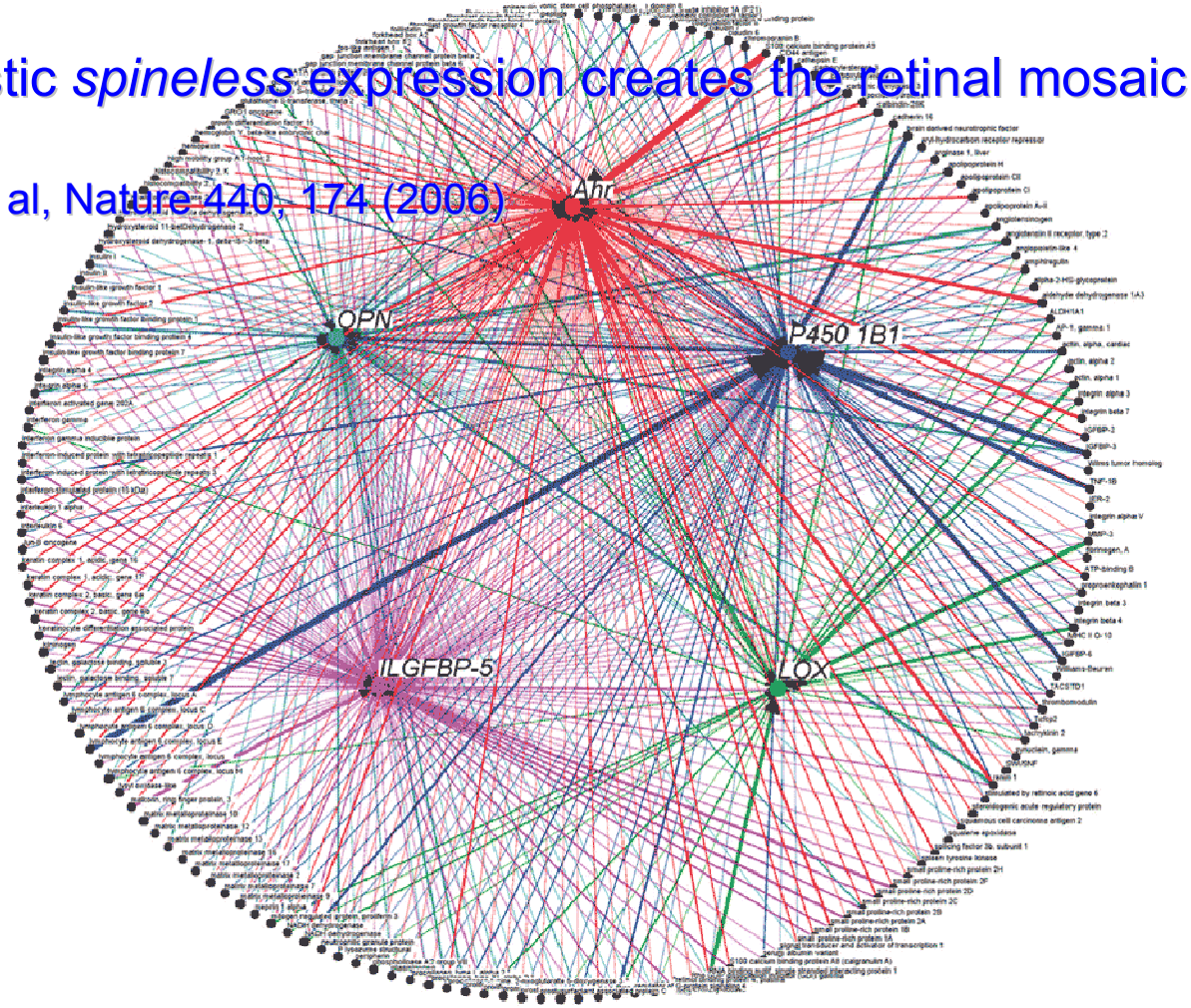


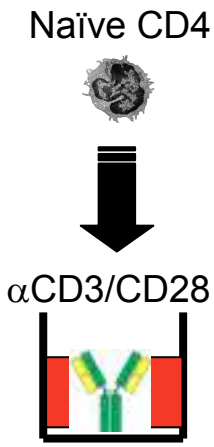
Figure 2. Gene–gene interaction networks activated by ligands of the *Ahr*. All three gene combinations for each target that met the cutoff of COD > 0.9 and error < 0.5 were individually plotted using a program developed by Breitkreutz et al. (2003). The thickness of the line denotes the selection frequency for individual gene for each target.

Role of AhR in the immune system ?

Research so far restricted to dioxin ➔ reports of widespread immune suppression

immunological phenotype of AhR knockout unclear

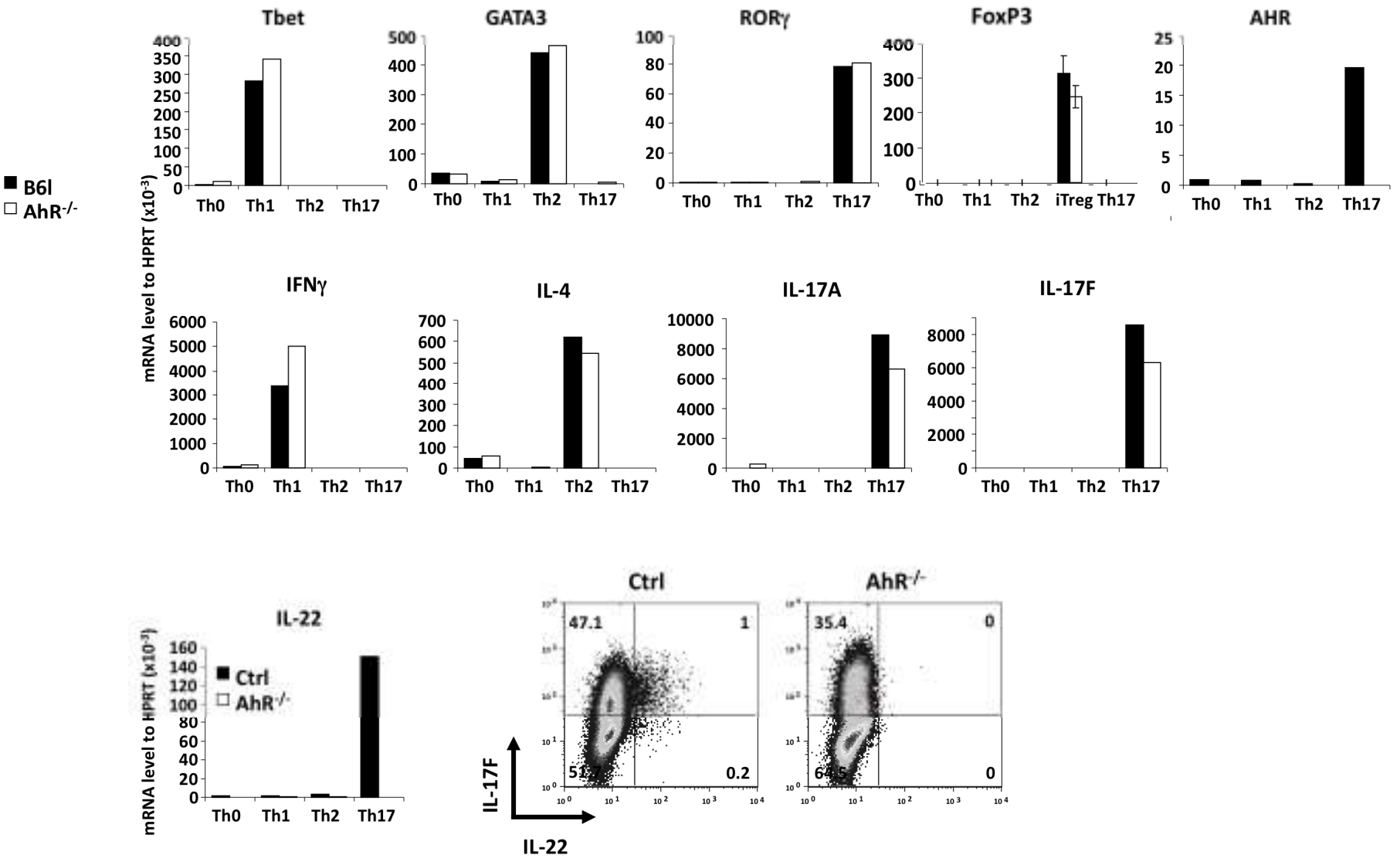
CD4 effector T cell differentiation in B6 vs AhR deficient mice



FACS sorted (CD44low, CD25-ve)
no APC

Day 5 RT-PCR and FACS analysis of polarized CD4 T cell subsets

CD4 effector differentiation in B6 vs AhR deficient mice



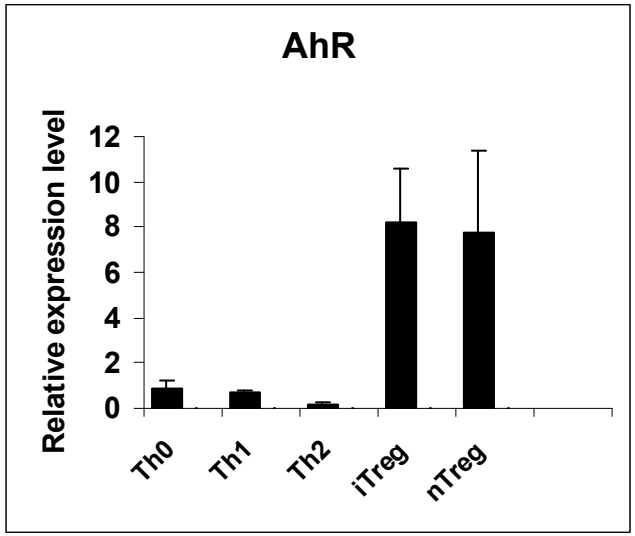
IL-22:

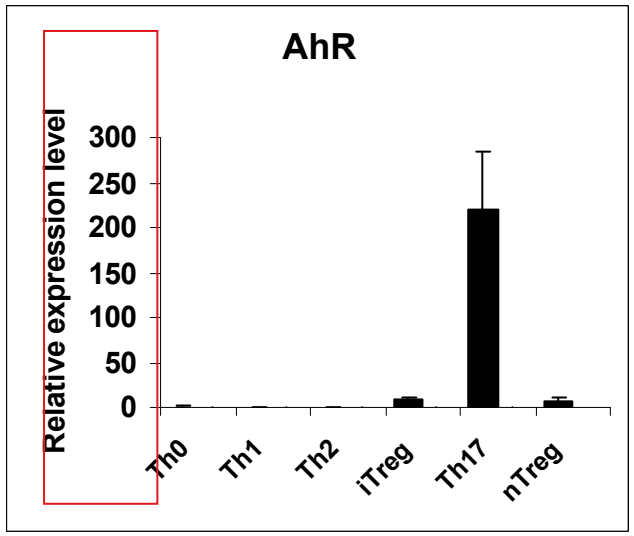
Interleukin-10 related T cell-derived inducible factor:

Laure Dumoutier, Emiel Van Roost, Didier Colau and Jean-Christoph Renauld
PNAS 97, 2000

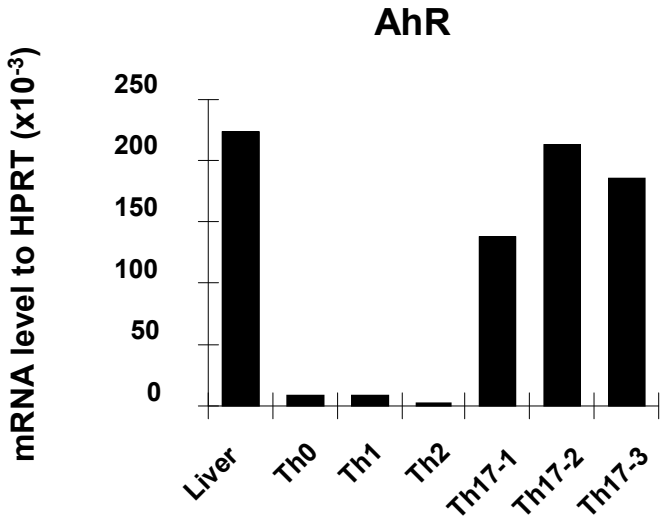
- defined as Th17 cytokine that mediates IL-23 induced dermal inflammation (Zheng et al. Nature 2007)
- induces pro-inflammatory gene expression in keratinocytes (Boniface et al, J.I. 2005)
- protects against liver injury in ConA induced hepatitis = survival factor for hepatocytes (Pan et al. Cell&Mol.Immunol.1, 2004), Zenewicz et al. Immunity 2007)
- mediates mucosal host defense (Aujila et al Nat.Med.2008, Zheng et al. Nat.Med.2008)

IL-22 receptor is expressed in liver, lung, skin, colon, pancreas, placenta but not in immune cells (Wolk et al Immunity 21, 2004)

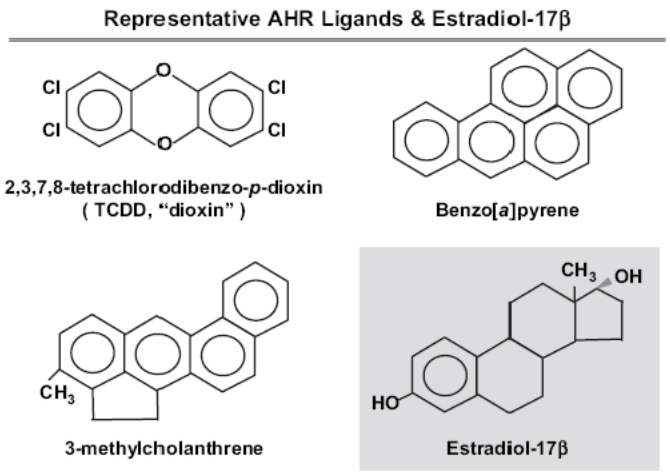


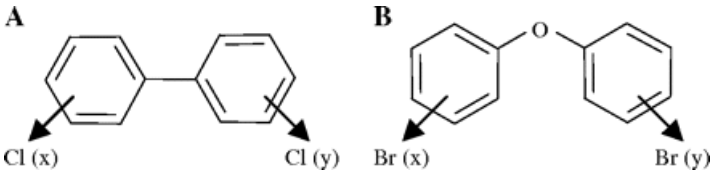


AhR expression in Th17 similar in magnitude to liver

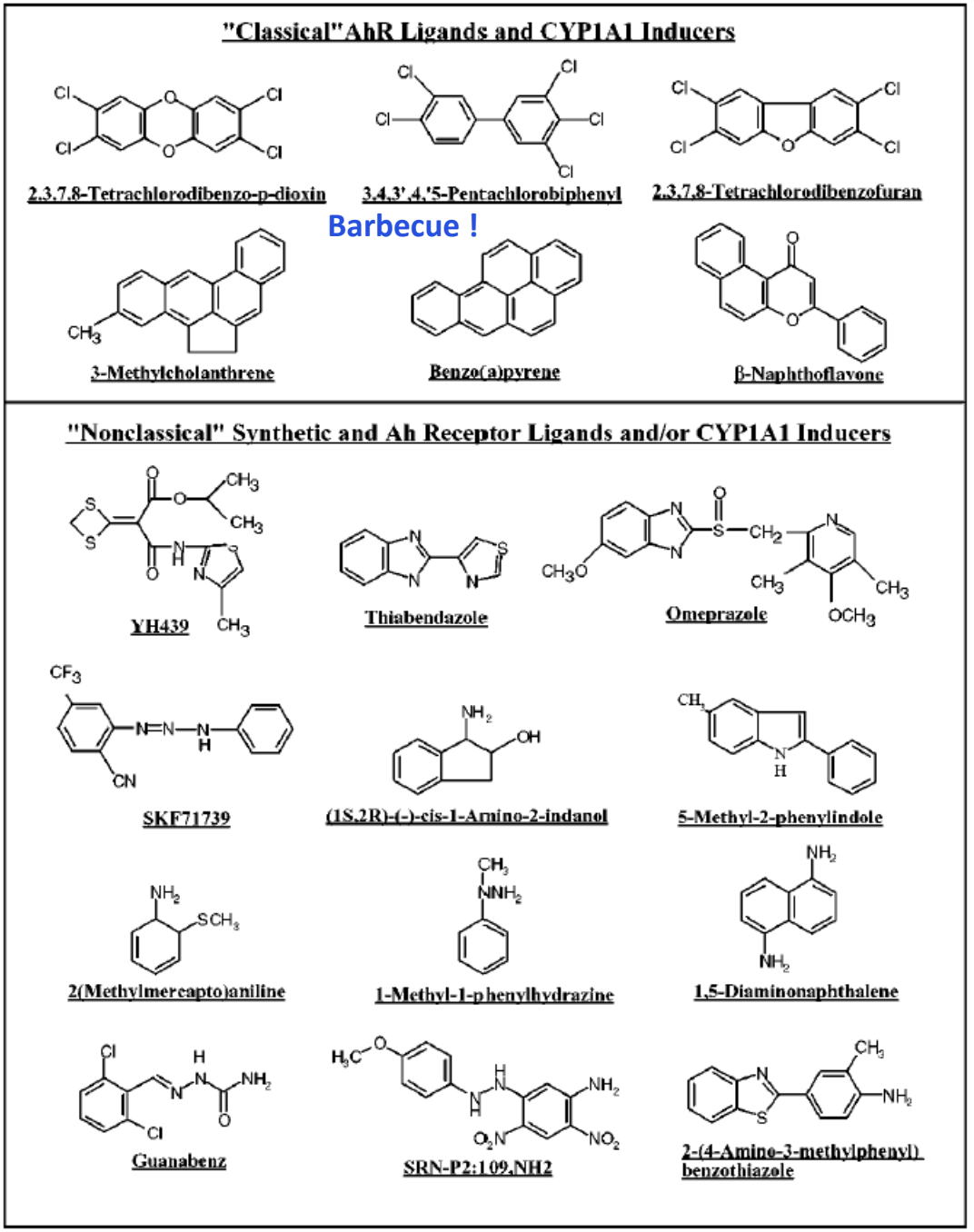


Examples of AhR ligands

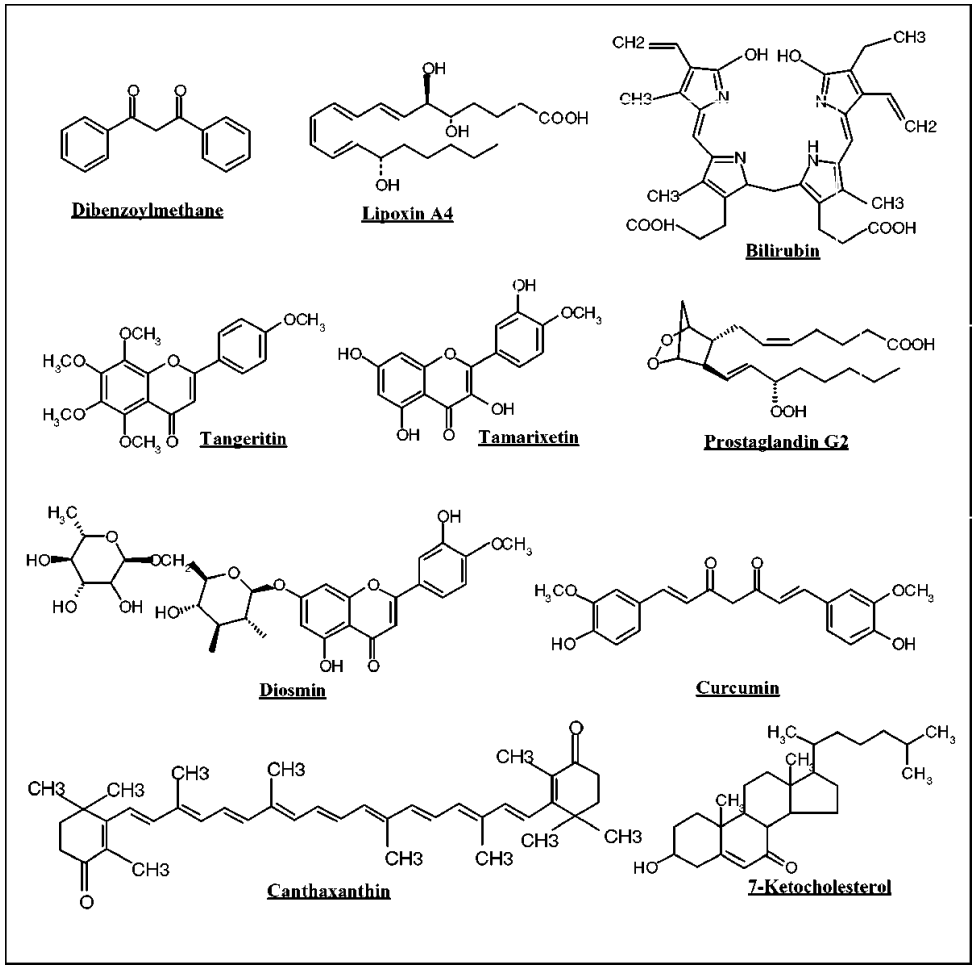




PCBs PBDEs



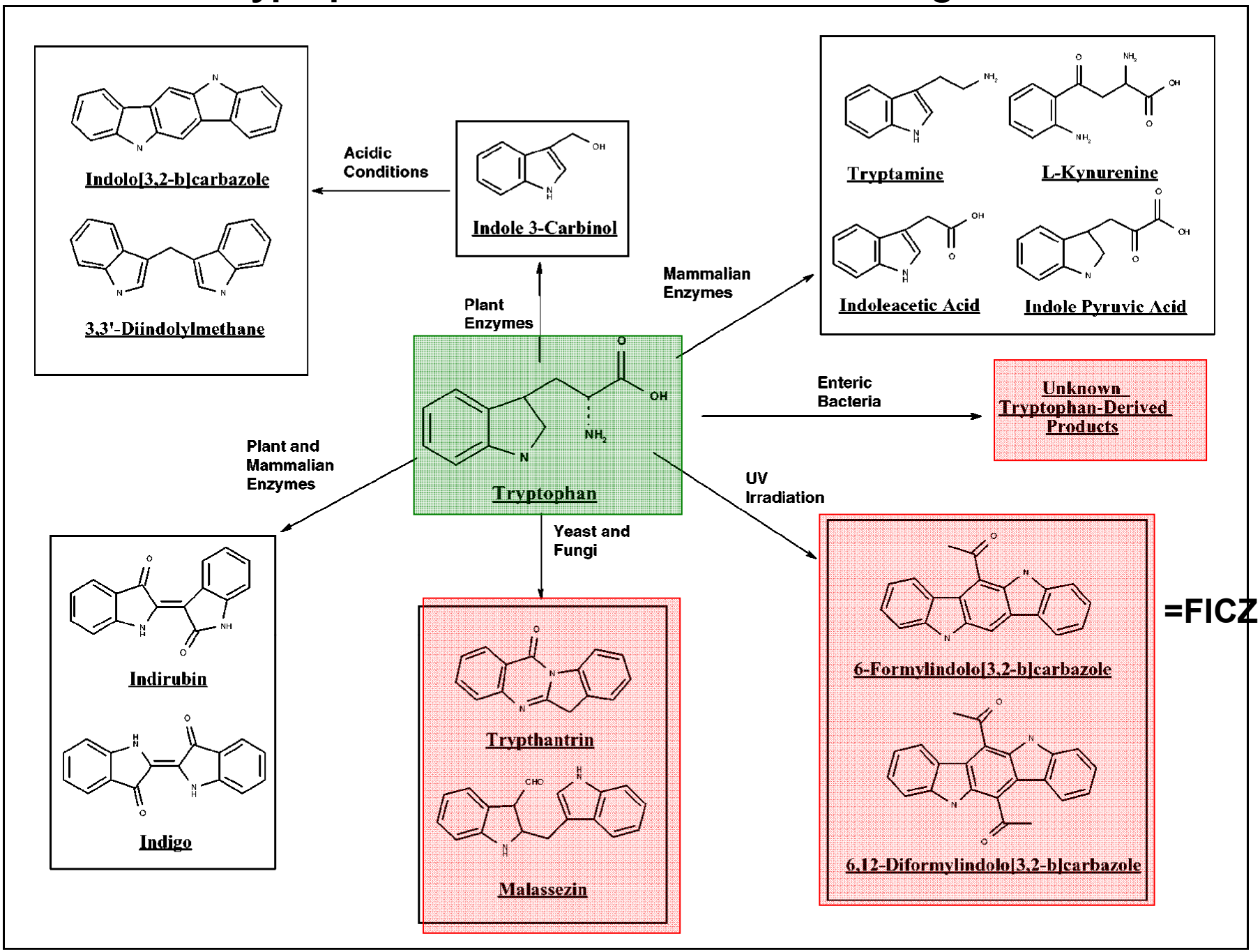
Naturally occuring AhR ligands and inducers of AhR dependent gene expression



The aryl hydrocarbon receptor is activated by modified low-density lipoprotein

Mc Millan and Bradfield, PNAS 104, 1412 (2007)

Tryptophan can be converted into AhR ligands

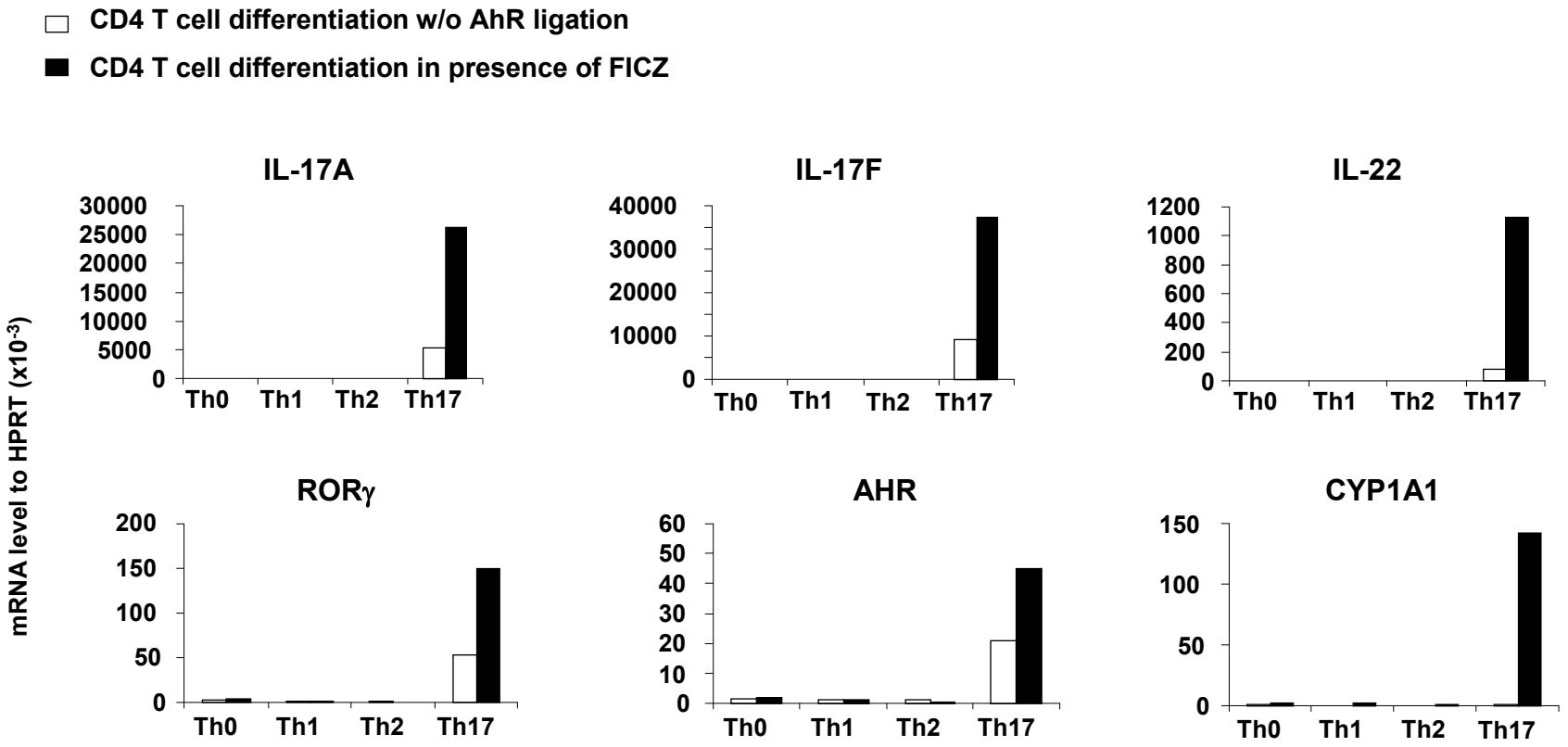


6-Formylindolo [3,2-b] Carbazole (FICZ)

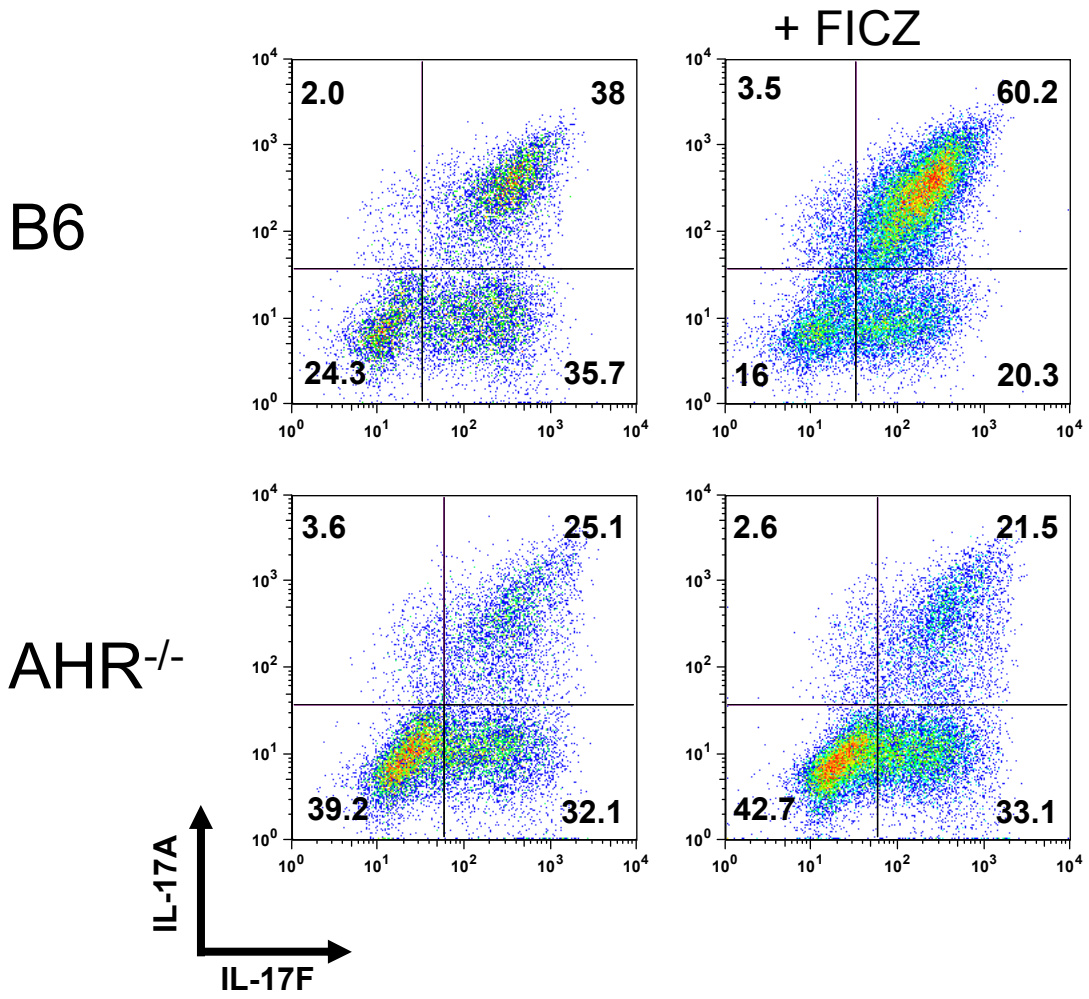
A tryptophan-derived, high affinity aryl hydrocarbon receptor ligand (Kd=7x10-11M).
Proposed to be an endogenous AhR ligand

Does FICZ influence expression of Th17 cytokines ?

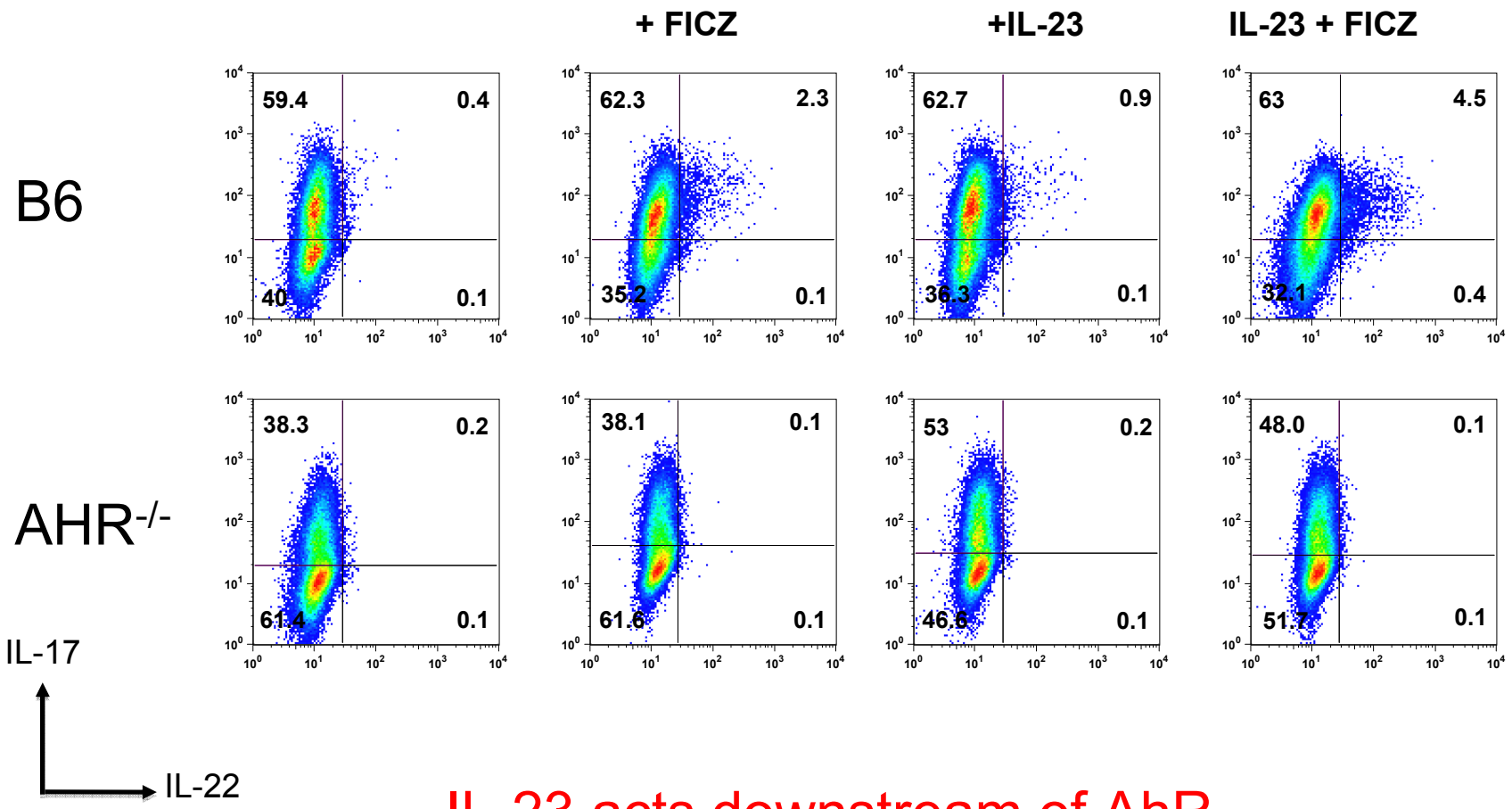
AhR activation increases expression of Th17 cytokines and induces CYP1A1 in Th17 T cells



AhR ligation promotes Th17 differentiation



AhR ligation induces IL-22 expression – what is the role of IL-23 ?



IL-23 acts downstream of AhR

Tryptophan not a stranger in immunology

Tryptophan is an essential amino acid because it is the only precursor for the endogenous biosynthesis of nicotinamide adenine dinucleotide (NAD).

Indoleamine 2,3-dioxygenase

tryptophan + O2 => N-formylkynurenine [IDO] (NAD)

IFNγ , regulatory T cells, CTLA4 –> induce IDO expression by macrophages
Tryptophan depletion is associated with immunosuppression involving T cell arrest and anergy.

Treg may deprive Th17 of potential AhR ligands by promoting tryptophan depletion

AhR polymorphisms that influence ligand binding affinity

AhR^{b-1}: B6
AhR^{b-2} : BALB/c, CBA, A/J…
AhR^{d}: DBA/2, 129, AKR, SWR….most humans

AhR^{d} type AhR encodes polypeptide of 848 aa (48 aa longer than B6)
Mutation Ala^{375} to Val (ligand binding site) and T to C mutation at termination codon resulting in elongated read-through (also present in AhR^{b-2})

Humans have equivalent Ala381 to Val mutation and elongation at C terminus

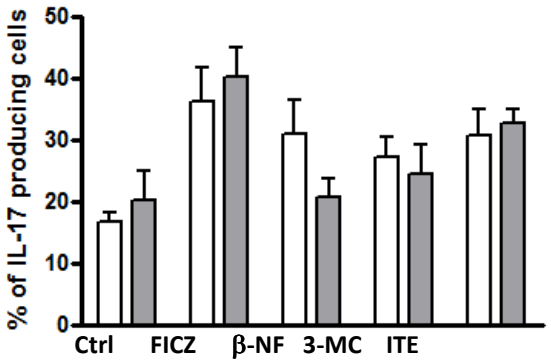
Reduced affinity (10 to 100 fold) measured in hepatic cytosol using specific binding of radioligand

A congenic mouse strain expressing the AhR^{d} version on a B6 background (B6.D2N.AhR) was generated by Bradfield et al.

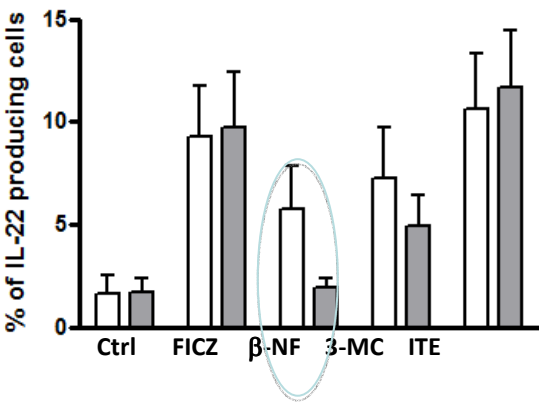
Potentiating Th17 differentiation with AhR ligands

Comparison of different AhR ligands in Th17 differentiation from B6 and B6 AhR^{d} mice

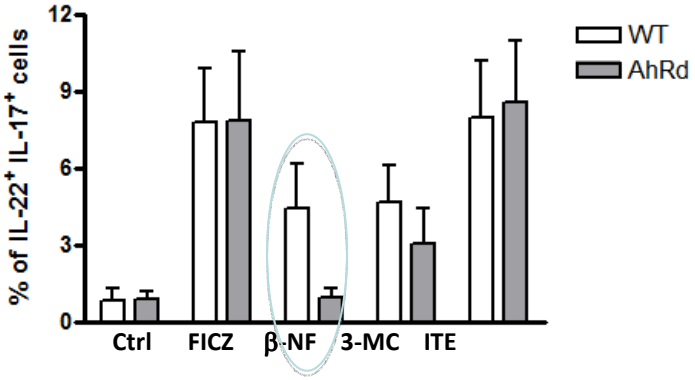
% IL-17 producers



% IL-22 producers

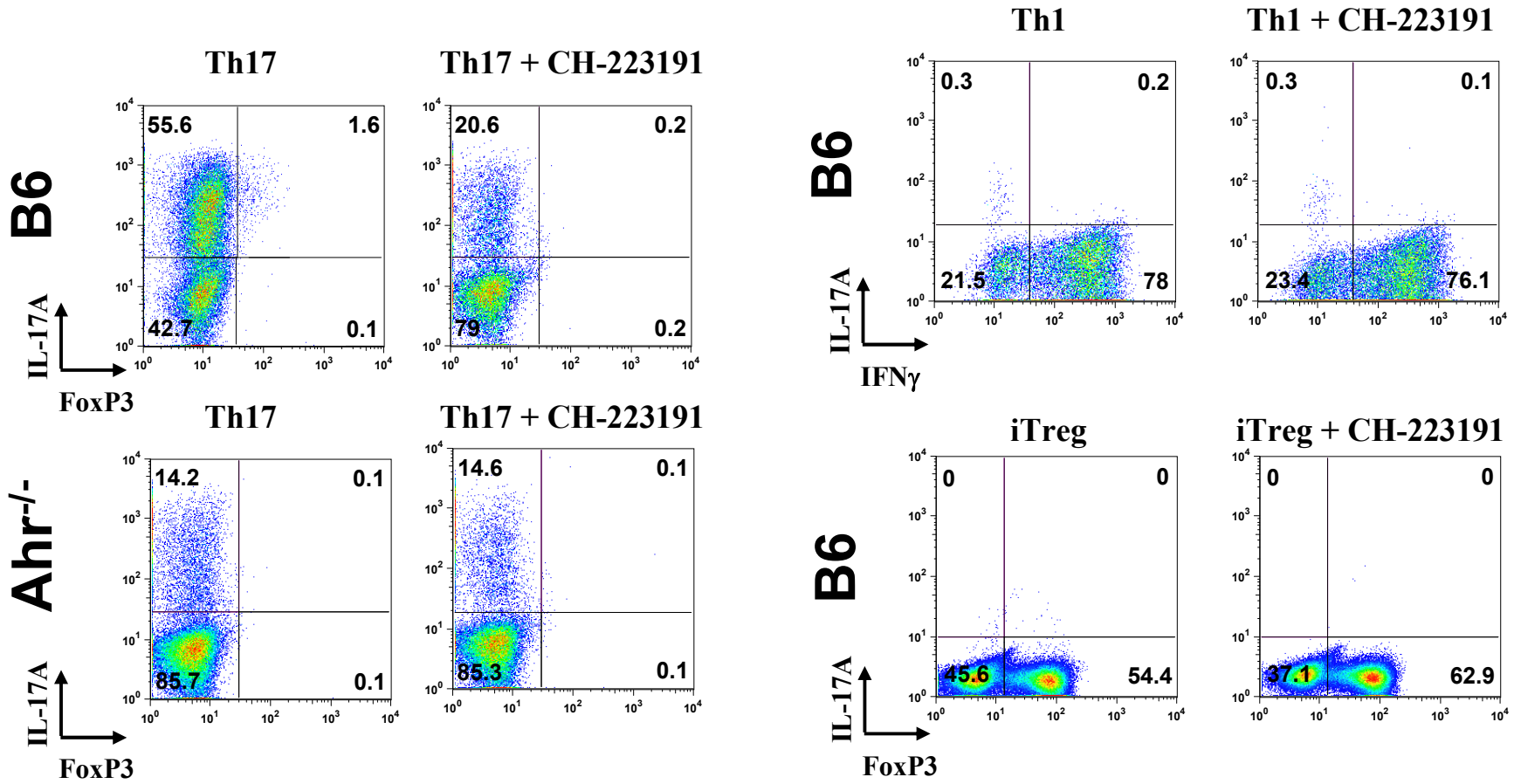


% double producers

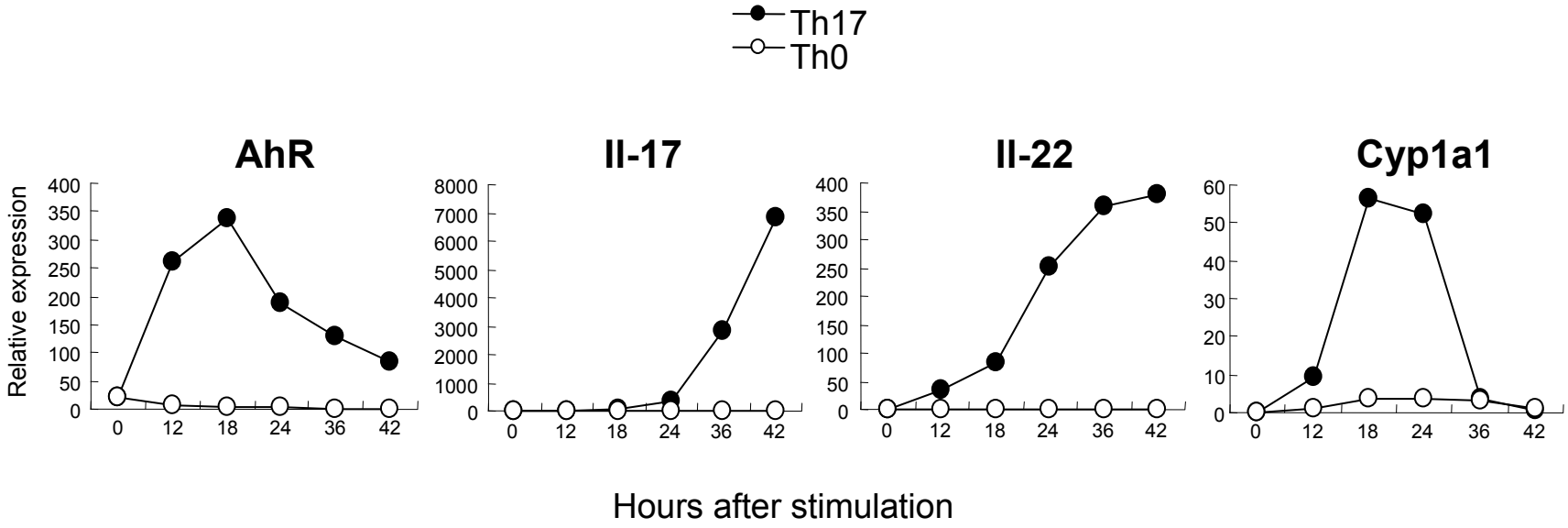


Natural AhR ligands shape Th17 differentiation in vitro

AhR antagonist dampens Th17 differentiation, but does not influence Th1 or iTreg differentiation



Natural AhR agonists in medium induce CYP1A1



How to make the best Th17 cells……

Identification of the Tryptophan Photoproduct 6-Formylindolo[3,2-*b*]carbazole, in Cell Culture Medium, as a Factor That Controls the Background Aryl Hydrocarbon Receptor Activity

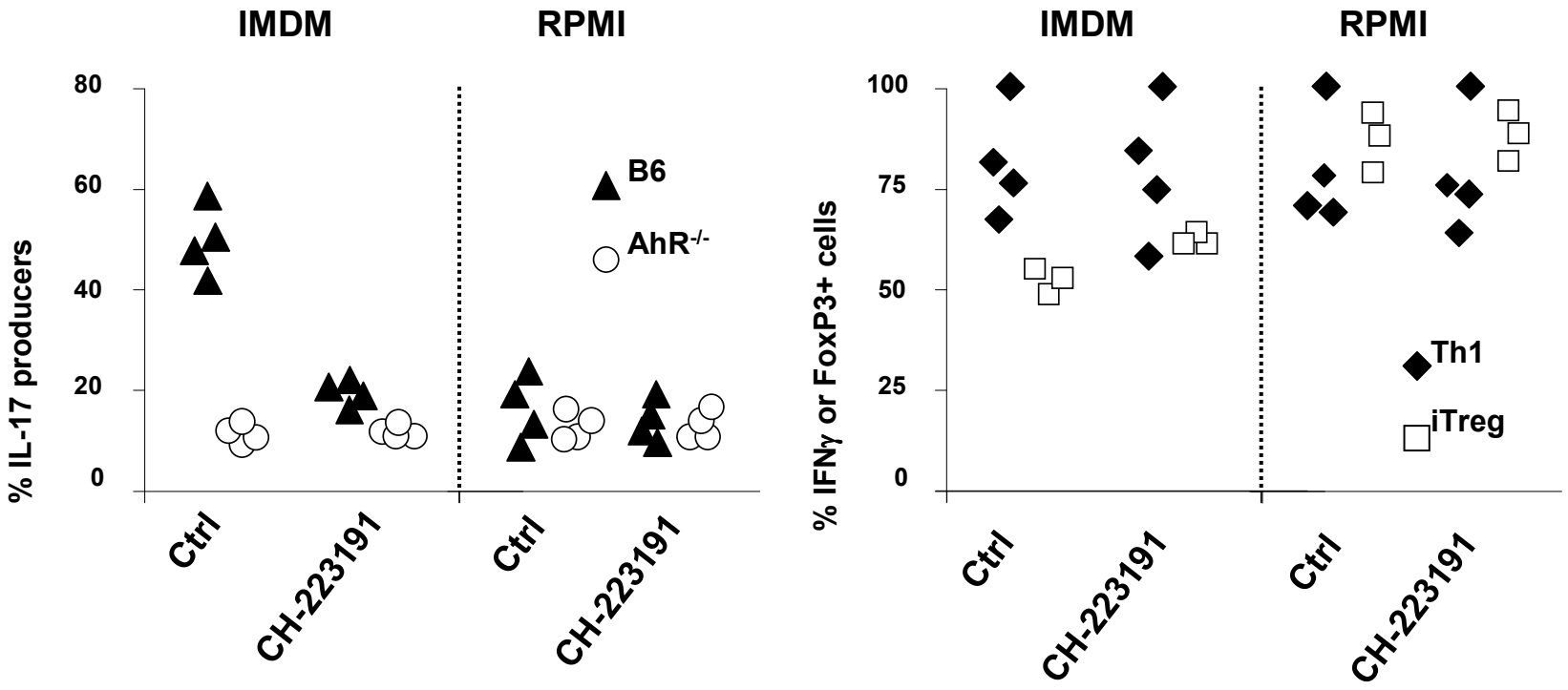
Mattias O¨ berg,[1,2,]* Linda Bergander,[1,]† Helen Ha°kansson,* Ulf Rannug,† and Agneta Rannug*
*Institute of Environmental Medicine, Karolinska Institutet, SE-171 77 Stockholm, Sweden, and †Division of Cellular and Genetic Toxicology, Stockholm University, Stockholm, Sweden

TOXICOLOGICAL SCIENCES 85, 935–943 (2005)

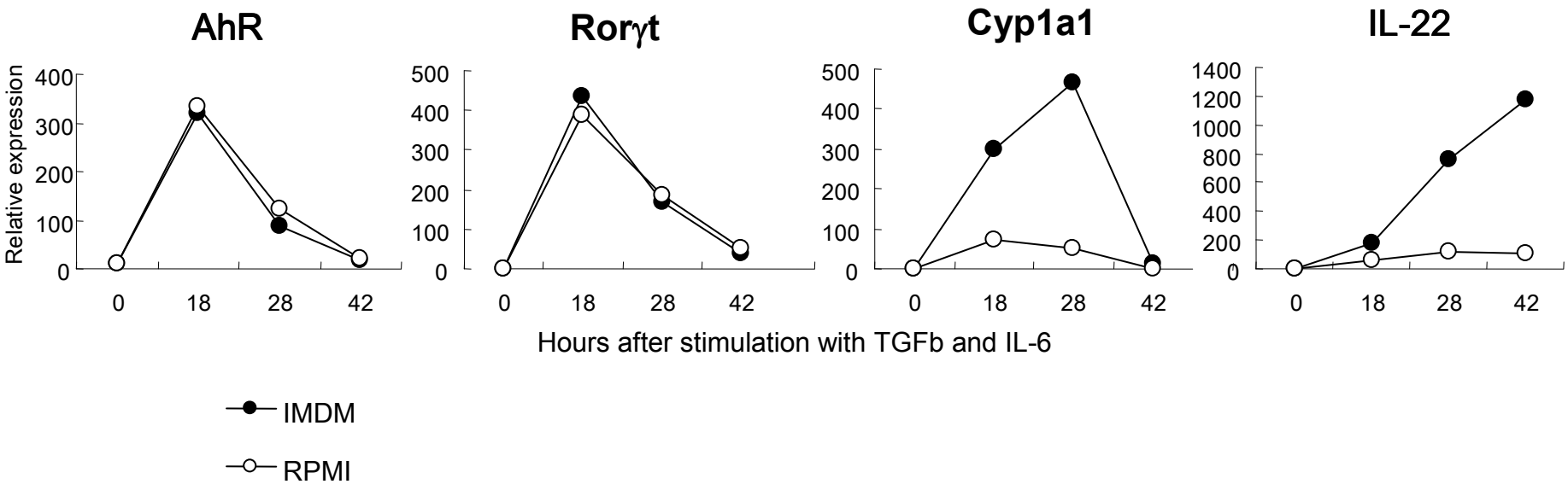
IMDM medium contains more potential AhR ligands than RPMI medium

	IMDM (conc.in mg/liter)	RPMI (conc. in mg/liter)
L-Tryptophan	16	5
L-Phenylalanine	66	15
L-Tyrosine	103	29
L-Histidine	42	15

Th17 differentiation is impaired in RPMI medium



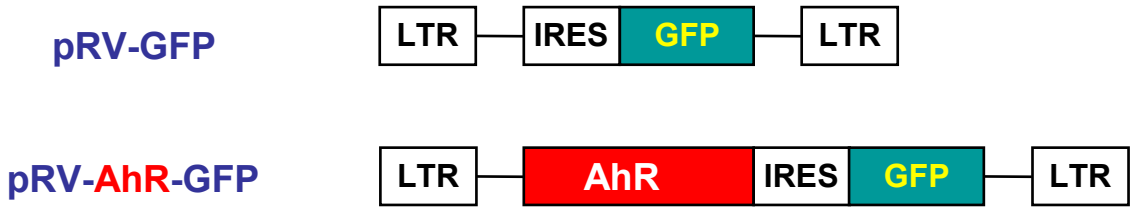
RPMI does not provide AhR activation



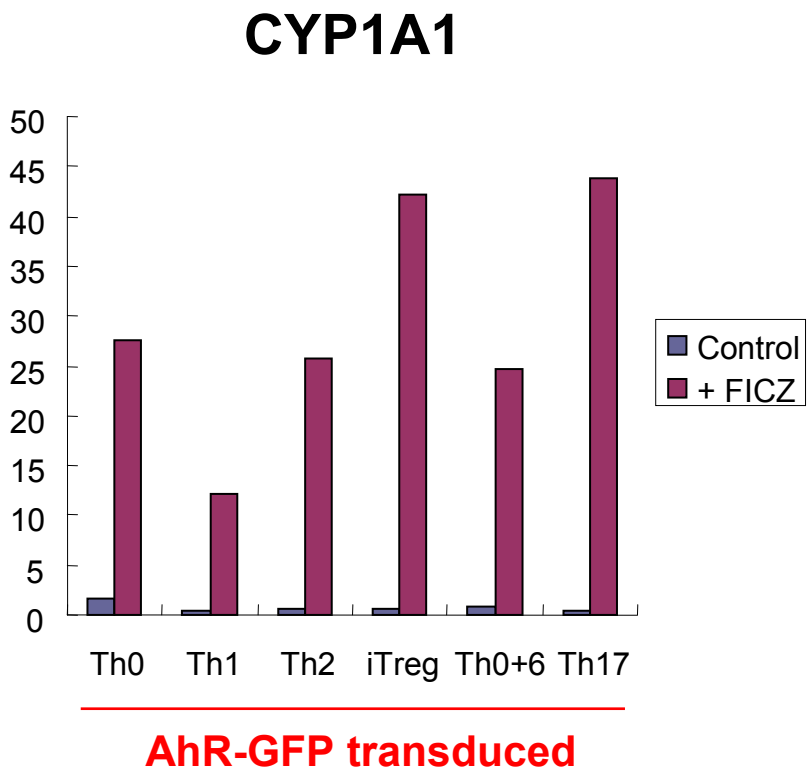
= don’t use RPMI if you want to look at Th17 responses !

Is expression of AhR necessary and sufficient for production of IL-22 and increased IL-17 ?

Retroviral transduction of *AhR*
into naïve CD4^{+} T cells from AhR-/- mice

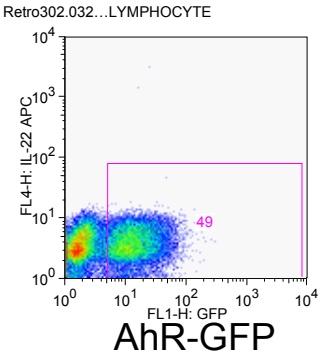


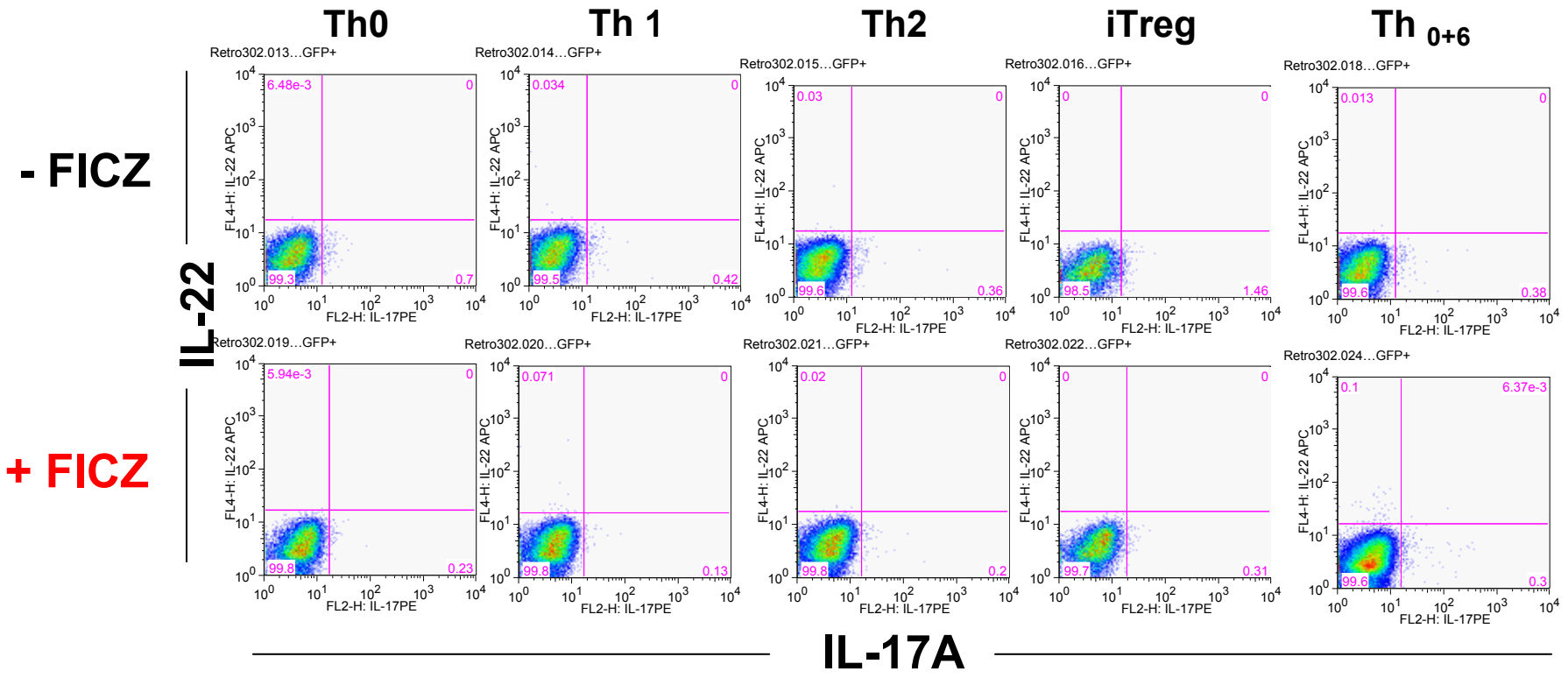
AhR transduction has functional consequences in all CD4 T cell subsets



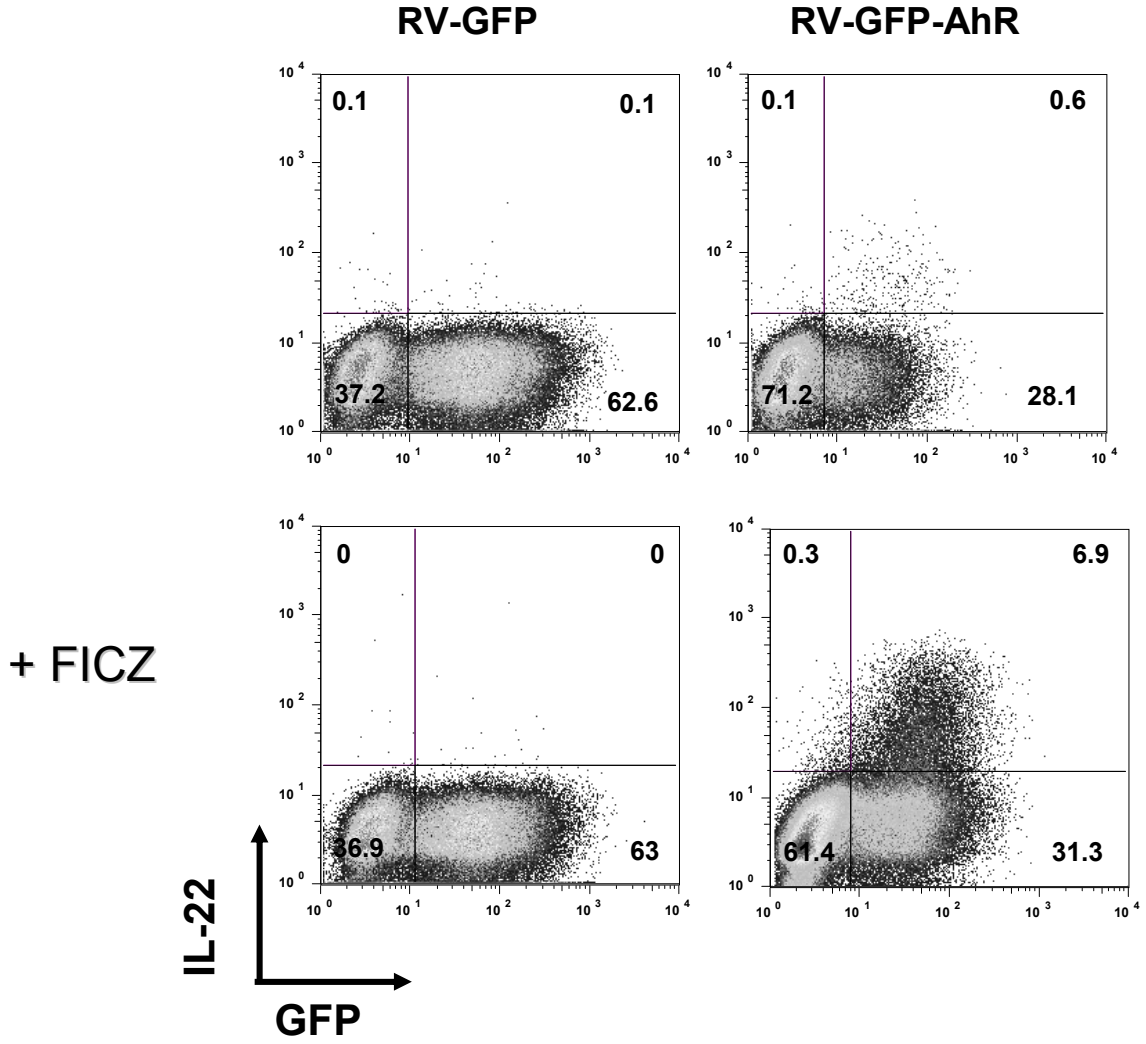
BUT :AhR expression alone does not induce IL-17 or IL-22

RV-AhR-GFP transfected; gated: live, GFP+

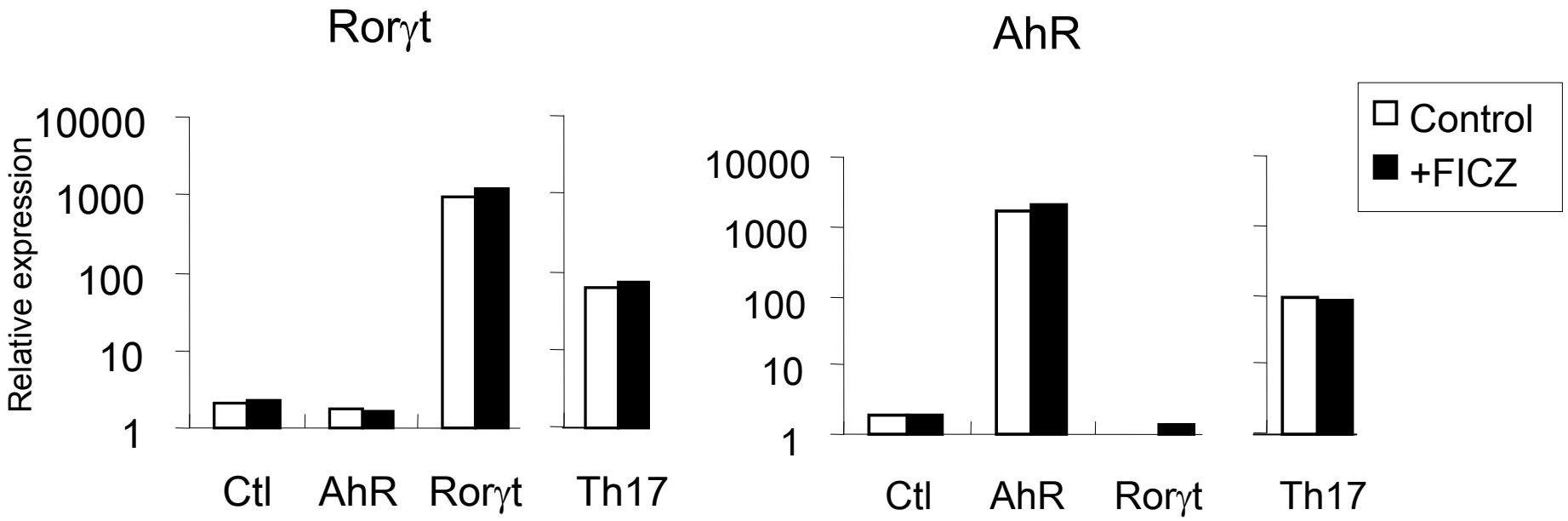




AhR transduction under Th17 conditions restores IL-22 production

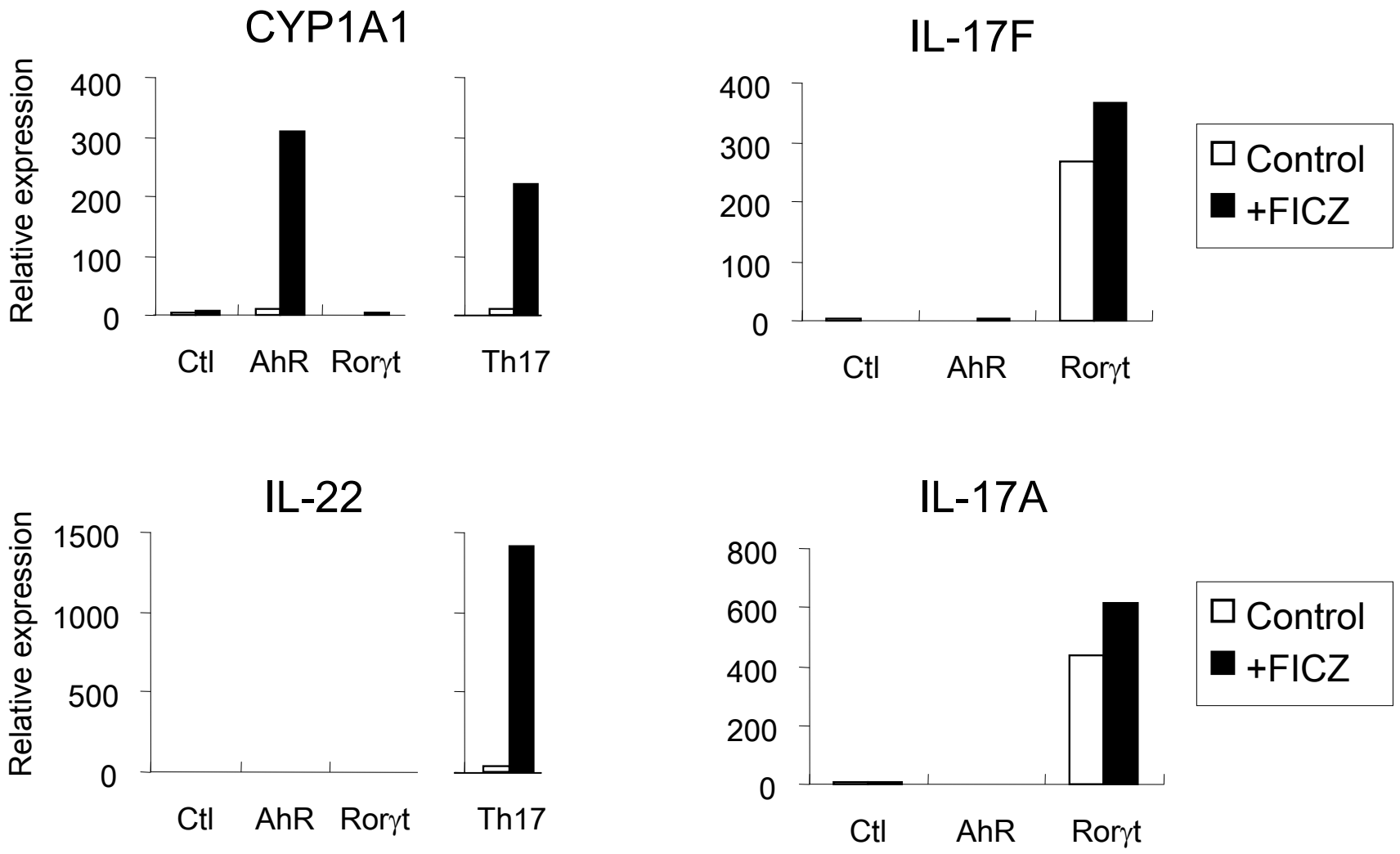


Retroviral transduction of naïve CD4$^+$ T cells with *AhR or Rorγt*

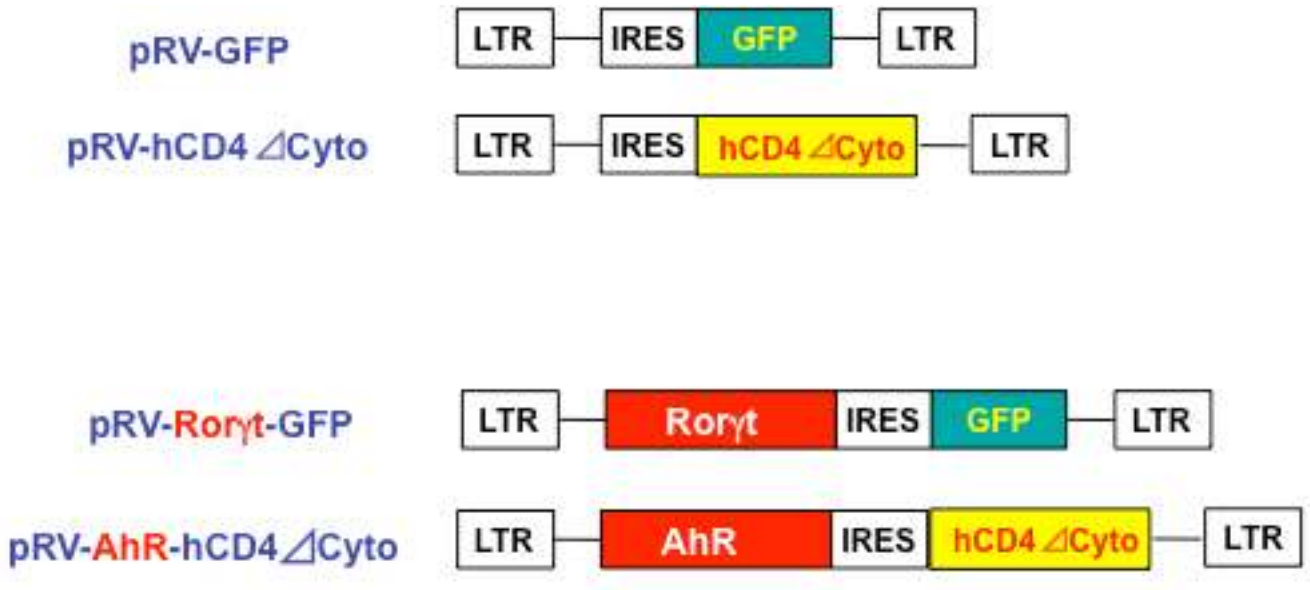


Although Th17 cells express both transcription factors, they are regulated independently.

Retroviral transduction of naïve CD4$^+$ T cells with *AhR or Rorγt*



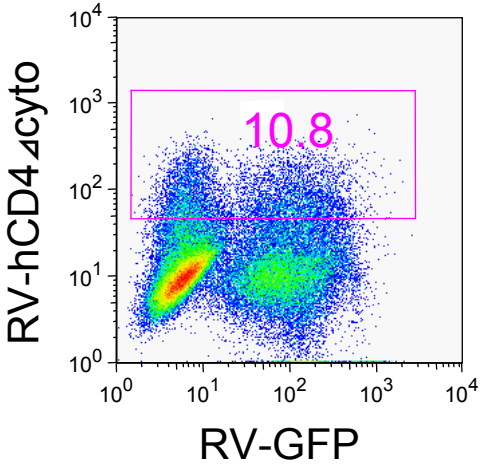
Can AhR and RORγt cooperate in naïve CD4 T cells for induction of IL-22 ?

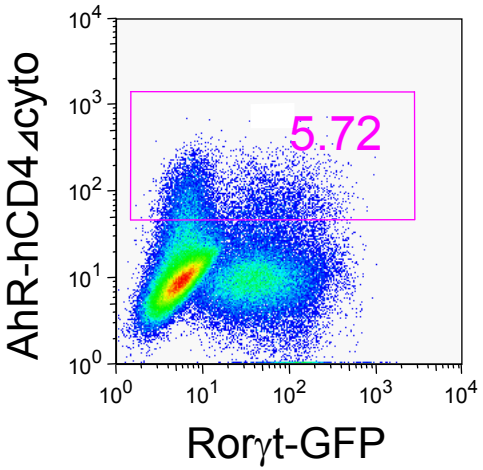


FACS sorted naïve wt CD4 T cells were double-transduced with AhR and Rorγt in the presence of FICZ.

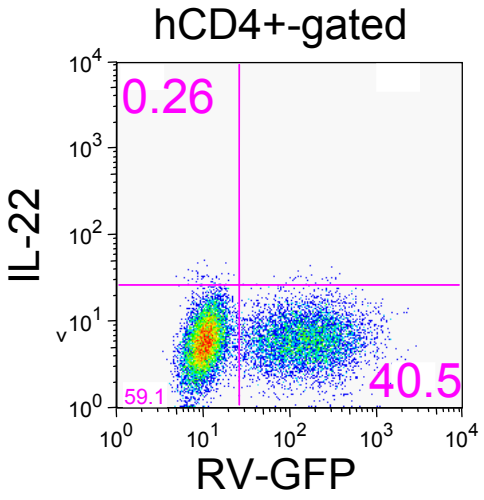
Retroviral co-transduction of *AhR and Rorgt*

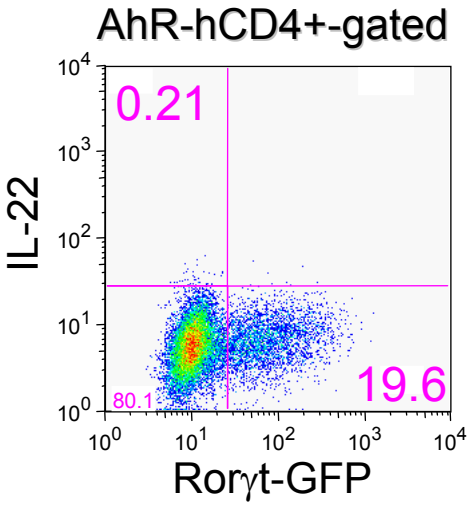
+FICZ





+FICZ





Conclusions

Rorγt does not control the expression of AhR

Forced expression of AhR or Rorγt is not sufficient for the production of IL-22 by Th0, Th1, and Th2 cells

Moreover, co-expression of AhR and Rorgt fails to upregulate IL-22, suggesting that a missing key molecule induced in Th17 cells may be important in the regulation of IL-22 expression

Rorγt seems to be involved in the IL-17-, but not IL-22-axis in Th17 cells.

Does AhR ligation play a role in the functionality of Th17 T cells in vivo ?

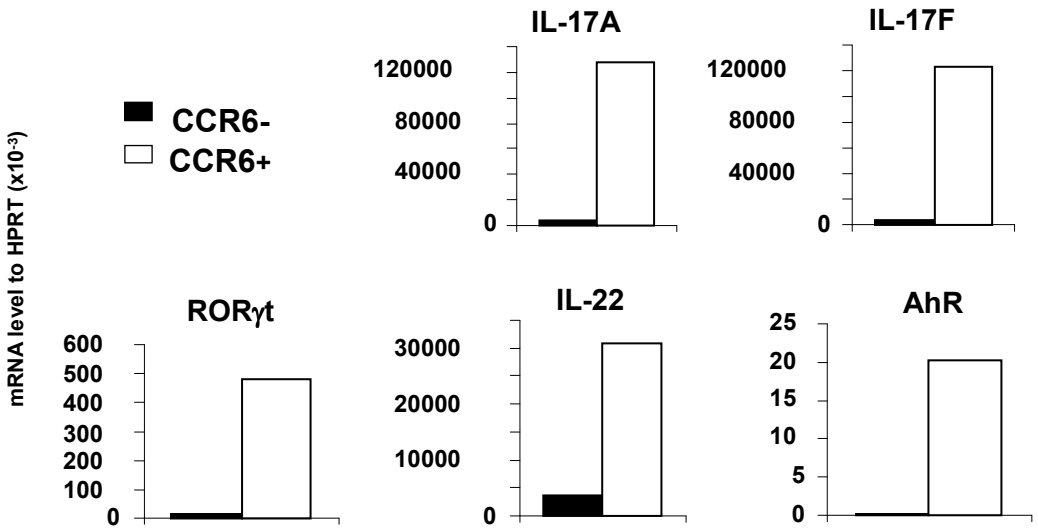
EAE (experimental autoimmune encephalomyelitis), a mouse model for multiple sclerosis in which Th17 play a major pathogenic role

Disease is induced by immunization with MOG

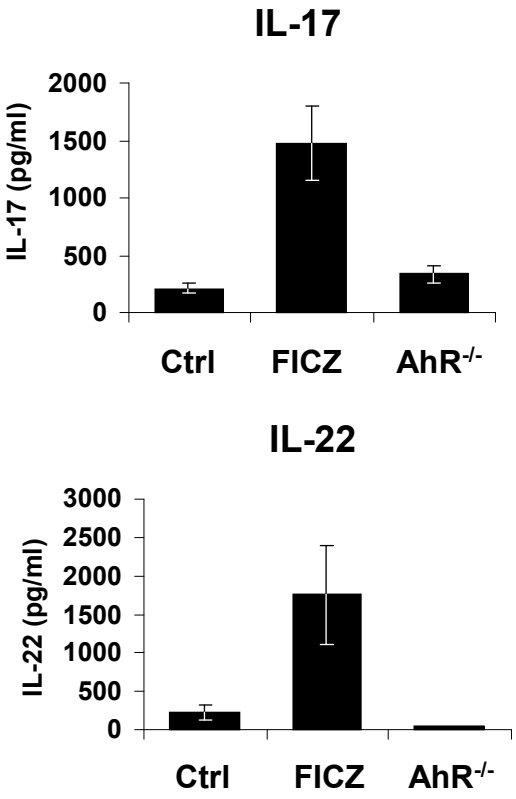
in presence or absence of FICZ

AhR is expressed and functional in Th17 cells in vivo

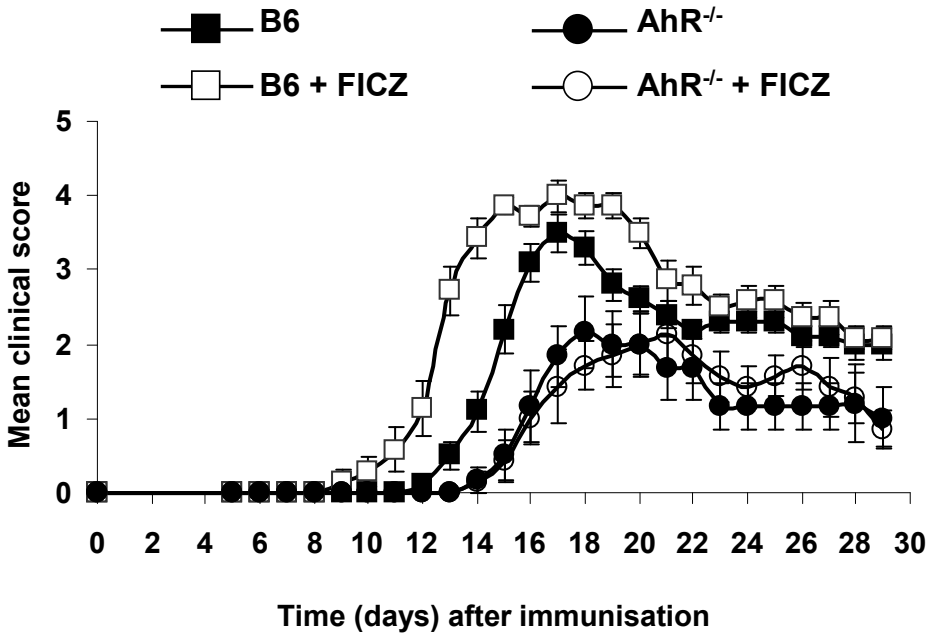
dLN of MOG/CFA immunised mice (d7)
FACS sorted for CCR6+ CD4 T cells



ELISA



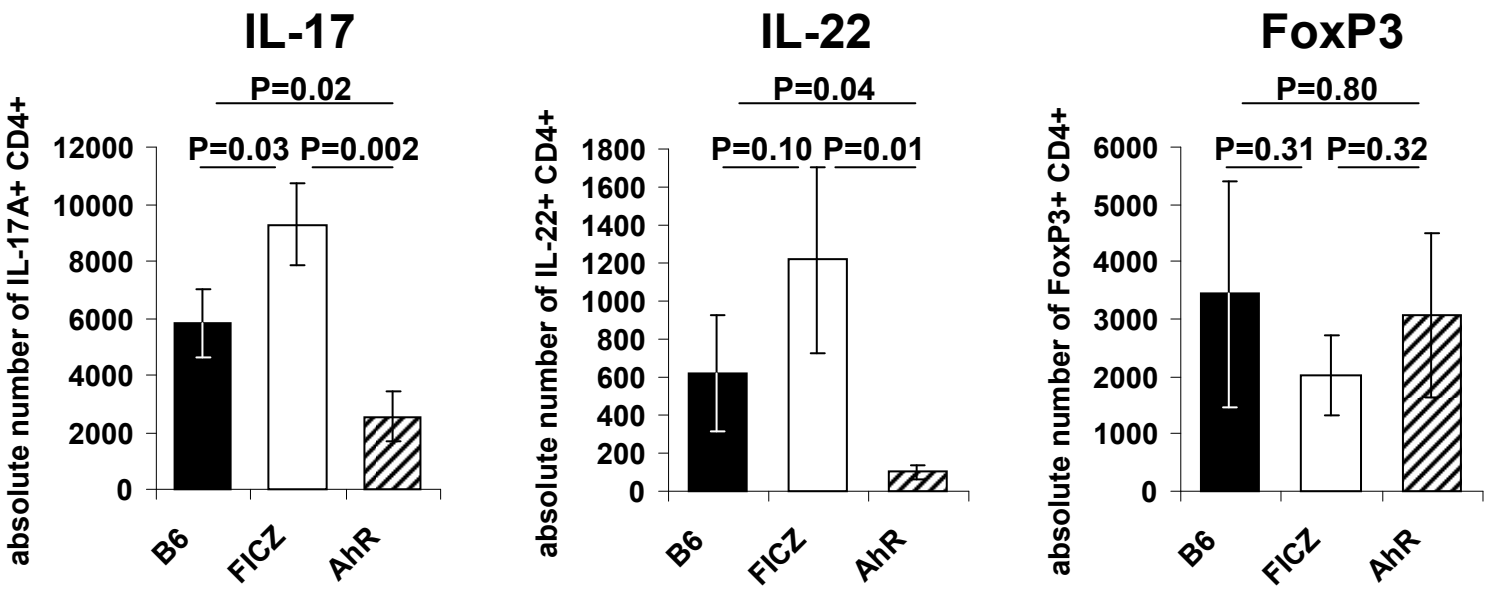
AhR ligation exacerbates EAE onset and severity



Group	Incidence	Mean day of onset	Mean Max score	Score >4
B6	12 of 12 (100%)	13.9 (±1.1)	3.6 (±0.5)	0 of 12 (0%)
B6 + FICZ	14 of 14 (100%)	11.7 (±1.2)	4.4 (±0.5)	5 of 14 (42%)
AhRko	12 of 14 (86%)	15.6 (±1.0)	2.3 (±1.0)	0 of 14 (0%)
AhRko + FICZ	7 of 8 (88%)	15.8 (±1.2)	2.3 (±1.1)	0 of 8 (0%)

AhR ligation results in increased numbers of IL-17 and IL-22 producers in spinal cord, but does not influence number of Treg

Spinal cord of MOG/CFA immunised mice

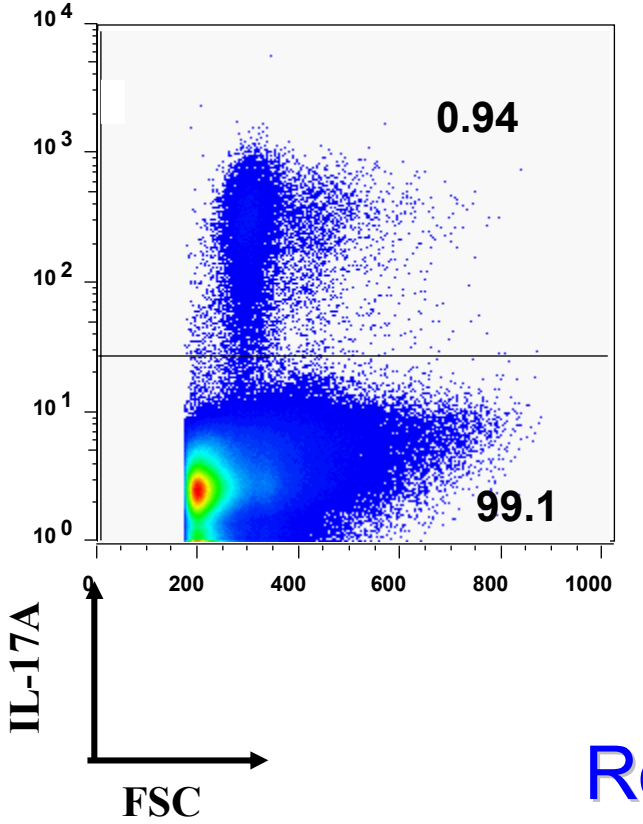


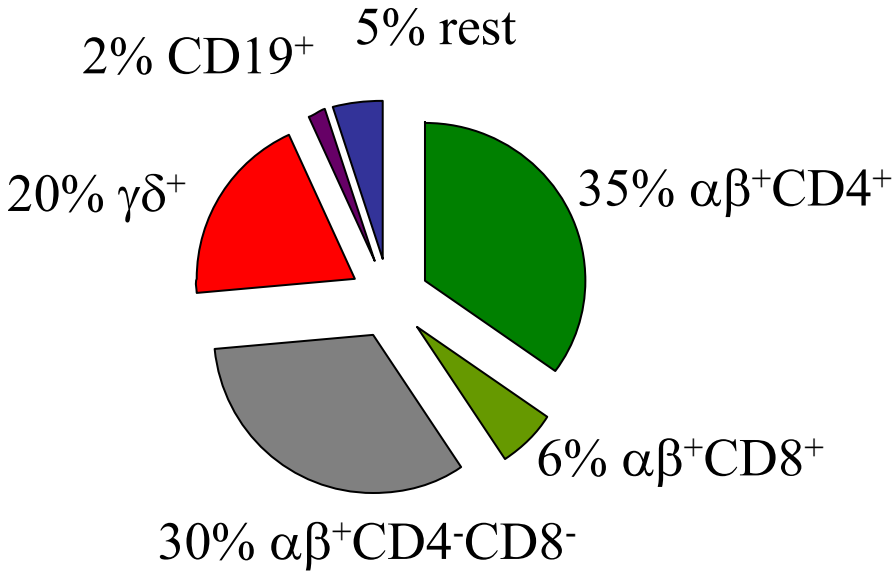
Autoimmune diseases are multifactorial:
intrinsic factors: genetics, hormones, age and gender
environmental factors: infections, diet, and chemicals

The link of AhR to the T_H17 program opens intriguing possibilities regarding the potential of environmental factors to initiate or augment autoimmune conditions

The involvement of AhR ligation by environmental toxins may explain the increasing prevalence of many autoimmune diseases in highly industrialized countries

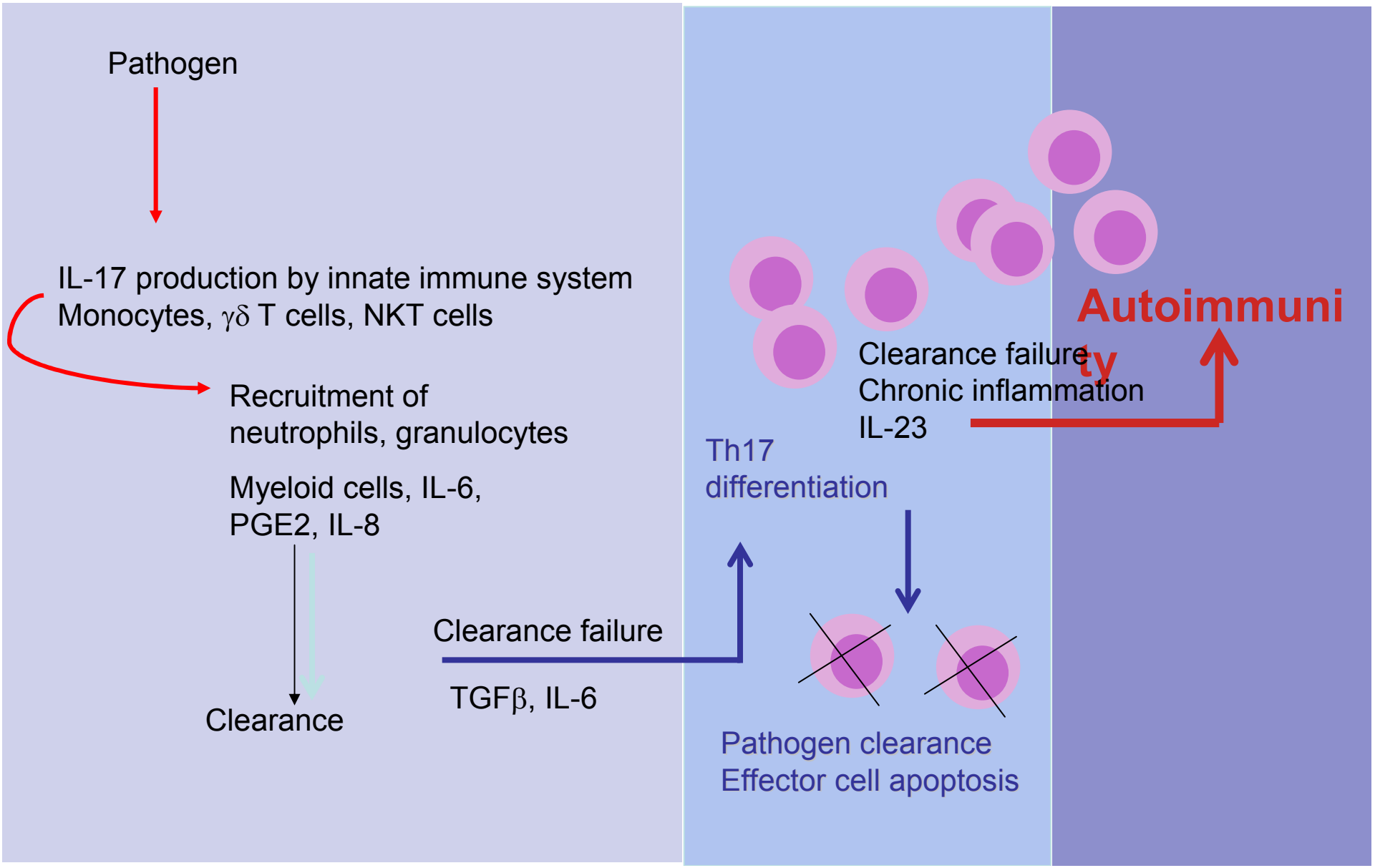
Many IL-17 producers in non-immune lymph node are not CD4 T cells….



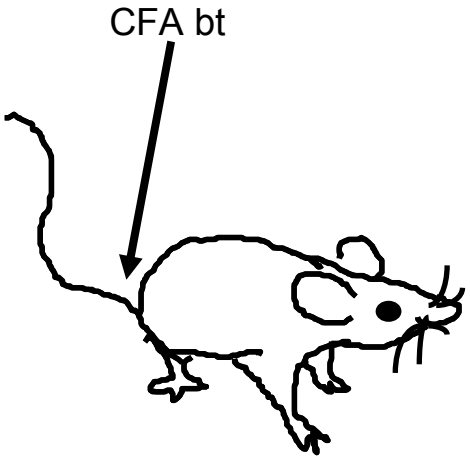


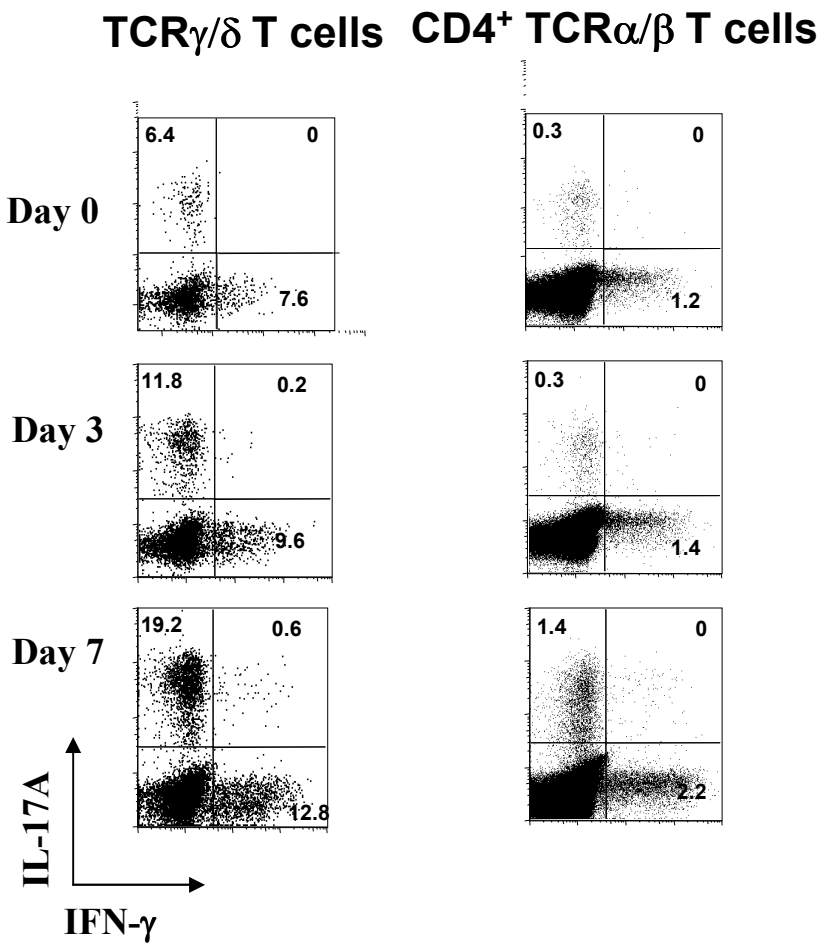
Role of AhR in TCRγδ T cells ?

Sequential stages of IL-17 mediated immune responses

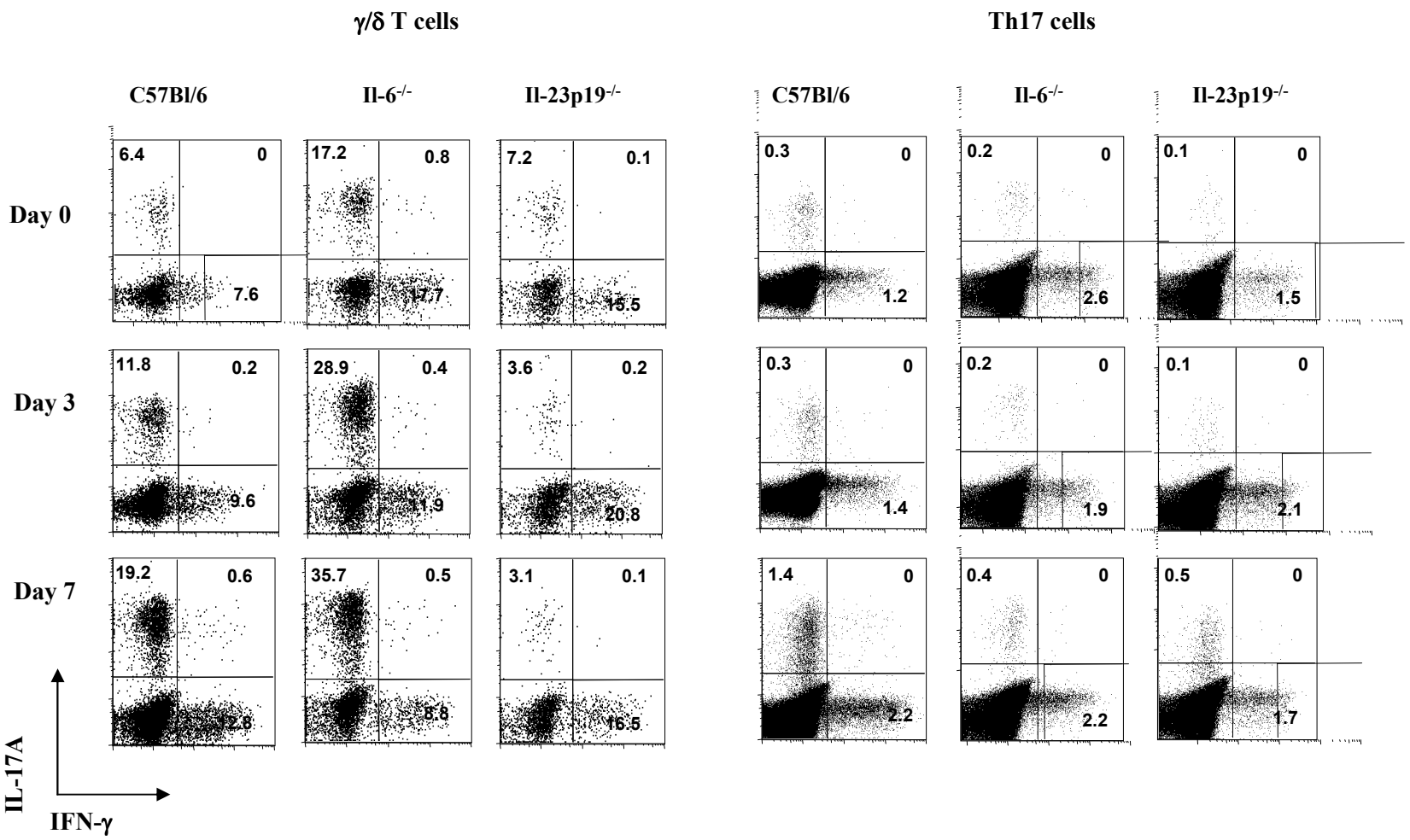


γδ T cells are poised to react to mycobacterial stimulus, whereas Th17 cells develop later

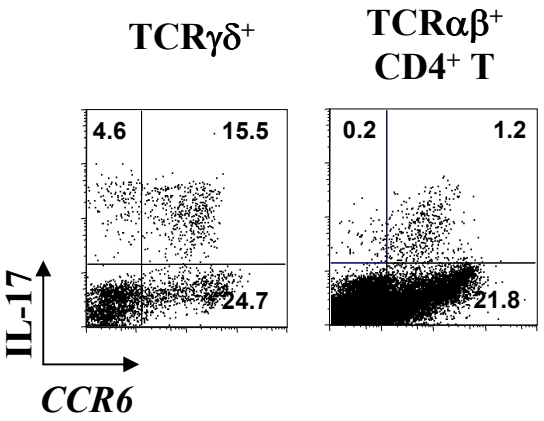


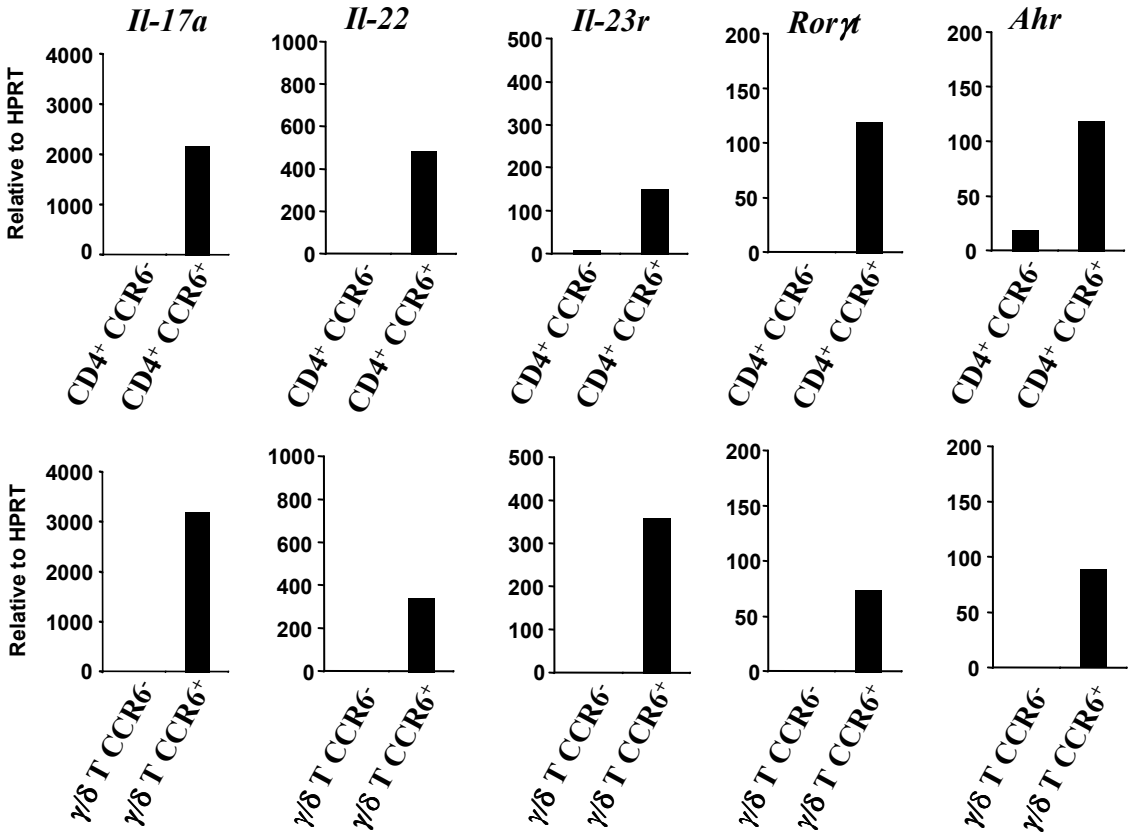


IL-6 is needed for the development of Th17 cells, but not IL-17 producing γδ T cells - IL-23 is crucial for both

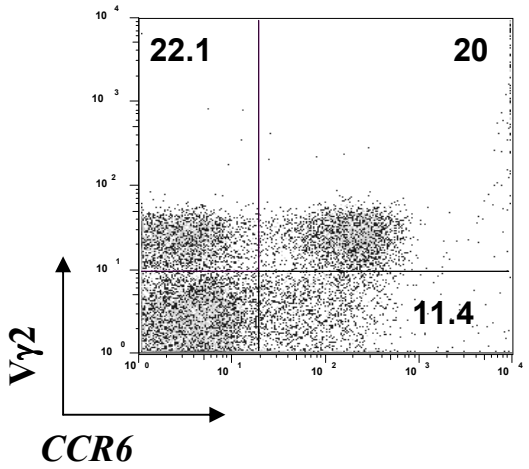


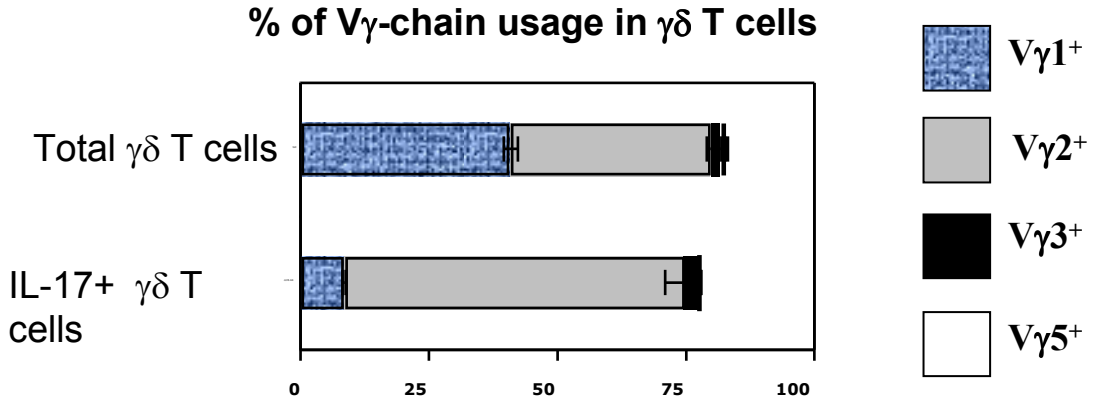
Gene expression profiles in IL-17 producing γδ T cells and Th17 are similar



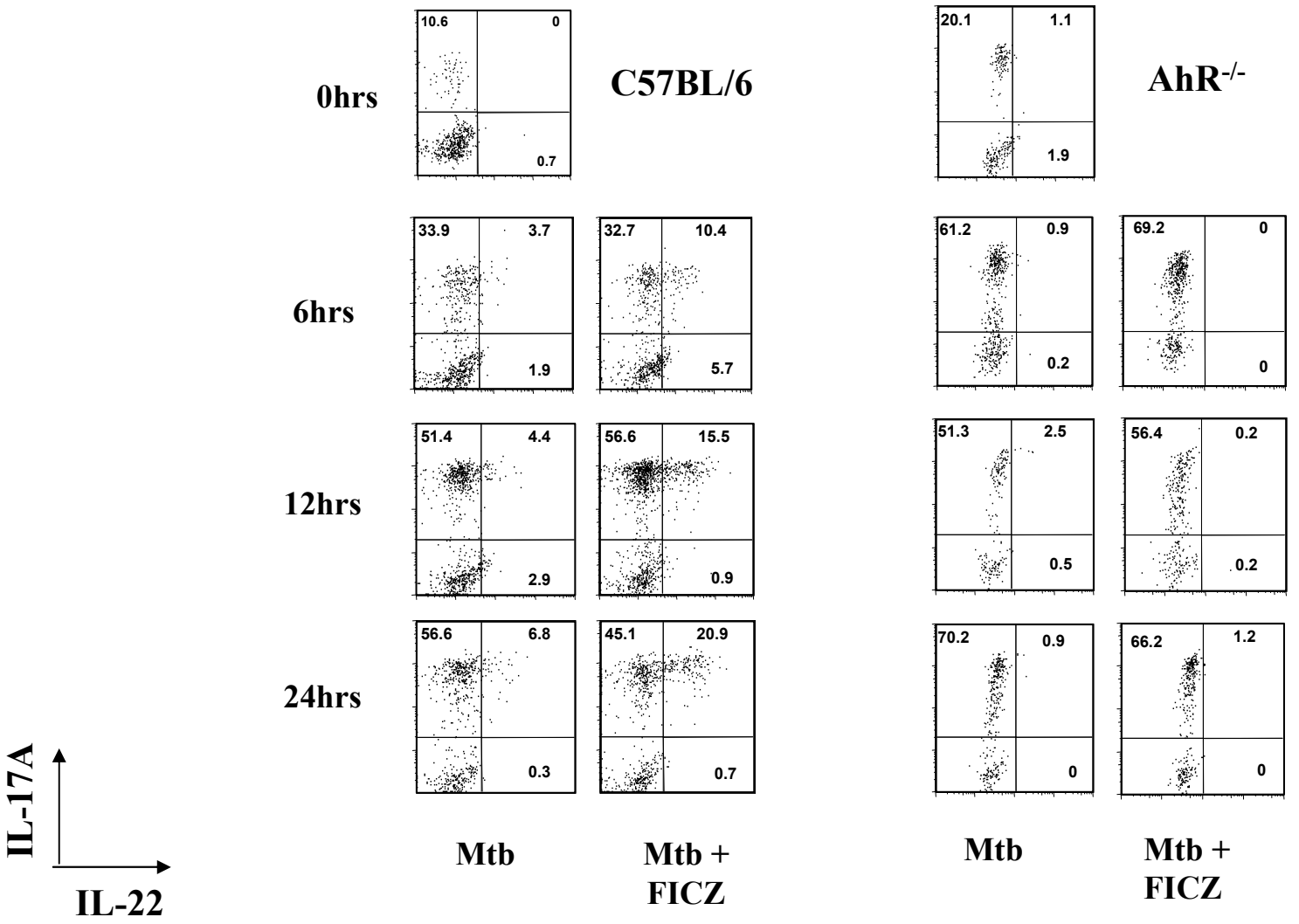


The majority of IL-17 producing γδ T cells express Vγ2





IL-17 producing γδ T cells depend on AhR ligation to produce IL-22



Further roles for AhR in the immune system….

Psoriasis: a T cell mediated (auto)immune disease

The connection of IL-22 to psoriasis preceded the discovery of Th17 cells

IL-22 Inhibits Epidermal Differentiation and Induces Proinflammatory Gene Expression and Migration of Human Keratinocytes

Katia Boniface*, François-Xavier Bernard, Martine Garcia*, Austin L. Gurney§, Jean-Claude Lecron*, and Franck Morel

The Journal of Immunology, 2005, 174: 3695-3702

The AhR ligand FICZ is generated by exposure to UVB in the skin

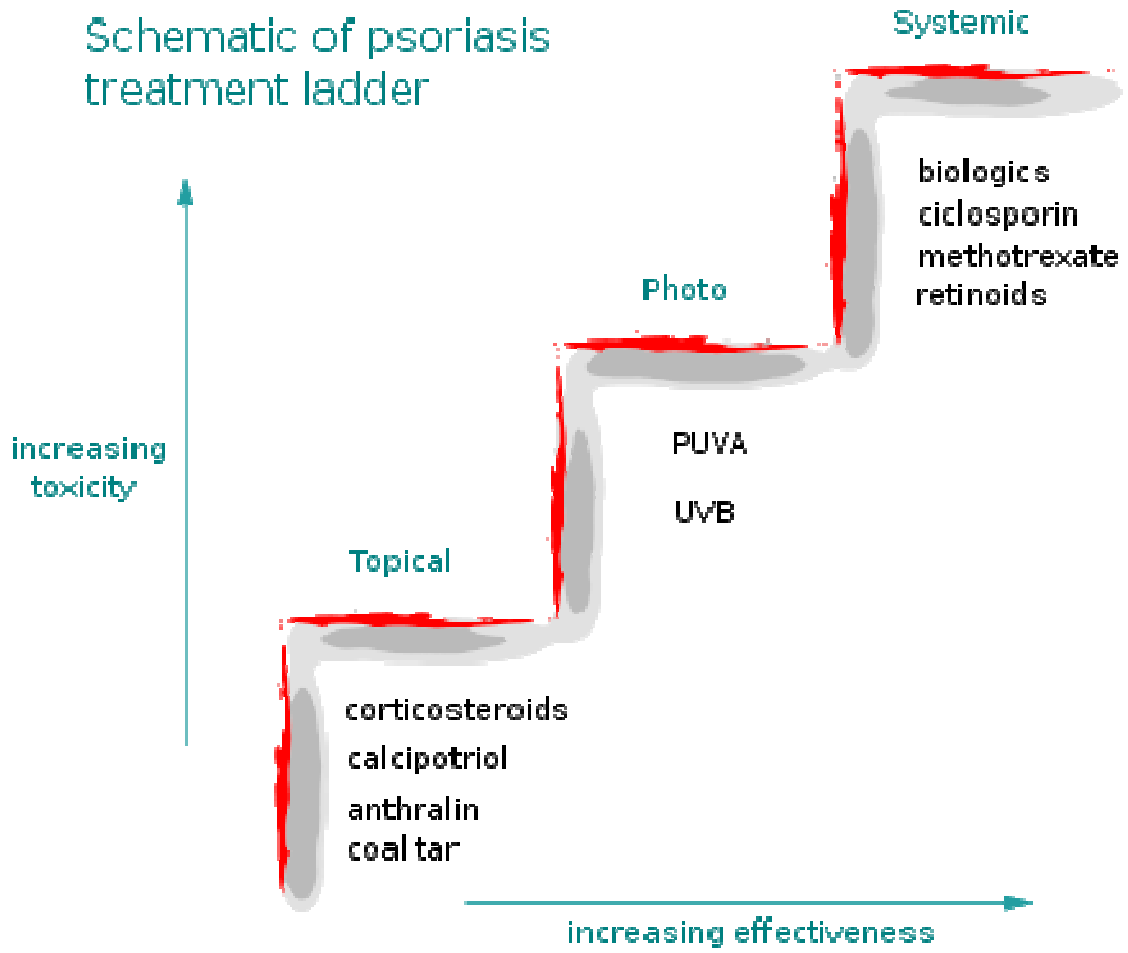
Lightening up the UV response by identification of the arylhydrocarbon receptor as a cytoplasmatic target for ultraviolet B radiation

Ellen Fritsche*, Claudia Scha¨ fer*, Christian Calles*, Thorsten Bernsmann†, Thorsten Bernshausen*, Melanie Wurm‡, Ulrike Hu¨ benthal*, Jason E. Cline*, Hossein Hajimiragha*, Peter Schroeder*, Lars-Oliver Klotz§, Agneta Rannug¶, Peter Fürst†, Helmut Hanenberg‡, Josef Abel*, and Jean Krutmann*

PNAS 104, 8851 (2007)

Typical immunological features of psoriasis:

•High levels of IL-22 and IL-17
• increased production of antimicrobial peptides (defensins)= no skin infections
• IL-23R a susceptibility gene



Clinical trial with anti-p40 highly successful

What is the role of AhR on different cell types ?
Which ones do we need to take into consideration in psoriasis ?

- Keratinocytes- what is their response to AhR ligands (other than CYP1A1..)
- Langerhans cells (express AhR, do not upregulate CYP1A1, but AhRR)
- resident TCR$\gamma\delta$ T cells (invariant type) and infiltrating TCR$\gamma\delta$ T cells ?
- infiltrating Th17 cells
- anyone else ? Fibroblasts ?

Collaborative project with Dr.U.Mrowietz and Dr. R. Lahesmaa within
ERC Advanced Investigator grant

The AhR is likely to have many diverse effects on the immune system and will influence different cell types in different ways

Immunosuppression is not its primary function !
(nor is the exacerbation of autoimmunity..)

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Biocity Turku

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Psoriasis Center Kiel

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