Aus dem Institut für Immunologie
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

Role of IFN-γ in the immunity and control of gastrointestinal nematode infections

Inaugural-Dissertation
zur Erlangung des Grades eines
Doctor of Philosophy (PhD)
in Biomedical Sciences
an der
Freien Universität Berlin

vorgelegt von
Bhavya Kapse
Biotechnologin aus Khargone, Indien

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LIST OF ABBREVIATIONS

°C:	degree Celcius
α:	Anti
µg:	microgram
µl:	microliter
A. vasorum:	*Angiostrongylus vasorum*
ANOVA:	Analysis of variance
APC:	Allophycocyanin
APC:	Antigen-presenting cell
Bcl-6:	B-cell lymphoma 6
BmDC:	Bone marrow-derived dendritic cells
BSA:	Bovine serum albumin
CD:	Cluster of differentiation
CD40-L:	CD40 ligand
CD62L:	leukocyte L-selectin
CFSE:	Carboxyfluorescein succinimidyl ester
CMF:	Calcium/magnesium free
CO_2:	Carbon dioxide
ctr:	control
d:	day
DC:	Dendritic cell
DCE:	Dead cell exclusion
DNA:	Deoxyribonucleic acid
DNaseI:	Deoxyribonuclease I
DTE:	Dithioreythritol
ELISA:	Enzyme-linked immunosorbent assay
Eos:	Eosinophil
ETDA:	Ethylenediamine tetraacetic acid
FACS:	Fluorescence-activated cell sorting
FCS:	Fetal calf serum
Fig:	Figure
FoxP3:	Forkhead box P3
FSC:	Forward scatter
FSC-A:	Forward scatter – area
FSC-H:	Forward scatter – height
GATA-3:	GATA binding protein 3
GI:	Gastrointestinal
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
h:	hour
hi:	high
HBSS:	Hank's balanced salt solution
HEPES:	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HES:	*H. polygyrus* excretory/secretory products
HES-DC:	Dendritic cells stimulated with *H. polygyrus* excretory/secretory products
H.p./*H. polygyrus*:	*Heligmosomoides polygyrus*
IC:	Intracellular
IFN-γ:	Interferon-gamma
Ig:	Immunoglobulin
IL:	Interleukin
IL-12Rβ:	Interleukin-12 receptor beta chain
IL-4Rα:	Interleukin-4 receptor alpha chain
ILC:	Innate lymphoid cell

iTreg:	Induced regulatory T cell
IVC:	Individually ventilated cages
L3:	Third stage larvae
L4:	Fourth stage larvae
lo:	low
M1:	Classically activated macrophages (CAM)
M2:	Alternatively activated macrophages (AAM)
mg:	milligram
min:	minute
ml:	milliliter
mLN:	Mesenteric lymph node
MHC:	Major histocompatibility complex
mMCP-1:	Mucosal mast cell protease-1
mo:	month
MP:	Memory phenotype
Muc2:	Mucin 2
MyD88:	Myeloid differentiation primary response 88
n:	number of mice
N. brasiliensis:	*Nippostrongyloides brasiliensis*
Na_2CO_3:	Sodium carbonate
$NaHCO_3$:	Sodium bicarbonate
NK:	Natural killer cells
nTreg:	Natural regulatory T cell
P value:	Probability value
PBS:	Phosphate buffer saline
PE:	Phycoerythrin
PerCP:	Peridinin Chlorophyll Protein Complex
PMA:	Phorbol 12-myristate 13-acetate
PS:	Parasite specific
qPCR:	Quantitative polymerase chain reaction
RELM-α:	Resistin-like molecule (RELM) alpha
RELM-β:	Resistin-like molecule (RELM) beta
rIFN-γ:	Recombinant interferon gamma
RORγt:	RAR-related orphan receptor gamma
RPMI:	Roswell Park Memorial Institute Medium
RT:	Room temperature
S. mansoni:	*Schistosoma mansoni*
SCID:	Severe combined immunodeficiency
SD:	Standard deviation
SI:	Small intestine
Siglec-F:	Sialic-acid-binding immunoglobulin-like lectin-F
siLP:	Small intestinal lamina propria
SPF:	Specific pathogen free
SSC:	Side scatter
STAT:	Signal transducer and activator of transcription
STH:	Soil-transmitted helminth
T. gondii:	*Toxoplasma gondii*
T. muris:	*Trichuris muris*
T. spiralis:	*Trichinella spiralis*
T. trichura:	*Trichuris trichura*
T-bet:	T box transcription factor
TCR:	T cell receptor
Teff:	T effector cell
Tfh:	T follicular helper cell
TGF-β:	Transforming growth factor beta

Th:	T helper cell
TLR:	Toll-like receptor
Treg:	Regulatory T cell
TSLP:	Thymic stromal lymphopoietin
U:	unit
Ym-1:	Chitinase-like protein 3 (Chil3)

1. INTRODUCTION

Tipping the balance in favor of Th1 response, either by experimental manipulation of type 1 cytokines or in models of co-infection with Th1 response inducing pathogens, leads to impaired protective type 2 immunity in nematode infections. Furthermore, inbred mice lines with an inherent Th1 bias display poor Th2 responsiveness and poor control of GI nematodes. A previous work from our group reported a rise in Th2/1 hybrid cells, which solely contribute to entire parasite specific IFN-γ pool upon early supplementation with rIFN-γ in *H. polygyrus* infection. The expansion of Th2/1 cells further led to poor parasite control in susceptible C57BL/6 mice (Affinass et al., 2018).
In light of the findings on memory phenotype (MP) T cells which increase in an age-dependent manner and rapidly release IFN-γ upon IL-12 triggers (Kawabe et al., 2017), we asked if the rise in age correlated with more Th2/1 cells and therefore, poor parasite control on account of increased proportions of IFN-γ competent cells in older mice. Working primarily with BALB/c mice displaying high resistance to the *H. polygyrus* infection, we assessed if age-dependent or experimentally induced differences in IFN-γ availability altered the resistance phenotype of the mice.
The specific aims of the study are as follows:

- To address if the proportion of Th2/1 cells generated upon *H. polygyrus* infection differed depending on host age.
- To assess if early blocking of type 1 cytokines leads to impaired instruction of Th2/1 cells and promotes resistance.
- To assess if early IFN-γ treatment skews the balance in favor of Th2/1 cells and promotes susceptibility in the otherwise resistant line.
- To assess if the differential susceptibility between C57BL/6 and BALB/c lines correlates with the differences in Th2/1 proportions determined by the intrinsic IFN-γ availability.
- To assess if the susceptible phenotype of C57BL/6 mice is contingent to the higher intrinsic IFN-γ availability.

2. LITERATURE

2.1 CD4+ T helper cells: differentiation, function, and regulation

2.1.1 Immune system: an overview

Immune system is a complex network of cells and molecules capable of differentiating self from non-self and eliminating harmful substances and infectious micro-organisms. Immune system is broadly divided between innate and adaptive systems. Innate immune system is evolutionarily conserved, forms the first line of defense, and acts rapidly against intruding microbes. However, innate immunity is non-specific and remains independent of the nature of invading pathogen. Innate response triggers adaptive immunity, which is specialized, more precise, long-lasting, and capable of 'memorizing' infectious agents. The adaptive immunity comprises of humoral response conveyed by B cells and antibodies and cell-mediated response driven by T cells. T cells predominantly include helper CD4+ T cells and cytotoxic CD8+ T cells which mediate effector functions via cytokine production or direct killing of infected cells.

2.1.2 CD4+ T cells: lymphopoiesis and activation

CD4+ T helper cells form a critical branch of cell-mediated adaptive immunity. These professional cytokine producing cells are crucial in aiding antibody production by B cells, recruitment of effector cells to the site of infection and enhancing cytotoxic features of innate cells (Zhu, 2018; Zhu & Paul, 2008).

CD4+ T cells derive from hematopoietic stem cells which differentiate into T cell progenitors in the bone marrow. These precursor cells migrate to thymus where they maturate into functional T cells. The developmental process predominantly involves T cell receptor (TCR) gene rearrangement and selection of precursor cells for survival (Luckheeram et al., 2012). T cell receptor comprises of $\alpha\beta$ or $\gamma\delta$ chains which interact with peptide MHC complex on antigen-presenting cells (APCs). TCR is linked with CD3 coreceptors which provide T cell activation signals (Luckheeram et al., 2012; Rudolph et al., 2006). The T cell precursors are selected for survival based on their affinity for self-antigen. The cells with very high or very low affinity for self-peptide MHC complex are killed by negative selection and death by neglect, whereas the cells with TCR binding to self-peptide MHC complex with an intermediate affinity survive (Klein et al., 2014; Luckheeram et al., 2012).

Recognition of foreign antigen because of the interaction of TCR with peptide-MHCII complex is the primary requisite for activation after mature T cells have left the thymus. MHCII is expressed by B cells, macrophages and dendritic cells (DC), of which DC are the most important activator of T cells (Jenkins et al., 2001). Next to TCR and CD3 activation, the delivery of costimulatory signals is necessary for CD4+ T cell activation. CD28 is an important co-stimulatory receptor expressed on T cells and interacts with ligands CD80 (B7-1) and CD86 (B7-2) expressed on DC (Luckheeram et al., 2012). The TCR activation further initiates a cascade of signaling pathways resulting in the proliferation of naïve CD4+ T cells and their subsequent differentiation into effector T cells of specific lineages(Luckheeram et al., 2012). The fate of differentiating CD4+ T helper cells depends on various factors including strength of TCR signal, the nature of antigen presenting cells, the type of costimulatory molecules and, importantly, the cytokines in the surrounding milieu (Lemos et al., 2003; Luckheeram et al., 2012; Oosterwegel et al., 1999; Ruterbusch et al., 2020; Tao et al., 1997; Zhu, 2018; Zhu & Paul, 2008)

2.1.3 Classification of CD4+ T helper cell lineages

CD4+ T helper cells are broadly classified into the following subsets:

Th1 cells: The differentiation of Th1 cells is conveyed by the cytokines IL-12 and IFN-γ (**Fig. 1**). IL-12 is released by APCs after sensing microbial signals and activates STAT4 in differentiating T helper cells(C. Hsieh et al., 1993; Kaplan et al., 1996; Thierfelder et al., 1996; Trinchieri & Sher, 2007), whereas IFN-γ is produced by Th1 cells among other

sources including CD8 T cells and NK cells and acts via STAT1 to promote Th1 differentiation (Afkarian et al., 2002; Lighvani et al., 2001). The master transcription factor implied in Th1 lineage commitment is T-bet (T box transcription factor) which directly regulates IFN-γ production (Szabo et al., 2000, 2002). T-bet expression is regulated by IFN-γ as well as IL-12 (Afkarian et al., 2002; Lighvani et al., 2001; Y. Yang et al., 2007). The main function of Th1 cells is to protect against intracellular bacterial, viral and protozoan infections (Mosmann & Coffman, 1989; Paul & Seder, 1994). Th1 response however, can contribute to the development of delayed type hypersensitivity and turn pathologic (Prete, 1992). IFN-γ released by Th1 cells contributes to the enhanced phagocytosis by innate mononuclear cells (Murray et al., 1985).

Th2 cells: IL-4 promotes the differentiation of Th2 cells (**Fig. 1**) by activating the transcription factor STAT6 (Kaplan et al., 1996; Shimoda et al., 1996; Takeda et al., 1996; Zhu et al., 2001). STAT6 is an important signal transducer in Th2 differentiation which promotes the upregulation of the transcription factor GATA-3 (GATA-binding protein-3) (Luckheeram et al., 2012). GATA-3 is the master regulator of Th2 differentiation pathway (Pai et al., 2004; D.-H. Zhang et al., 1997; W. Zheng & Flavell, 1997; Zhu et al., 2004). GATA-3 deficiency in CD4+ T cells results in the abrogation of Th2 commitment (Pai et al., 2004; W. Zheng & Flavell, 1997; Zhu et al., 2004). GATA-3 binds to the promoters of genes associated with Th2 cytokines and is crucial for their production in differentiating as well as committed Th2 cells (Pai et al., 2004; Tanaka et al., 2011; Wei et al., 2011; Yamashita et al., 2002; W. Zheng & Flavell, 1997). GATA-3 further induces the proliferation of Th2 cells (Wei et al., 2011; W. Zheng & Flavell, 1997). Of note, pathways other than IL-4/STAT6 signaling also contribute to the development of Th2 cells. Activation of STAT5 by IL-2 promotes the differentiation of Th2 cells *in vitro* (Cote-Sierra et al., 2004; Yamane et al., 2005; Zhu et al., 2003). TSLP is another cytokine implicated in differentiation of Th2 cells by acting on dendritic cells (Ito et al., 2005; Liu, 2006; B. Zhou et al., 2005).

Functionally, Th2 cells are protective against extracellular parasites but however, also contribute to the development of asthma and allergic disorders(Mosmann & Coffman, 1989; Paul & Seder, 1994; Prete, 1992; Sokol et al., 2009). The principle cytokines released by Th2 cells are IL-4, -5, -9 and -13 (Luckheeram et al., 2012). IL-4 is essential for the class switch and production of IgE by B cells (Kopf et al., 1993). IL-4 also mediates the release of active molecules including histamine and serotonin from innate cells (Zhu & Paul, 2008). IL-4 further contributes to the pro-inflammatory response by inducing the production of IL-6, GM-CSF and adhesion molecules (Doucet, 1998). IL-5 is importantly involved in the recruitment of eosinophils (Coffman et al., 1989). IL-13 is protective in driving the expulsion of GI nematodes as mentioned below and drives pathologic response in individuals with allergic asthma (Doran et al., 2017; Marone et al., 2019; Urban et al., 1998; Wynn, 2003).

Th17 cells: TCR activation in presence of TGF-β and IL-6 results in the differentiation of Th17 cells(Bettelli et al., 2006; Mangan et al., 2006; Veldhoen et al., 2006). The master transcription factor regulating Th17 lineage fate is RORγt induced by STAT3 activation by IL-6/-21/-23 (Ivanov et al., 2006; Korn et al., 2009; X. O. Yang et al., 2008; Zhu & Paul, 2008). Th17 cells provide protective immunity against extracellular bacteria and fungi (Weaver et al., 2006; Zhu & Paul, 2008). The effector cytokines produced by Th17 cells are IL-17A, IL-17F, IL-21 and IL-22 (Zhu & Paul, 2008). IL-22 promotes the inflammatory response in skin and involved in protective immunity in certain bacterial infections (Aujla et al., 2008; Liang et al., 2006; Y. Zheng et al., 2007, 2008).

Regulatory T (Treg) cells:

Regulatory T cells can be classified as induced Tregs (iTregs) which are generated in response to antigen presentation and natural Tregs (nTregs) which are FoxP3+ cells released from thymus as a separate lineage (Chen et al., 2003; Luckheeram et al., 2012). iTregs are polarized in response to TGF-β in absence of pro-inflammatory cytokines (Bettelli et al., 2006; Chen et al., 2003). The main transcriptional regulator of Treg differentiation is FoxP3 which is induced by TGF-β (Chen et al., 2003; Yoshimura & Muto, 2010). FoxP3 is critical for the differentiation and function of Tregs (Fontenot et al., 2003;

Wan & Flavell, 2007; Williams & Rudensky, 2007; H. Yagi et al., 2004). Tregs produce IL-10, TGF-β and IL-35 (Zhu & Paul, 2008) and are importantly implied in maintenance of self-tolerance and regulating effector responses. Manipulation of the frequencies and functional activity of Tregs has beneficial effects in autoimmunity, allograft rejection, tumors and chronic infections (Joffre et al., 2008; Zhu & Paul, 2008).

Follicular T helper (Tfh) cells:

Tfh cells are different from other subsets of conventional T effector cells. The main function of Tfh cells is to aid the development of antigen-specific humoral immunity by supporting the differentiation of B cells into plasma cells, promoting antibody production, Ig class switching and generation of memory B cells (Breitfeld et al., 2000; Luckheeram et al., 2012; Vinuesa et al., 2005; Zhu, 2018). The master transcriptional regulator of Tfh differentiation is Bcl6 (Nurieva et al., 2008, 2009; Vogelzang et al., 2008). Tfh cells are categorized into IFN-γ producing Tfh1 cells, IL-4 producing Tfh2 cells and IL-10 producing Tfh10 cells.

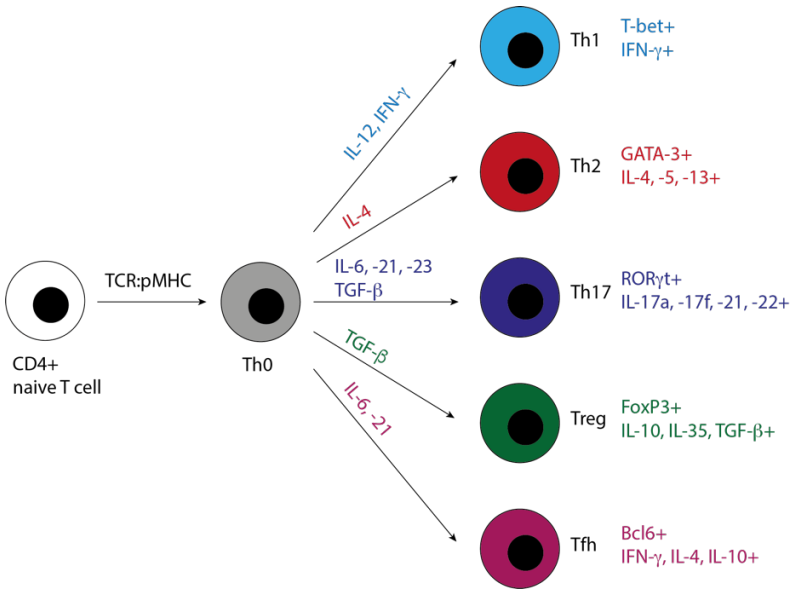


Fig. 2.1. Differentiation of naïve CD4+ T cells into diverse T helper lineages

Naïve CD4+ T cells are activated upon TCR: peptide MHCII interaction and further differentiate into Th1, Th2, Th17, Treg and Tfh cells depending on the cytokine milieu. IL-12 and IFN-γ promote T-bet+ IFN-γ producing Th1 cells, IL-4 promotes GATA-3+ IL-4, -5, 13 producing Th2 cells. IL-6, -21, -23 with TGF-β promote RORγT+ IL-17a, -17f, -21, 22 producing Th17 cells. FoxP3+ Tregs are induced in response to TGF-β and produced IL-10, -35 and TGF-β. Bcl6+ Tfh cells are induced in response to IL-6 and IL-21 and differentiate into IFN-γ producing, IL-4 producing or IL-10 producing subsets.

2.1.4 Cross-regulation between T helper cell lineages

An important step of Th lineage commitment is an active suppression of differentiation into alternative lineages. Such cross-inhibition may occur by direct interaction between master transcription factors, competition for DNA-binding sites and transcriptional control of cytokines and other critical regulators (Zhu, 2018; Zhu & Paul, 2008).

T-bet, the master transcriptional regulator of Th1 lineage inhibits endogenous Th2 differentiation program and represses the expression of Th2-related genes (Zhu et al., 2012). T-bet downregulates GATA-3 expression and function, thereby preventing the commitment to Th2 program and ensuring the differentiation and commitment of Th1 cells (Hwang, 2005; Usui et al., 2006; Zhu et al., 2012). T-bet expression by retroviral gene transduction in polarized Th2 cells results in their conversion to Th1 cells associated with increased IFN-γ production and repressed IL-4 and IL-5 expression (Szabo et al., 2000).

T-bet is also involved in the silencing of IL-4 gene in Th1 cells (Djuretic et al., 2007). Next to T-bet, IL-12 inhibits Th2 differentiation by suppressing IL-4 production *in vivo* (McKnight et al., 1994). Furthermore, IFN-γ suppresses the proliferation of Th2 cells (Oriss et al., 1997). IFN-γ is also required for suppression of IL-4 production by primed Th1 cells, thereby stabilizing Th1 lineage commitment (Y. Zhang et al., 2001). IFN-γ induced transcription factor STAT1 represses activation of STAT6 and inhibits IL-4 induced cell proliferation (Yu et al., 2004).
Similarly, GATA-3 is reported to exert suppressive effects on Th1 differentiation and function. GATA-3 expression in differentiating Th1 cells leads to downregulation of STAT4 further leading to suppressed IFN-γ production (Usui et al., 2003). Conditional deletion of GATA-3 permits the differentiation of Th1 cells in an IL-12/ IFN-γ independent manner (R. Yagi et al., 2010; Zhu et al., 2004). Furthermore, IL-4 inhibits IFN-γ production and diverts the differentiating Th1 cells towards Th2 lineage in a STAT6 dependent manner (Wurtz, 2004).
Besides Th1-Th2 cross-regulation, TGF-β, the polarizing cytokine for Tregs and in combination with IL-6 for Th17 cells, inhibits Th2 development by repressing GATA-3 (Gorelik et al., 2000). T-bet represses the development of Th17 cells by inhibiting the transcription of RORγt (Lazarevic et al., 2011). Th17 differentiation is also suppressed by IFN-γ and IL-4 (Harrington et al., 2005; Park et al., 2005). Th17 and Treg lineages also mutually suppress each other due to antagonistic effects between the key transcription factors (X. O. Yang et al., 2008; L. Zhou et al., 2008).

2.1.5 CD4+ T helper cell heterogeneity and plasticity

Despite the substantial evidence of mutual cross-inhibition between different Th subsets, transcription factors of these supposedly dichotomous lineages are often co-expressed, challenging the idea of fixed, noninterchangeable Th subsets (Brucklacher-Waldert et al., 2014; Zhu, 2018). The revised paradigm of Th differentiation therefore suggests that CD4+ T helper cells are not permanently imprinted but exhibit potential of altering their expression programs according to the need of immune response (Bluestone et al., 2009; Brucklacher-Waldert et al., 2014). CD4+ T helper cells may acquire a heterogenous phenotype by expressing transcription factors of multiple lineages and exhibiting functional properties of each lineage. Alternatively, Th cells of a particular lineage may undergo a complete switch to an alternate cell fate while extinguishing the features of previous lineage. The heterogeneity and plasticity of CD4+ Th cells is useful in maintaining the balance between tolerance and inflammation (Brucklacher-Waldert et al., 2014).
T helper cell plasticity is more frequently observed in Th17 and Treg cells compared to other T cell subsets (L. Zhou et al., 2009). In absence of TGF-β, Th17 cells under the influence of IL-12 and IL-23 upregulate IFN-γ via STAT4/T-bet signaling pathway (Lee et al., 2009). Th17 cells generated in a model of bacterial-induced intestinal inflammation convert into ex-Th17 cells with extinguished IL-17 production and upregulated IFN-γ expression after passing an intermediary IL-17A+ IFN-γ+ stage (Morrison et al., 2013). Th17 cells also acquire an anti-inflammatory phenotype by transdifferentiating into Tregs which highlights their therapeutic potential in inflammatory disorders (Gagliani et al., 2015). Additionally, Th17 cells are also capable of acquiring Tfh phenotype further aiding in high-affinity IgA release (Hirota et al., 2013). Tregs acquire a Th2-like phenotype and contribute to impaired oral tolerance in a model of food allergy (Noval Rivas et al., 2015). A subset of Tregs with unstable FoxP3 expression convert into 'exFoxP3' T cells with an inflammatory phenotype and potential implications in autoimmunity (X. Zhou et al., 2009). Tregs are also reported to switch towards Th17 as well as Tfh phenotype owing to their instability and plasticity (Tsuji et al., 2009; Xu et al., 2007). Similarly, *Leishmania major* specific Th1 cells cultured with IL-4 extinguished IFN-γ production and upregulated IL-4 and IL-10 expression (Mocci & Coffman, 1995). Apart from cells completely switching their phenotype, heterogenous T helper cell populations displaying the features of more than

one lineage are often reported. These include T-bet/RORγt co-expressing Th1/Th17 cells, IL-4+IL-17+ Th17/Th2 cells, IL-17+FoxP3+ Treg/Th17 cells and T-bet+GATA-3+ Th2/1 hybrid cells (Califano et al., 2014; Hovhannisyan et al., 2011; Morrison et al., 2013; Peine et al., 2013). The current work is majorly focused on the regulation and functional properties of Th2/1 hybrid cells.

2.1.6 Heterogenous T-bet+ GATA-3+ Th2/1 hybrid cells

Despite the extensive evidentiary support of Th1 – Th2 cross-inhibition, the synergy between Th1 and Th2 signaling is importantly manifested by the existence of Th2/1 hybrid cells, which co-express intermediate levels of key transcription factors T-bet and GATA-3 and produce moderate levels of IFN-γ and type 2 cytokines IL-4, -5 and -13 (Affinass et al., 2018; Bock et al., 2017; Peine et al., 2013). The intermediary features of Th2/1 cells further manifest in the attenuated immunopathology conveyed by these cells in models of type 1 and type 2 inflammation. The phenotype of Th2/1 cells is highly stable as restimulation of differentiated Th2/1 cells under Th1 or Th2 polarizing conditions results in quantitative modulation of Th1 or Th2 features, while neither phenotype is entirely extinguished (Peine et al., 2013). The Th2/1 cells can be generated *in vitro* upon stimulation with polarizing cytokines IFN-γ, IL-12 and IL-4 and are naturally induced *in vivo* in response to infections by parasites *Schistosoma mansoni, Strongyloides ratti* and *Heligmosomoides polygyrus* in murine hosts and *Strongyloides stercolaris* in human hosts (Affinass et al., 2018; Bock et al., 2017; Peine et al., 2013). Th2/1 cells in *H. polygyrus* infected mice are identified as the main source of parasite-specific IFN-γ. Mice with defective IFN-γ signaling display diminished proportions of Th2/1 cells. Whereas early supplementation with IFN-γ results in increased Th2/1 bias and stronger parasite-specific IFN-γ production. The bias in favor of Th2/1 cells and increased parasite-specific IFN-γ is associated with poor control of the infection in the susceptible C57BL/6 mice (Affinass et al., 2018).

2.2 Th2 response in GI nematode infections

2.2.1 *Heligmosomoides polygyrus* – model for chronic helminthiasis

Chronic GI helminth infections are a global burden heavily impacting humans as well as livestock. *Heligmosomoides polygyrus*, a natural mouse parasite has been successfully modeled in laboratory settings. Primary infections with *H. polygyrus* can be tolerated for several months by susceptible mice. This is in contrast to the GI nematode models *Nippostrongyloides brasiliensis* and *Trichinella spiralis* which are cleared in the span of 2-3 weeks. The long-term patency of *H. polygyrus* renders it to be a suitable model for studying chronic human helminthiasis (Behnke et al., 2009; Johnston et al., 2015; Reynolds et al., 2012).

H. polygyrus has a direct life cycle, i.e., the entire lifespan of the parasite is spent in a single host. The parasite is introduced by oral gavage in laboratory settings, whereas *in natura*, the parasite is taken up by fecal ingestion or from the host fur while grooming (Hernandez & Sukhdeo, 1995; Johnston et al., 2015; Reynolds et al., 2012). The ingested larvae exsheath in the stomach and later migrate to the duodenum and invade the sub-mucosal layer. The larvae localize to the intestinal walls by day 3 post infection. After undergoing two developmental molts, the larvae mature into adult worms and emerge back into the intestinal lumen at about 8 days post infection. The worms loop around intestinal villi, mate and produce eggs which hatch in the environment after being excreted with the feces. The larvae undergo two developmental molts to form L3 larvae capable of infecting the rodent hosts (Behnke et al., 2009; Johnston et al., 2015; Reynolds et al., 2012).

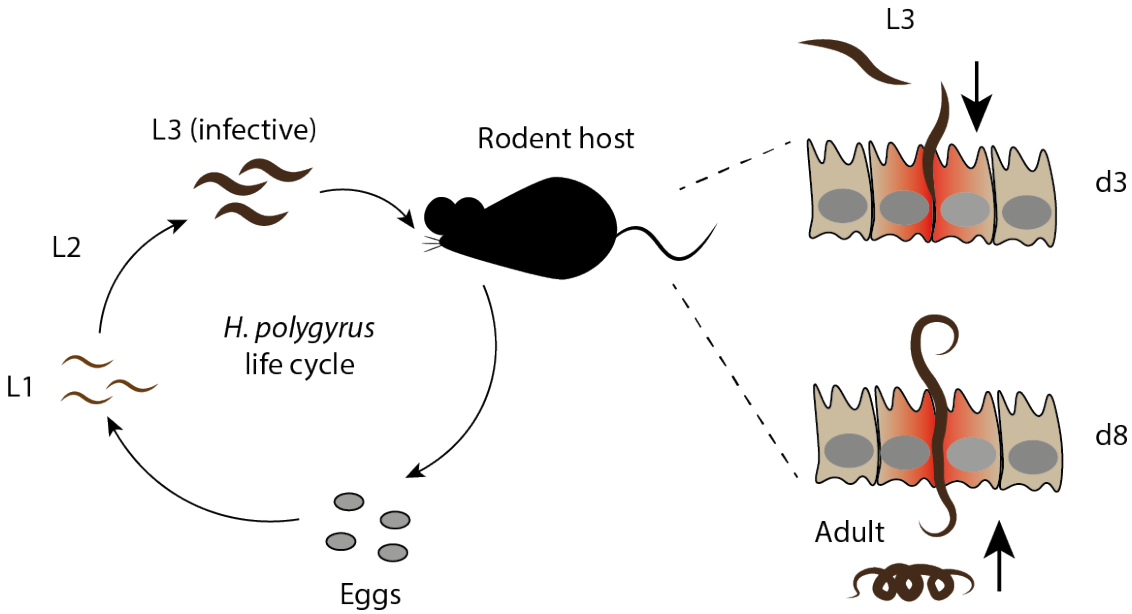


Fig. 2.2. Life cycle of *Heligmosomoides polygyrus*
The parasite eggs hatch into larvae in the environment. The larval development undergoes from L1 to L3 stage before they are taken up by the host. The infective L3 larvae are ingested orally by the rodent host. The L3 larvae invade the small intestinal sub-mucosa at day 3 post infection. The larvae undergo development into L4 and L5 stages and ultimately into adult worms, which emerge back into the intestinal lumen at day 8 post infection. The worms produce eggs which are excreted in the feces starting at day 10 post infection.

2.2.2 Immune response in GI nematode infections

Epithelial cells form the first contact with the invaded larvae. The activation of intestinal epithelium is critical for the generation of protective immunity (Zaph et al., 2007). Mice lacking tuft cells – specialized chemosensory cells in intestinal epithelium, display poor control of GI nematode *N. brasiliensis* whereas tuft cell hyperplasia induced by IL-4 and IL-13 results in the expulsion of the parasite (Gerbe et al., 2016; von Moltke et al., 2016). The activated epithelial cells release cytokines IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) which strongly intercommunicate to activate the innate type 2 immunity (Sorobetea et al., 2018). IL-25 is released primarily by tuft cells which is necessary for the optimum clearance of GI nematodes (Fallon et al., 2006; Pei et al., 2016). Similarly, early supplementation with IL-33 promotes parasite expulsion in *T. muris* infected mice (Humphreys et al., 2008).
The innate responders in nematode infection are necessary for the initiation of adaptive immunity as well as driving terminal expulsion mechanisms. These primarily involve ILC2s, basophils, mast cells, eosinophils, neutrophils, macrophages and hematopoietic progenitor cells (Inclan-Rico & Siracusa, 2018). ILC2 are innate counterparts of Th2 cells which are critical for the early instruction of Th2 effector cells in *N. brasiliensis* infection and are also implied in the generation of Th2 memory (Halim et al., 2016; Oliphant et al., 2014). Basophils form a rare population at steady state which expand following helminth infection. The role of basophils in nematode infection is context dependent. While dispensable in case of certain nematode infections basophils are known to provide protective immunity against *T. spiralis* and *T. muris* (Inclan-Rico & Siracusa, 2018).
Mast cells are tissue residing granulocytes activated in response to IL-3, IL-9, IL-33 and TSLP. The activated mast cells degranulate and release effector molecules which promote the parasite expulsion by M2 polarization, smooth muscle contraction, increased intestinal permeability and mucus production (Inclan-Rico & Siracusa, 2018). Mast cells are critical players in the expulsion of nematodes *H. polygyrus* and *T. muris* (Sorobetea et

al., 2018). Alternative activation of macrophages is a hallmark of helminth infection and is characterized by the elevated expression of Ym-1, RELM-α, IL-4Rα, CD206 and arginase-1 (Reynolds et al., 2012). Alternatively activated macrophages are crucial in generating protective immunity against challenge *H. polygyrus* infection and are also possibly involved in mediating smooth muscle contraction necessary for the expulsion of *N. brasiliensis* (Anthony et al., 2006; Zhao et al., 2008).
Next to the innate immune cells, B cells are also crucially involved in anti-nematode responses. B cells promote the expansion of effector and memory Th2 cells and generate protective class switched and affinity maturated antibodies in mice infected with *H. polygyrus* (Wojciechowski et al., 2009). B cells are also involved in generating protective immunity in *T. muris* infected mice mounting a mixed Th1/Th2 response to the infection (Sahputra et al., 2019). IgG is the major class-switched antibody mediating protective immunity against *H. polygyrus*, while IgA and IgE have minor or no effects. Transfer of serum with higher parasite specific IgG1 activity leads to the generation of protective immunity in *H. polygyrus* infected mice (Reynolds et al., 2012).
The terminal mechanisms involved in the worm expulsion involve enhanced mucus production, release of neutralizing mediators, hyperproliferation of epithelial cells and enhanced intestinal peristalsis (Sorobetea et al., 2018). Goblet cells are specialized cells in intestinal epithelium primarily involved in secretion of mucus, which is suggested to interfere with the helminth establishment in the intestinal niches and feeding of small intestine. Goblet cell hyperplasia is a hallmark of intestinal helminth infections. Besides mucus production, goblet cells release anti-microbial factor RELM-β which is critical for the expulsion of *N. brasiliensis* and *H. polygyrus* (Herbert et al., 2009; Marillier et al., 2008; Sharpe et al., 2018).

2.2.2.1 Importance of CD4+ T cell response

Nematode infections elicit a strong Th2 response characterized by the expression and release of cytokines IL-3, -4, -5, -9 and -13 (Reynolds et al., 2012; Yasuda & Nakanishi, 2018). IL-4 is paramount for conferring protective immunity against primary as well as challenge infection with *H. polygyrus*. IL-13 is involved in protection against challenge *H. polygyrus* infection while IL-5 is considered dispensable (Urban et al., 1991).
CD4+ T cell responses are highly critical for the efficient type 2 protective immunity. *H. polygyrus* infected SCID (T and B cell deficient) mice and athymic nu/nu mice displayed increased parasite load associated with compromised goblet cell hyperplasia and Muc2 expression (Hashimoto et al., 2009). Adoptive transfer of CD4+ T cells confers resistance to otherwise fully susceptible SCID mice in a *T. muris* infection model (Else & Grencis, 1996). Thymus deprived mice exhibit higher morbidity due to trichiniasis and display longer persistence of parasite. Congenitally athymic mice infected with *N. brasiliensis* displayed lifelong inability to clear the worm infection (Jacobson & Reed, 1974). Thymectomized mice are unable to generate protective immunity to primary *T. muris* infection, an effect reversed upon injection of mLN lymphocytes and thymocytes (Wakelin & Selby, 1974).

2.2.2.2 T cell response in human helminthiasis

Infections with soil-transmitted helminths (STH) are a major health and economic burden in low to middle income countries. The long-living parasites benefit from their ability to immunomodulate host responses. Infections in natural systems dramatically differ from laboratory settings. For instance, wild mice experiencing more antigenic exposure display higher immune activation and more diverse repertoire of effector cells compared to lab mice. Furthermore, infections in nature occur in a repeated and variable dose as opposed to the single bolus infection in laboratory. Various approaches including rewilding of laboratory mice as well as employing trickle infections are employed to efficiently model STH in wild settings. The parasite infection *in natura* does not ensure sterilizing immunity to subsequent challenges, instead the infection persists throughout the lifetime due to inadequate development of protective immunity (Colombo & Grencis, 2020). Th2 immunity,

however, has a functional consequence in human helminthiasis as seen by negative correlations between IgE and type 2 cytokines with infection intensity in *Trichuris trichura* and *Ascaris lumbricoides* infections respectively (Faulkner et al., 2002; Turner et al., 2003). Furthermore, PBMC derived IL-5 in infected cohort is positively correlated with resistance to reinfection post deworming in a model of human necatoriasis (Quinnell et al., 2004). T cell memory formed after high dose of STH infection provides protective immunity against secondary infections (Colombo & Grencis, 2020).

2.2.3 Regulation of anti-nematode Th2 response by Th1 cytokines

2.2.3.1 Cross-inhibition by Th1 cytokines and type 1 infections

Several studies have reported the inhibitory effects of Th1 cytokines on the type 2 immunity in nematode infections. Mice deficient in NLRP3 inflammasome harboring diminished levels of type 1 cytokine IL-18 display strong protective type 2 response in *T. muris* infection (Alhallaf et al., 2018). IL-18 is also negatively correlated with production of Th2 cytokines IL-10 and IL-13 and parasite expulsion in the context of *T. spiralis* infection (Helmby & Grencis, 2002). Effective Th2 immunity to *H. polygyrus* is impaired by IL-12 produced by CD103+ migratory DCs (Everts et al., 2016). Exposure to IL-12 in resistant mice infected with *T. muris* impairs parasite control and promotes chronicity (Bancroft et al., 1997). IL-12 suppresses Th2 responses generated upon schistosome egg inoculation in an IFN-γ dependent manner (Oswald et al., 1994). *T. spiralis* infected mice exposed to IL-12 display increased larval and worm burden and poor induction of Th2 response (Helmby & Grencis, 2003). BALB/c mice infected with *N. brasiliensis* display reduced eosinophilia and impaired parasite control upon exposure to IFNs. In a model of mixed type 1 and type 2 inflammation, IFN-γ confines the niches of ILC2 and Th2 cells leading to ameliorated mortality associated with type 1 pathogen *Listeria monocytogenes* (Cautivo et al., 2022).

Co-infections or preceding infections with type 1 response inducing pathogens also diminish the effective type 2 immunity in nematode infections. Infection with protozoan *Toxoplasma gondii* resulted in dramatic decline in Th2 response in mice co-infected with *H. polygyrus* or *N. brasiliensis* (Ahmed et al., 2017; Liesenfeld et al., 2004). Similarly, Th2 responses are compromised by type 1 cytokines IFN-γ and IL-12 in a helminth/ plasmodium co-infection model resulting in impaired protective immunity against the helminth *H. polygyrus* (Coomes et al., 2015).

2.2.3.2 Differential resistance across inbred mice lines

The default Th differentiation program differs in inbred mice lines from different genetic backgrounds. T cell activation *in vitro* under neutral conditions leads to varying degrees of Th1 phenotype in cells from BALB/c and B10.D2 mice in accordance with their *in vivo* Th responses to Leishmania infection (C. S. Hsieh et al., 1995). The difference in Th1 responsiveness between the two strains is correlated with the maintenance of IL-12Rβ2 chain and STAT4 signaling in B10.D2 but not BALB/c mice (Güler et al., 1997). Similarly, resistance to *H. polygyrus* infection differs considerably across mice lines where BALB/c mice are more resistant than the fully susceptible C57BL/6 mice. The resistance phenotype correlates with the higher sustenance of IL-10 and IL-4 production, activation of innate cells including ILC2 and alternatively activated macrophages, broader range of specificity of antibody responses and granuloma formation. Resistant strains further display poorer IFN-γ production in response to HES stimulation compared to more susceptible strains (Filbey et al., 2014).

2.3 Memory phenotype CD4+ T cells

Under homeostatic conditions, CD4+ T cells comprise of naïve and memory T cells, which are further divided between pathogen-specific memory and non-specific memory phenotype (MP) T cells. MP cells develop independent of foreign antigen exposure as these are seen in germ-free, antigen-free and specific pathogen-free mice. MP cells

derive from peripheral naïve precursors in TCR and CD28 dependent manner and proliferate rapidly in steady state.
MP cells are present right at the birth and continue to increase in an age-dependent manner. MP cells comprise of a T-bet^{hi} subset which is induced in response to IL-12 produced by CD8α+ type 1 DCs in TLR-MyD88 dependent and foreign-antigen independent manner. These cells release IFN-γ in an innate IL-12 dependent fashion and contribute to early protective response in the context of *T. gondii* infection (Kawabe et al., 2017, 2020).

3. MATERIAL AND METHODS

3.1 MATERIAL

3.1.1 Laboratory equipment

Table 3.1 List of laboratory equipment and suppliers

Equipment	Supplier
CASEY cell counter	TT Innovatis, Roche, Mannheim, Germany
Cell incubator	Thermo Scientific, Schwerte, Germany
Centrifuge 5810/5810R	Eppendorf, Hamburg, Germany
Centrifuge 5454R	Eppendorf, Hamburg, Germany
FACSAria™ III cell sorter	BD Bioscience, San Jose, CA, USA
FACSCanto™ II flow cytometer	BD Bioscience, San Jose, CA, USA
Lamina flow	Scanlaf, Mars Safety 2 LaboGene, Lynge, Denmark
Lamina flow	Heraeus LB-732-C Thermo Scientific, Boston, MA, USA
Microscope (camera)	Leica ICC50 HD Leica Microsystems, Wetzlar, Germany
Microscope (compound)	Leica DM750 Leica Microsystems, Wetzlar, Germany
Pipette adjustable (10-1000 µl)	Eppendorf, Hamburg, Germany
Pipette multichannel (20 – 200 µl)	Brand, Wertheim, Germany
Temperature regulator for FACSAria™ III	Thermo Fisher Scientific, Boston, MA, USA
Vacuum pumping unit, PC 3004	Vario Vacuubrand, Wertheim, Germany
VortexGenie 2	Scientific Industries, Bohemia, NY, USA
Waterbath	Lauda-Brinkmann, Delran, NJ, USA
Waterbath shaker	Brunswick Innova 3100 Eppendorf, Hamburg, Germany

3.1.2 Laboratory plasticware

Table 3.2 List of laboratory plasticware and suppliers

Plasticware	Supplier
Cell strainers	BD Bioscience, San Jose, CA USA
Eppendorf tubes	Eppendorf, Hamburg, Germany
Falcon tubes	Greiner bio-one, Frickenhausen, Germany
Flat-bottomed 6-well plates	Costar, Corning Inc., Corning, NY, USA
Flat, round and V-bottomed 96-well plates	Costar, Corning Inc., Corning, NY, USA
Flow cytometry tubes	Sarstedt, Nümbrecht, Germany
Petridishes	Eppendorf, Hamburg, Germany
Pipette tips (10 - 1000 µl)	Greiner bio-one, Frickenhausen, Germany
Steripettes (10 - 50 ml)	Costar, Corning Inc., Corning, NY, USA
Syringes (1 - 10 ml)	Braun, Melsungen, Germany
Transfer pipettes	Sarstedt, Nümbrecht, Germany

3.1.3 Reagents

Table 3.3 List of reagents and suppliers

Reagent	Supplier
BSA	AppliChem, Darmstadt, Germany
DNase I	Sigma-Aldrich, Steinheim, Germany
DTE	Sigma-Aldrich, Steinheim, Germany
Ethanol	AppliChem, Darmstadt, Germany
EDTA	AppliChem, Darmstadt, Germany
FACS lysis solution	BD Bioscience, San Jose, CA, USA

Fixation/Permeabilization concentrate and diluent	eBioscience, San Diego, CA, USA
FCS	PAN Biotech, Aidenbach, Germany
FCS	HY Clone GE Healthcare, South Logan, UT, USA
Gentamycin	AppliChem, Darmstadt, Germany
HEPES	PAN Biotech, Aidenbach, Germany
IC Fixation buffer	eBioscience, San Diego, CA, USA
Isofluorane	Abbott, Ludwigshafen, Germany
Isopropanol	Carl Roth, Karlsruhe, Germany
L-glutamine	PAN Biotech, Aidenbach, Germany
Penicillin/Streptomycin	PAN Biotech, Aidenbach, Germany
Percoll	GE Healthcare, Uppsala, Sweden
Permeabilization buffer (10x)	eBioscience, San Diego, CA, USA
Sodium bicarbonate (NaHCO3)	Carl Roth, Karlsruhe, Germany
Sodium carbonate (Na_2CO3)	Carl Roth, Karlsruhe, Germany
Sodium pyruvate	PAN Biotech, Aidenbach, Germany
TL Liberase	Roche, Basel, Switzerland
Water, Millipore	Millipore, Darmstadt, Germany
Water, molecular biology grade	AppliChem, Darmstadt, Germany

3.1.4 Growth factors, inhibitors and stimulators

Table 3.4 List of growth factors, inhibitors and stimulators and their suppliers

Growth factor/ inhibitor/ stimulator	**Supplier**
GM-CSF	Peprotech, Hamburg, Germany
Brefeldin A	eBioscience, San Diego, CA, USA
Ionomycin	Sigma-Aldrich, Steinheim, Germany
PMA	eBioscience, San Diego, CA, USA

3.1.5 Cytokines and neutralizing antibodies for *in vivo* administration

Table 3.5 List of cytokines and neutralizing antibodies and their suppliers

Cytokine/ antibody	**Supplier**
rIFN-γ	Peprotech
Ultra-LEAF™ Purified anti-mouse IL-12/23 p40 (C17.8)	Biolegend
Ultra-LEAF™ Purified anti-mouse IFN-γ(XMG1.2)	Biolegend

3.1.6 Buffers and media

Table 3.6 List of buffers and media and their suppliers

Buffers/ media	**Supplier**
HBSS	PAN Biotech, Aidenbach, Germany
PBS	PAN Biotech, Aidenbach, Germany
RPMI 1640	PAN Biotech, Aidenbach, Germany

3.1.7 Single cell preparation

Table 3.7 List of buffers and media used in single cell preparation

Buffers/ media	**Composition**
CMF (Ca, Mg-free)	HBSS (w/o Ca2+ and Mg2+) 2% FCS 10mM HEPES 25mM NaHCO3
CMF/DTE	CMF 8% FCS (final 10%) 0.154 mg/mL DTE

HBSS/EDTA	HBSS 10% FCS 15mM HEPES 5mM EDTA
HBSS/HEPES	HBSS 15mM HEPES
RPMI, complete	RPMI 1640 10% FCS 2mM L-glutamine 100U/mL penicillin + 100U/mL streptomycin
RPMI, complete (siLP)	RPMI (w/o NaHCO3) 5% FCS 15mM HEPES 100ug/mL gentamycin
RPMI, digest	complete RPMI siLP 0.1mg/mL TL liberase 0.1mg/mL DNase I
RPMI, fecundity analysis	RPMI 1640 200U/mL penicillin + 200U/mL streptomycin
RPMI, serum free	RPMI 1640 100U/mL penicillin + 100U/mL streptomycin
RPMI, wash	RPMI 1640 1% FCS 100U/mL penicillin + 100U/mL streptomycin

3.1.8 Flow cytometry (buffers)

Table 3.8 List of buffers used in flow cytometry

Buffers	Composition
FACS buffer	PBS 1% BSA 2mM EDTA
FACS buffer (for storing sampled blood)	PBS 1% BSA 5mM EDTA
Lysis solution	1-part 10x FACS lysis solution + 9-part water
Fixation/ Permeabilization buffer	1-part Fixation/permeabilization concentrate + 3-part Fixation/ permeabilization diluent
IC Fixation buffer, working	1-part IC Fixation buffer + 1-part water
Permeabilization buffer	1-part Permeabilization buffer (10x) + 9-part water

3.1.9 Flow cytometry (antibodies, dyes and other reagents)

Table 3.9 List of antibodies used in flow cytometry

Antibody	Clone	Color conjugate
CD4	RM4-5	Alexa 700 Brilliant Violet 510 PerCP
FoxP3	FJK-16s	PE-eFluor610 eFluor 450 Alexa 488 PerCP-Cy5.5
T-bet	4B10	PE
GATA-3	TWAJ	eFluor 660
IFN-γ	XMG1.2	eFluor 450 Alexa 700

		PE-eFluor610
IL-4	BVD6-24G2	Biotin
IL-13	eBio13A	Alexa 488
CD154	MR1	Biotin
CD44	IM7	PerCP
CD62-L	MEL-17	APC-eFluor780
RELM-α		Biotin
F4/80	BM8	eFluor 450
Siglec-F	E50-2440	PerCP-Cy5.5

Table 3.10 Dyes, secondary conjugates and blocking antibodies used in flow cytometry

	Clone	**Color conjugate**
Fixable Viability Dye	--	eFluor 780 eFluor 506
Streptavidin	--	PE PE-Cy7 APC
FcγRII/III blocking antibody	93	**--**

3.1.10 Software

Table 3.11 List of software used and companies.

Software	**Company**
Adobe Illustrator	Adobe Systems Incorporated, San Jose, USA
FlowJo	Tree Star, Ashland, OR USA
GraphPad Prism	GraphPad Software, San Diego, USA

3.2 METHODS

3.2.1 Infection model, *in vivo* treatments, and samplings

3.2.1.1 Ethics statement

All experiments performed in this study are compliant with National Animal Protection Guidelines and approved by German Animal Ethics Committee for the protection of animals (Licence no. G0176/16, G0113/15).

3.2.1.2 Mice and parasites

Male and female C57BL/6 and BALB/c mice of different age groups were purchased from Janvier, St. Berthevin, France. Mice were housed under standard SPF conditions in individually ventilated cages (IVCs). The *H. polygyrus* life cycle was maintained by serial passage in C57BL/6 mice.

3.2.1.3 *H. polygyrus* infection

Mice were infected with 200 *H. polygyrus* (H.p.) L3 larvae. The L3 larvae maintained at 4°C were collected, counted, and adjusted for 225 larvae in a volume less than 200 µl. The infection dose per mouse (225 L3) was aliquoted in separate test tubes. The L3 larvae were administered by oral gavage with the help of gavage needle and 1 ml syringes. To terminate the experiment, mice were first anesthetized by isoflurane inhalation and later sacrificed by performing cervical dislocation.

3.2.1.4 *In vivo* treatments

Mice were treated with recombinant cytokine IFN-γ at the dose of 2,5 µg, twice a day, from days 0-4 post infection. *In vivo* cytokine neutralizing antibodies αIFN-γ (XMG1.2) and

αIL-12 (C17.8) were given at the dose of 0,5 mg on days 0, 3 and 6 post infection. All the treatments were administered by intraperitoneal injections using U-40 1 ml insulin syringes.

3.2.1.5 Intermediate blood and fecal sampling

Intermediate blood sampling was performed without anesthesia, where blood was collected from submandibular vein using lancets. At the time point of dissection, blood was collected from orbital sinus under general anesthesia with the help of glass capillary tubes. The blood was stored in tubes containing FACS buffer until further processing.
The feces were sampled by placing the mice in glass beakers for some minutes until they defecated. Fecal pellets (2-3) were collected and used for parasite egg counting or stored for additional assays.

3.2.2 Parasitological assays

3.2.2.1 L4 migration assay

L4 migration assay was performed at day 6 post infection. A segment of duodenum of approx. 2-3 cm in length was cut from freshly retrieved small intestine and tied at both the ends without disturbing the luminal contents. Individual segments were cultured in serum free RPMI in 6-well plates at 37°C, 5% CO_2. After 4 h, the larvae egressed out in the medium and those still present in the tissue segment were counted separately under the microscope. The larval egress was determined by calculating the percentage of egressed larvae from the total larvae present in the tissue segment before starting the culture.

3.2.2.2 Parasite egg counting from feces.

Parasite egg numbers in the feces were estimated using floatation technique. Briefly, fecal pellets were soaked in 1 ml water and homogenized using a glass rod. The fecal material was diluted using 6 ml of saturated solution of NaCl. The sample was taken into McMaster counting chamber and the number of eggs were determined under microscope. The number of eggs per gram of feces was determined by the formula:

$$\text{Eggs per gram feces} = \frac{7 * 6{,}67 * \text{Egg count}}{\text{Weight of feces (in grams)}}$$

3.2.2.3 Adult worm counting

Adult worms were counted at day 14 or day 28 post infection. The freshly isolated small intestine was opened by cutting along the length of the intestine. The adult worms were collected using forceps into NaCl containing Petri dishes. The total number of adult worms isolated from the small intestinal lumen were counted under the microscope with the help of glass pipette.

3.2.2.4 Female worm fecundity analysis

To determine the fecundity of individual female worms, eight female worms (where possible) were collected and placed in individual wells in 96-well flat-bottomed plate containing 200 µl RPMI with 200 U/ml penicillin and 200 U/ml streptomycin. The female worms were cultured for 24 h at 30°C and 5% CO_2. After the culture period, the media was resuspended well to gain a uniform suspension of the eggs. A fourth of the total media was immediately collected into a separate 96-well plate and the number of eggs were counted under the microscope. The total number of eggs was estimated by extrapolating the number of eggs counted in the smaller volume.

3.2.3 Assessment of immunological parameters

3.2.3.1 Preparation of single cell suspensions

Spleen and mLN

The spleen and mLN were isolated and collected in cold wash RPMI. The spleen was cut into smaller pieces. The organs were meshed and forced through 70 µm cell strainer using

wash RPMI. The cell suspension was centrifuged at 300xg, 10 min, 4°C. The spleen cells were resuspended in cold erylysis buffer and incubated for 2 min on ice to lyse contaminating erythrocytes. The erylysis was stopped by adding complete RPMI. The cells were centrifuged and finally both spleen and mLN cells were resuspended in complete RPMI. The cells were counted using CASEY cell counter and adjusted to desired concentration.

Small intestinal lamina propria

The small intestine was retrieved and placed in Petri dishes containing cold CMF buffer. After cutting off Peyer's patches, the intestine was opened by cutting across the length. The intestinal contents including mucus, faecal material, and worms in case of infected mice were removed. Worms were collected in NaCl solution if needed. The intestine was washed thrice in HBSS/HEPES buffer, cut into pieces of 1 cm, and stored in HBSS/DTE buffer (20 ml). The small intestinal pieces were placed in an incubator/ shaker at 200 rpm, 15 min, 37°C. The material was transferred into HBSS/EDTA buffer (20 ml) followed by vigorous shaking on a highspeed shaker for 15 min, RT. The intestinal pieces were rinsed in RPMI to remove EDTA and placed in RPMI digest medium (10 ml) at 37°C. This was followed by incubation at 200 rpm, 30 min, 37°C. The intestinal pieces were then vortexed to disrupt and homogenize the tissues into single cell suspensions. The cells were forced through 20G needle through 70 μm cell strainer and washed twice with HBSS/HEPES buffer. Cells were layered on a Percoll gradient and centrifuged. The lamina propria cells were collected from 40/70% interface. The cells were washed once and resuspended in complete RPMI (0,5 – 1 ml) and counted using CASEY cell counter.

3.2.3.2 Isolation of lymphocytes from peripheral blood

Blood (2-3 drops) was collected in tubes containing FACS buffer (1 ml). The samples were centrifuged at 340xg, 2 min, RT. Following the careful aspiration of supernatant, the pelleted blood was resuspended in FACS lysis solution (1-part 10x FACS Lysis solution + 9-part water) and incubated for 2 min, RT to lyse erythrocytes. The lysis was stopped by adding excess volume of FACS buffer. The cells were centrifuged, and lymphocytes were resuspended in FACS buffer for subsequent staining procedures.

3.2.3.3 Generation of bone marrow derived dendritic cells

Mouse tibia and femur bones were harvested and placed in cold wash RPMI. The tips of the bones were cut, and the marrow was flushed with wash RPMI using 27G needle and forced through 70 μm cell strainer. The cell suspension was centrifuged at 300xg, 10 min, 4°C. The erythrocytes were lysed by resuspending the cells in lysis buffer and placing on ice for 5 min. The lysis was stopped by adding excess volume of complete RPMI. The cells were washed once, resuspended in complete RPMI, and adjusted to 0.5-0.6 millions/ ml. The cell suspension was transferred in Petri dishes and GM-CSF (20 ng/ml) was added. After 3 days, 10 ml of fresh complete RPMI containing GM-CSF (20 ng/ml) was added to each Petri dish. The cells were harvested at day 7 post isolation.

3.2.3.4 Detection of parasite specific CD4+ T cell responses

Dendritic cells generated as described earlier were loaded with 5-10 μg/ml of adult *H. polygyrus* excretory/secretory products (HES). The cells were incubated at 37°C, 5% CO_2 for 5-6 h. The freshly isolated spleen and mLN cells were added to the HES pulsed dendritic cells followed by a brief spinning at 300 rpm, 30 sec, 4°C to allow the cells to form contact with dendritic cells. After 2-3 h of incubation in conditions as earlier, Brefeldin A (3 μg/ml) was added. The cells were incubated overnight at 37°C, 5% CO_2 followed by intracellular staining.

3.2.3.5 Polyclonal stimulation for detecting intracellular cytokines

The cells were stimulated with PMA and Ionomycin (1 μg/ml each) and incubated at 37°C, 5% CO_2 for 4 h. After the first 30 min of incubation, Brefeldin A (3 μg/ml) was added to the cells to inhibit the release of intracellular cytokines.

3.2.3.6 Cell staining for flow cytometry

Surface staining

The cells in complete RPMI were transferred to 96-well V-bottomed plate and washed with FACS buffer to remove the traces of culture medium. The cells were resuspended in surface staining mix comprising of FcγRII/III blocking antibody (1:40), fixable viability dye (1:1000) and antibodies against the target surface markers in FACS buffer. The cells were incubated at 4°C in dark for 15 min. After washing once with FACS buffer, the cells were acquired on the flow cytometer or used for intracellular staining as needed.

Intracellular staining (transcription factors)

After staining surface markers as described, the cells were resuspended in fixation/permeabilization buffer (80 µl) and incubated at RT in dark for 30 min. The cells were washed twice with permeabilization buffer. The intracellular staining mix containing antibodies against target transcription factors in permeabilization buffer was added to the cells followed by incubation at 4°C in dark for 30 min. The cells were washed twice with permeabilization buffer and kept as pellets on ice until acquisition on flow cytometer.

Intracellular staining (cytokines)

Post surface staining, the cells were fixed using IC fixation buffer (100 µl) and incubated at RT in dark for 30 min. The fixed cells were stained as described earlier and acquired on flow cytometer.

3.2.3.7 Flow cytometric analysis

The lymphocytes were gated based on their size and granularity in FSC/SSC region. The doublets were excluded from the gated lymphocytes using FSC-A/FSC-H parameters. The dead cells were gated out by eliminating DCE+ cells. The CD4+ T cells were identified in the live cells and assessed for T-bet/GATA-3 or IFN-γ/ IL-4/IL-13 expression as needed.

3.2.3.8 Statistical analysis

Statistical analyses were performed using GraphPad Prism Software. Normality was determined using Shapiro–Wilk test. Based on the normality analysis, ordinary one-way-ANOVA and Tukey's multiple comparisons test or Kruskal–Wallis test and Dunn's multiple comparison test was performed in case of more than two groups. Comparisons of two groups were performed with an unpaired t test or Mann–Whitney test. Correlation analysis was performed using Pearson or Spearman correlation depending on the normality tests. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$.

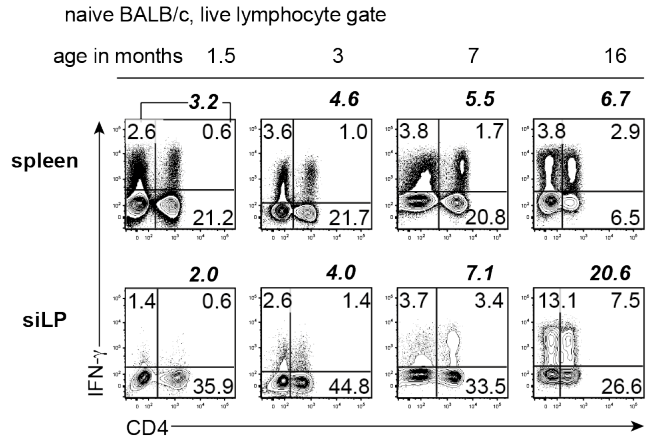
4. RESULTS

4.1 IFN-γ rises in an age-dependent manner in naïve and H.p. infected BALB/c mice.

4.1.1 IFN-γ competent cells increase with age in uninfected BALB/c mice.

IFN-γ is identified as a cardinal factor driving the *in vitro* generation of Th2/1 cells which breaks the dominance of IL-4 over IL-12 signaling (Peine et al., 2013). A previous study from our group reported that the differentiation of Th2/1 cells following H.p. infection is importantly regulated by IFN-γ (Affinass et al., 2018). Increased Th2/1 bias is further associated with impaired parasite control in susceptible C57BL/6 mice. As MP T cells, which develop independent of foreign antigenic exposure and rapidly release IFN-γ upon IL-12 stimulus are reported to rise in an age-dependent manner, it is conceivable that older mice with more MP T cells at steady state are predisposed to generate more Th2/1 cells post H.p. infection and thereby display higher susceptibility. To test this hypothesis, we worked with genetically resistant BALB/c mice and compared the IFN-γ competence and the proportions of steady state T effector/ memory cells (Teff/mem) in mice aged from 1.5 to 18 months. To assess if IFN-γ competence is regulated in an age-dependent manner, spleen or siLP-derived lymphocytes were stimulated using PMA/Ionomycin followed by intracellular IFN-γ detection. Total IFN-γ production post polyclonal stimulus was progressively increased from 1.5 months to 16 months old mice in both spleen as well as small intestine where a two-fold rise was seen in spleen and a ten-fold rise in small intestine. The trend was cogently reflected in scatter graphs depicting strong positive correlation between IFN-γ producing lymphocytes and age of the mice **(Fig. 4.1.1A – B)**. Upon further deciphering the cells based on CD4 expression, CD4+IFN-γ+ cells were increased from the youngest (1.5 months) to oldest group (16 months) by a factor of five in case of spleen and by a factor of twelve in case of small intestine. Accordingly, IFN-γ producing cells in CD4+ T cells were positively correlated with the age in both the organs. In addition, a positive association between IFN-γ production potential and the age of the mice was seen in small intestinal CD4- cells. Overall, our findings suggest that immune maturation with age resulted in increased potential for non-specific IFN-γ release in in BALB/c mice.

A



B.

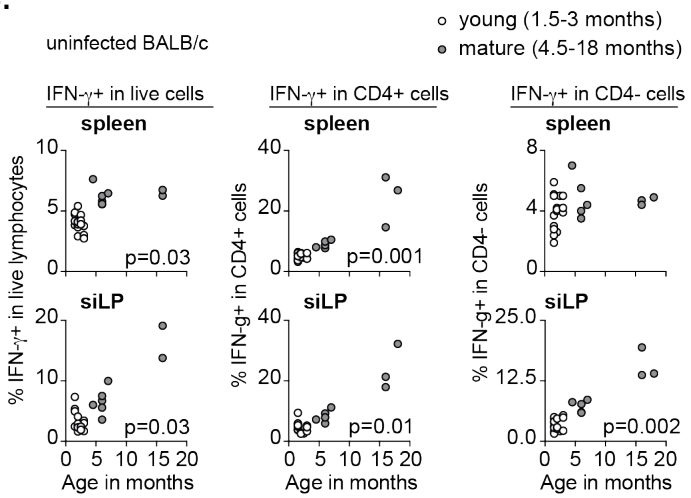


Fig. 4.1.1. IFN-γ competent cells increase with age in uninfected BALB/c mice.
A Contour plots depicting PMA/ionomycin-induced IFN-γ expression by CD4+, CD4- and total lymphocytes derived from the spleen and small intestinal lamina propria (siLP) of naïve BALB/c mice at the age of 1.5, 3, 7 and 16 months. **B** Graphs depict correlation between the percentage of IFN-γ+ cells detected in total, CD4+ and CD4- lymphocytes and mouse age. Circles correspond to individual mice. Data from multiple independent experiments are pooled (n=23-24 mice). P value was determined using Pearson correlation or nonparametric Spearman correlation analysis depending on the normality tests.

4.1.2 Rise in age is associated with increased effector/memory like T cells at steady state.

Previous studies have reported that MP T cells, which rapidly release IFN-γ in an innate fashion accumulate with age in uninfected C57BL/6 mice (Kawabe et al., 2017). As we observed the age-dependent rise in IFN-γ production post non-specific polyclonal stimulus in naïve BALB/c mice, it is conceivable that IFN-γ competent MP T cells also accumulate in an age-dependent manner in BALB/c mice. To address this possibility, we assessed the abundance of CD4+CD44+CD62L- T eff/meM like cells in differentially aged naïve BALB/c mice and further identified the T-bet expressing Th1 subset in the gated population of Teff/mem like cells. CD44+CD62L- Teff/mem like population in CD4+ T cells was indeed more prominent in 5.5 months old compared to 3 months old mice in spleen, mLN and small intestine **(Fig. 4.1.2)**. Furthermore, Teff/mem like cells in older age group were more enriched in T-bet expressing cells compared to the younger cohort. Hence, the rise in age correlated with increased proportions of Th1 Teff/mem like cells at steady state in naïve BALB/c mice which may contribute to the observed rapid IFN-γ release upon polyclonal stimulation.

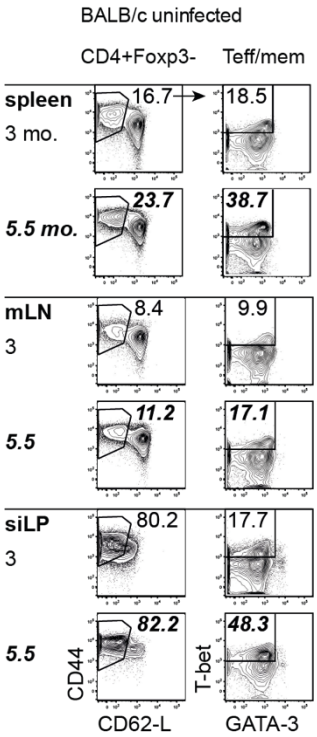


Fig. 4.1.2. Rise in age is associated with increased effector/memory like T cells at steady state.
Left: Contour plots depicting CD44 vs. CD62L expression in CD4+FoxP3- T cells in spleen, mLN and small intestine in uninfected BALB/c mice. Right: Corresponding plots depicting T-bet vs. GATA-3 expression in CD44+CD62L- T cells.

4.1.3 Age-dependent rise in IFN-γ is maintained post H.p. infection.

H.p. infection induces a strongly polarizing Th2 response in BALB/c mice followed by expulsion of adult worms within four weeks of infection (Filbey et al., 2014). To ensure if the age-dependent rise in IFN-γ is not overrun by robust H.p.-induced Th2 responses in BALB/c mice, we evaluated PMA/Ionomycin driven IFN-γ release at day 7 post infection in the mesenteric lymph node. Mature mice displayed a highly prominent rise in T-bet or IFN-γ expressing T cells in mLN compared to the younger cohort at day 7 post infection, which was even more pronounced compared to the age-dependent rise in uninfected controls **(Fig. 4.1.3A)**. Similarly, IFN-γ expressing CD8+ T cells were also increased with age in naïve mice **(Fig. 4.1.3B)**. Hence, the age-dependent rise in type 1 activity is also seen post H.p. infection in BALB/c mice.

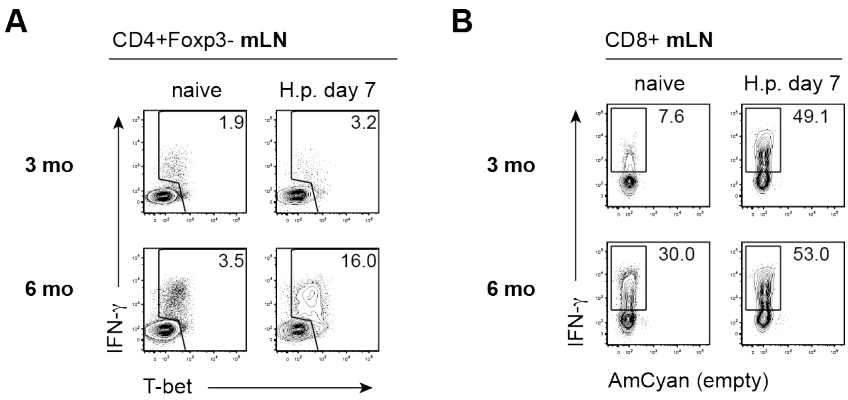


Fig. 4.1.3. Age-dependent rise in IFN-γ is maintained post H.p. infection.
Contour plots depicting IFN-γ producing cells in response to stimulation with PMA/Ionomycin in **A** CD4+Foxp3- T cells and **B** CD8+ T cells in mLN in mice aged 3 months (top) and 6 months (bottom). Plots on the left correspond to naïve controls and those on the right represent H.p. infected mice at day 7 post infection.

4.2 Mature mice accumulate more Th2/1 cells and display poor parasite control.

4.2.1 Mature mice display increased frequencies of Th2/1 hybrid cells.

As IFN-γ competence increases in an age-dependent manner in BALB/c mice, we asked if the H.p. infected mature mice (5-10 months old) displayed an increased Th2/1 bias compared to young adult (1.5-3 months old) mice. Assessing the circulating GATA-3+ T cells in blood, increased proportions of T-bet co-expressing cells were seen in mature compared to young adult mice at the early stage of infection **(Fig. 4.2.1A)**. The early shift towards Th2/1 cells in blood was reflected in the Teff response in small intestine at the chronic stage of infection, where mature compared to young adult mice displayed increased accumulation of mucosal Th2/1 cells **(Fig. 4.2.1B)**. Systemic Teff response in spleen mirrored small intestine in displaying elevated proportions of Th2/1 cells in mature compared to young adult mice **(Fig. 4.2.1B)**. Classical Th2 cells expanded post H.p. infection in both spleen and small intestine. However, no age-dependent effects on the systemic as well as mucosal accumulation of classical Th2 cells were seen **(Fig. 4.2.1B)**. Similarly, splenic as well as intestinal Th1 cells were increased in trend with the age of the mice **(Fig. 4.2.1B)**. Overall, age-dependent rise in IFN-γ correlated with increased accumulation of Th2/1 cells in mature compared to young adult mice.

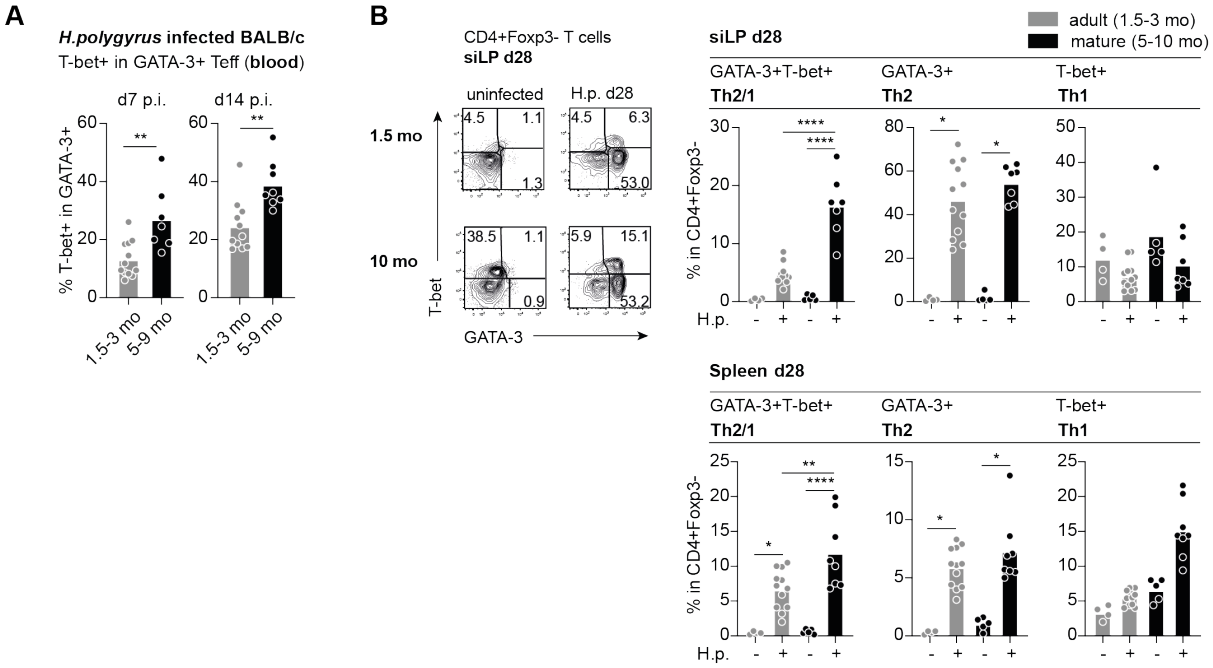


Fig. 4.2.1. Mature mice display increased frequencies of Th2/1 hybrid cells.
A Frequencies of T-bet expressing cells in circulating CD4+GATA-3+ T cells at day 7 and day 14 post H.p. infection. Data from two independent experiments are pooled (n young adult=11-12; mature=7-8 mice). **B** Representative flow cytometry plots depicting T-bet vs. GATA-3 expression in siLP-derived CD4+FoxP3- T cells at day 28 post H.p. infection. Bar graphs report the frequencies of Th2/1, Th2 and Th1 cells in CD4+FoxP3- T cells in siLP (top) and spleen (bottom). Data from two independent experiments are combined (n young adult naïve=4; mature naïve=5; infected young adult=12; infected mature=7-8 mice). Bars represent mean and circles correspond to individual mice. Statistical analysis was performed using unpaired t test or Mann-Whitney test in A and one-way ANOVA with Tukey's multiple comparisons test

or Kruskal-Wallis test with Dunn's multiple comparisons test in B. *$p \leq 0.05$, **$p \leq 0.01$, ****$p \leq 0.0001$.

4.2.2 Higher Th2/1 bias in mature mice correlates with poor parasite control.

Young adult as well as mature mice displayed low worm burden four weeks post infection, where some individuals reported complete infection clearance **(Fig. 4.2.3B)**. BALB/c mice are expected to clear the infection within four-six weeks (Filbey et al., 2014), which suggests that age dependent rise in IFN-γ does not alter the expulsion phenotype in the resistant strain. However, worms isolated from mature mice displayed higher reproduction potential. Parasite egg shedding in the feces determined over the course of infection was elevated in mature mice in the week following the onset of egg deposition (**Fig. 4.2.3A**). Furthermore, the female worms isolated from mature compared to young adult mice at chronic stage displayed higher reproduction fitness, as determined by the egg released *ex vivo* in the 24 h culture (**Fig. 4.2.3C**). Hence, the small intestinal accumulation of Th2/1 cells in mature compared to young adult mice possibly promoted the maturation of more fecund female worms.

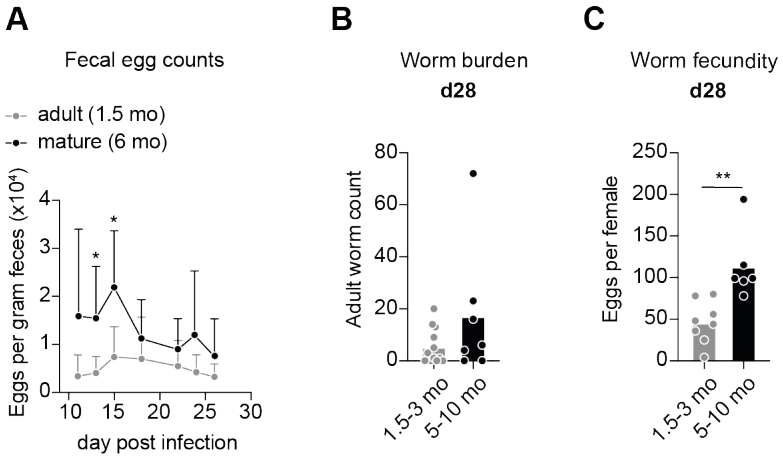


Fig. 4.2.2. Higher Th2/1 bias in mature mice correlates with poor parasite control.
A Kinetics of fecal egg counts per gram feces over the course of infection in young adult and mature mice. Data from one out of two experiments with similar results are shown (n young adult=6; mature=4 mice) as mean ± SD. **B** Adult worm counts at day 28 post infection. Data derive from two independent experiments (n young adult=12; mature=7 mice). **C** Average number of eggs produced by individual female worms (typically eight worms per mouse, minimum 3) isolated from each mouse at day 28 post infection and cultured for 24 h. Data from two independent experiments are shown (n adult=8; mature=6 mice; note that expulsion was completed in 4 young adult and 2 mature mice). Bars represent mean and circles correspond to individual mice. Statistical analysis was performed using unpaired t test or Mann-Whitney test. In A, statistical analysis to compare young adult and mature mice was separately performed at each time point. *$p \leq 0.05$, **$p \leq 0.01$.

4.2.3 Intestinal IFN-γ competence positively correlates with worm fecundity.

To ascertain if the age-dependent rise in parasite egg production was resulted by the increased local IFN-γ competence, we correlated the IFN-γ producing or T-bet expressing CD4+ T cells with average number of eggs released by typically eight female worms isolated per mouse. Indeed, IFN-γ producing and T-bet expressing CD4+ T cells displayed a strong positive correlation with the eggs released by female worms **(Fig. 4.2.6A, B)**, thereby suggesting that age-dependent rise in fecundity is in fact associated with differential local IFN-γ competence.

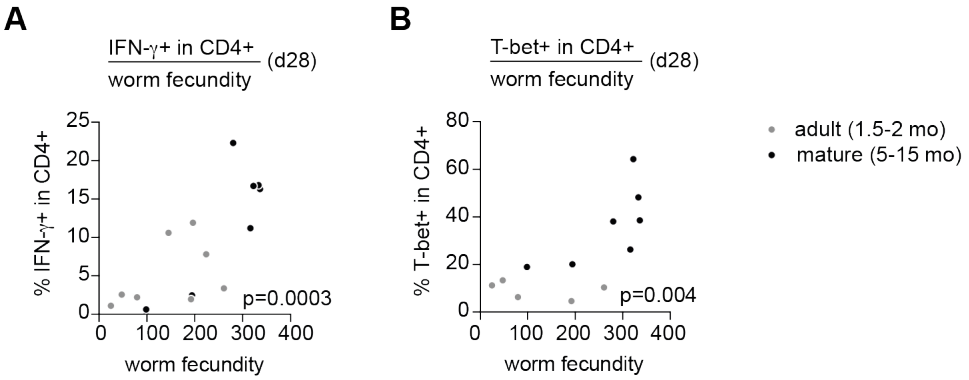


Fig. 4.2.3. Intestinal IFN-γ competence positively correlates with worm fecundity. Scatter graphs depict the correlation of the percentage of IFN-γ+ cells determined after PMA/ionomycin stimulation **(A)** and T-bet expressing cells **(B)** in small intestinal CD4+ T cells with the mean egg counts produced by worms from the same mouse at day 28 post infection. Data derive from three-four experiments (n=13-16 mice). Circles correspond to individual mice. P value was determined using Pearson correlation or nonparametric Spearman correlation analysis depending on the normality tests.

4.2.4 Early Th2 responses in H.p. infected young adult vs. mature mice

To assess if the early expansion of Th2/1 cells seen in blood at the early stage of infection occurred at the expense of classical Th2 cells, we compared the Teff response in lymphoid organs at day 6 post infection. Th2 polarization in mLN as well as spleen remained comparable between young adult and mature mice **(Fig. 4.2.6)**. However, fewer Th2 cells accumulated in small intestine in mature compared to young adult mice. Reflective of the Teff responses at chronic stage, mature mice displayed an early rise in Th2/1 cells in mLN and spleen. Increased Th2/1 polarization in mLN correlated with higher accumulation of Th2/1 cells in small intestine at day 7 post infection. Importantly, the findings suggest that the Th2 polarization remained unaffected by the age-dependent rise in IFN-γ competence. However, fewer Th2 cells home to small intestine in mature compared to young adult mice.

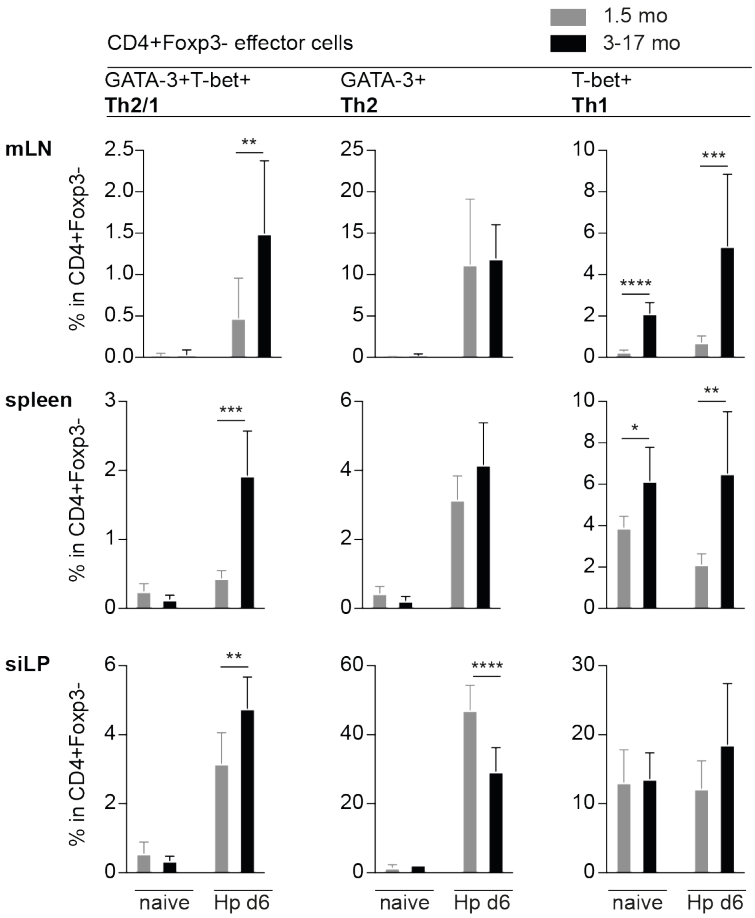


Fig. 4.2.4. Early Th2 responses in H.p. infected young adult vs. mature mice
Bar graphs report the frequencies of Th2/1, Th2 and Th1 cells in mLN (top), spleen (mid) and small intestine (bottom) in CD4+FoxP3- T cells in young adult and mature BALB/c mice. Data from six independent experiments with n=3-10 mice/group are reported as mean + SD. Statistical analysis was separately performed between young adult and mature naïve mice and between young adult and mature H.p. infected mice using unpaired t test or Mann-Whitney test. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$.

4.3 Early blocking of type 1 cytokines leads to increased parasite control.

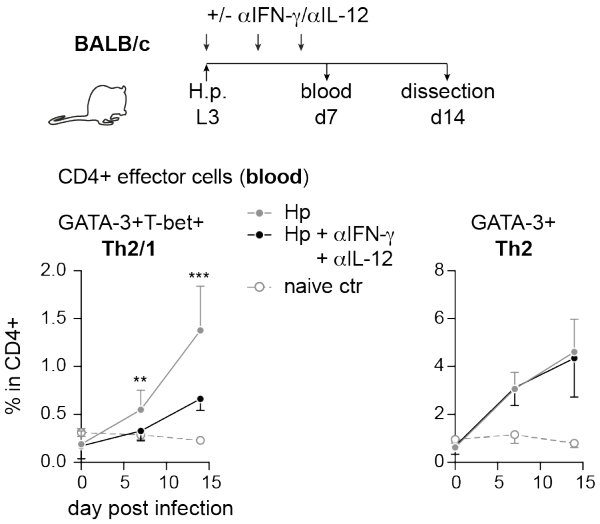
Next, we aimed to substantiate if the increased Th2/1 bias and impaired resistance in mature mice was indeed resulted by increased IFN-γ availability and not a consequence of additional physiological or immunological changes associated with the rise in age of mice. To address this hypothesis, we treated young adult BALB/c mice (1.5 months old) with *in vivo* cytokine neutralizing antibodies against IFN-γ and IL-12 **(Fig. 4.3.1A)**. IL-12 was blocked next to IFN-γ in order to prevent IL-12 induced polarization of IFN-γ producing Th1 cells (C. Hsieh et al., 1993). Following a weekly blood survey, mice were dissected at day 14 post infection.

4.3.1 Early IFN-γ/IL-12 blocking leads to restricted generation of Th2/1 cells.

IFN-γ/IL-12 blocked mice displayed selectively diminished frequencies of circulating Th2/1 cells in blood evident from the early stage of infection **(Fig. 4.3.1A)**. Poor early systemic Th2/1 response in blood correlated with reduced accumulation of small intestinal Th2/1 cells at day 14 post infection in IFN-γ restricted mice **(Fig. 4.3.1B)**. IFN-γ/IL-12 blocking also resulted in diminished proportions of Th2/1 cells in spleen **(Fig. 4.3.1C)** showing that IFN-γ/IL-12 blocking had a prominent effect on mucosal as well as systemic sites. H.p. infection led to a dramatic rise in small intestinal Th2 cells. However, similar to the

observation in young vs. mature mice, IFN-γ/IL-12 blocking had no effect on the accumulation of small intestinal classical Th2 cells. Splenic and small intestinal Th1 cells were not significantly affected by the blocking regimen **(Fig. 4.3.1B-C)**. Hence, early restriction of endogenous IFN-γ availability resulted in selectively diminished systemic and mucosal Th2/1 cells.

A



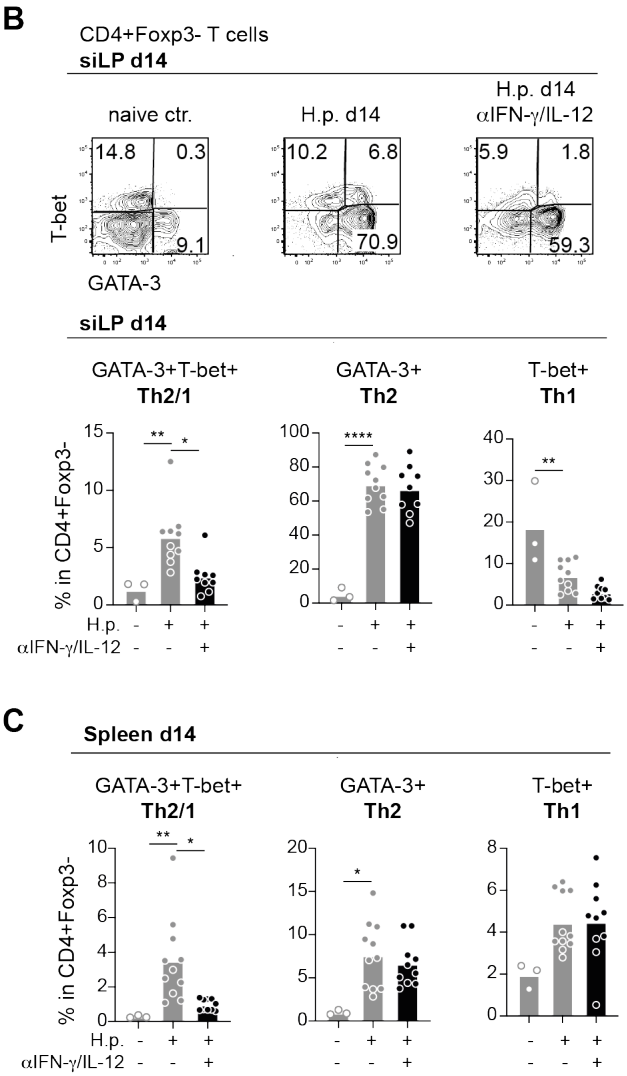


Fig. 4.3.1. Early IFN-γ/IL-12 blocking leads to restricted generation of Th2/1 cells.
A Experimental scheme: BALB/c mice (1.5 months old) infected with H.p. were treated with blocking antibodies against IL-12 and IFN-γ (0.5 mg each) at days 0, 3 and 6 and dissected at day 14 post infection. Graphs depict the frequencies of Th2/1 and Th2 cells determined in CD4+ T cells in peripheral blood on day 0, 7 and 14. Data from three independent experiments are shown as mean ± SD. (n naive=2; H.p. ctr=11; αIFN-γ/αIL-12=10 mice). Samples with low PBMC counts were excluded. **B** Representative flow cytometry plots depicting T-bet vs. GATA-3 expression in siLP-derived CD4+FoxP3- T cells isolated on day 14 post infection. Graphs depict the frequencies of Th2/1, Th2 and Th1 cells in CD4+FoxP3- T cells in siLP (**B**) and spleen **(C)**. Data from three independent experiments are pooled (n naïve=3; H.p. ctr=10-11; αIFN-γ/αIL-12=9-10 mice) in B and C. Samples with poor viability were excluded. Bars represent mean and circles correspond to individual mice. Statistical significance was determined between infected groups at each time point was performed using unpaired t test or Mann-Whitney test in A. Statistical analysis comparing all the groups were reported using one-way ANOVA with Tukey's multiple comparisons test or Kruskal-Wallis test with Dunn's multiple comparisons test in B and C. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$.

4.3.2 Diminished Th2/1 response correlates with higher resistance.

Diminished proportions of small intestinal Th2/1 cells expectedly coincided with higher resistance, characterized by lowered parasite egg shedding in the feces and reduced fecundity of female worms isolated from the gut of IFN-γ restricted mice **(Fig. 4.3.2A, B)**.

Worm burden remained unaffected by the blocking regimen **(Fig. 4.3.2C)**. In summary, poor generation of Th2/1 cells because of restricted IFN-γ availability contributes to higher resistance, which corroborates the IFN-γ-Th2/1-susceptibility axis observed when comparing young adult vs. mature mice.

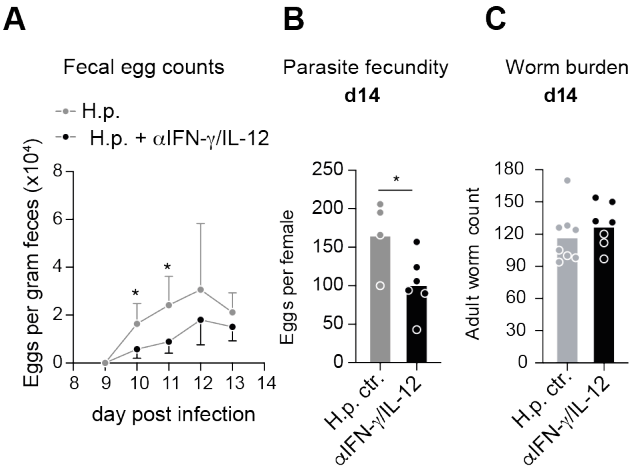


Fig. 4.3.2. Diminished Th2/1 response correlates with higher resistance.
A Fecal egg counts determined over the course of infection expressed as mean ± SD. Data derive from one out of three experiments with similar results (n H.p. ctr=4; αIFN-γ/αIL-12=6 mice). **B** Circles report the average fecundity of 8 individual female worms per mouse within 24 h of culture. Data derive from one out of three independent experiments with similar results (n H.p. ctr=4; αIFN-γ/αIL-12=6 mice). **C** Adult worm burden at day 14 post infection. Data from two independent experiments are shown (n H.p. ctr=8; αIFN-γ/αIL-12=7 mice). Bars represent mean and circles correspond to individual mice. Statistical analysis was performed using unpaired t test or Mann-Whitney test. Statistical analysis in A between untreated and αIFN-γ/αIL-12 treated mice was separately performed at each time point. *$p \leq 0.05$.

4.4 Early exposure to IFN-γ promotes Th2/1 cells and leads to poor resistance.

In addition to restricting early endogenous IFN-γ availability, a converse approach was performed where young adult BALB/c mice aged 1.5 months were infected with H.p. and treated with recombinant IFN-γ. The treatment was performed over the first five days of infection at the dose of 2.5 μg/mouse administered intraperitoneally twice a day. Following the weekly blood survey to determine the phenotype of Teff cells, experiment was terminated at day 28 post infection.

4.4.1 Early IFN-γ treatment leads to expansion of local and systemic Th2/1 cells

Early exposure to IFN-γ promoted the expansion of Th2/1 cells in blood which was evident from the early stage of infection. The expanded Th2/1 cells further increased over time in IFN-γ treated mice. **(Fig. 4.4.1A)**. The early rise in circulating Th2/1 cells was reflected in higher mucosal accumulation of Th2/1 cells at the chronic phase of infection **(Fig. 4.4.1B, C)**. Classical Th2 cells were drastically reduced upon IFN-γ exposure at the early stage of infection **(Fig. 4.1.1A)**. However, the early paucity of Th2 cells was eventually compensated as indicated by the comparable levels of Th2 cells in IFN-γ treated vs. untreated mice at day 28 post infection **(Fig. 4.1.1A-C)**. Th1 cells remained unaffected by the exposure to IFN-γ **(Fig. 4.1.1C)**. In brief, early exposure to IFN-γ promotes the expansion and systemic and mucosal accumulation of Th2/1 cells.

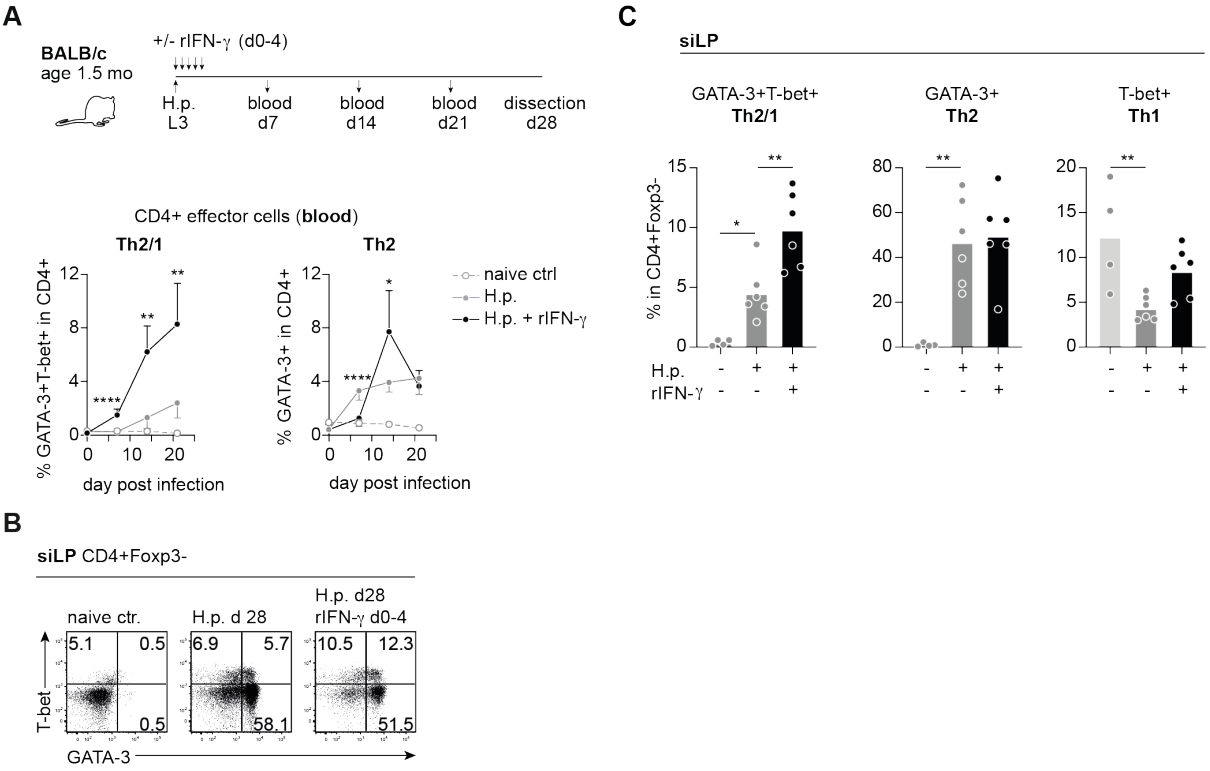


Fig. 4.4.1. Early IFN-γ treatment leads to expansion of local and systemic Th2/1 cells.
A Experimental scheme: BALB/c mice (1.5 months old) were infected with H.p. and treated with rIFN-γ (2.5 μg twice a day) from day 0 to 4 post infection followed by dissection at day 28 post infection. Graphs report the frequencies of Th2/1 hybrid and Th2 cells in CD4+ T cells in blood over the course of infection. Data from two independent experiments are pooled (n naïve=3, infected ctr/ rIFN-γ treated=6 mice) and depicted as mean ± SD. **B** Representative flow cytometry plots depicting T-bet vs. GATA-3 expression in siLP-derived CD4+FoxP3- T cells at day 28 post H.p. infection. **C** Bar graphs report the frequencies of Th2/1, Th2 and Th1 cells in siLP CD4+ T cells. Data from two independent experiments are combined (n naïve=4; infected ctr/ rIFN-γ treated=6 mice). Bars represent mean and circles correspond to individual mice. Statistical analysis in A was performed between untreated and IFN-γ treated mice at each time point using unpaired t test or Mann-Whitney test. Statistical significance was determined using one-way ANOVA with Tukey's multiple comparisons test or Kruskal-Wallis test with Dunn's multiple comparisons test in C. *$p \leq 0.05$, **$p \leq 0.01$, ****$p \leq 0.0001$.

4.4.2 More Th2/1 cells correlate with poor parasite control in IFN-γ treated mice.

Small intestinal milieu populated by increased proportions of Th2/1 cells significantly affected parasite fitness in IFN-γ treated mice **(Fig. 4.4.2)**. Parasite egg shedding was increased right from the beginning of egg deposition and was further maintained at significantly higher levels in IFN-γ treated compared to untreated mice at majority of surveyed time points **(Fig. 4.4.2A)**. While the expulsion phenotype four weeks post infection remained unaffected with most individuals displaying very few or no worms in both the groups **(Fig. 4.4.2B)**, female worm fecundity in IFN-γ treated mice was significantly higher than untreated controls **(Fig. 4.4.2C)**. IFN-γ treated mice therefore reflected mature mice in displaying increased worm fitness. In addition, early exposure to IFN-γ led to significantly reduced granuloma formation in mice typically displaying pronounced granulomatous response post H.p. infection (Filbey et al., 2014). In brief, small intestinal accumulation of Th2/1 cells in IFN-γ exposed mice correlates with poor resistance accounted for by the development of worms with higher reproductive potential.

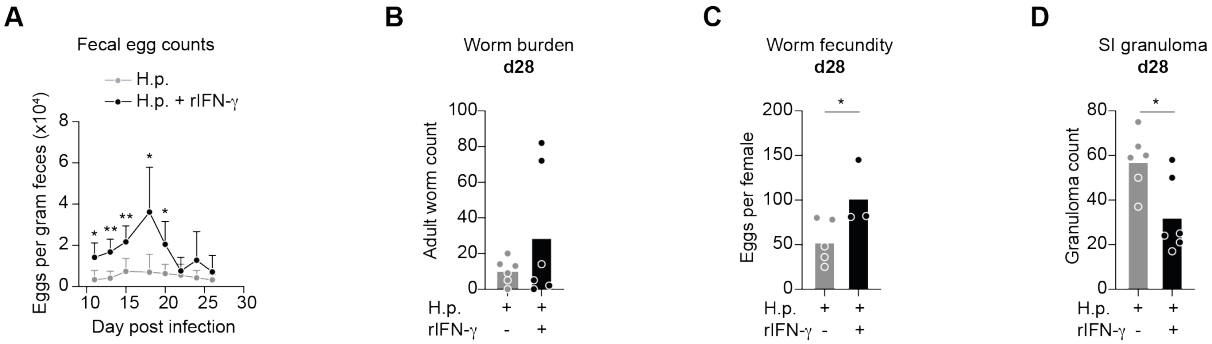


Fig. 4.4.2. More Th2/1 cells correlate with poor parasite control in IFN-γ treated mice. **A** Kinetics of fecal egg shedding over the course of infection. Data from two independent experiments are shown as mean ± SD (n=6 mice/group). **B** Adult worm counts at day 28 post infection. Data derive from two independent experiments (n=6 mice/group). **C** Average number of eggs produced by individual female worms (typically eight worms per mouse, minimum 3) isolated from each mouse. Data from two independent experiments are pooled (n untreated infected ctr=5; rIFN-γ treated=3 mice). Note that expulsion was completed in one infected ctr and three rIFN-γ treated mice. **D** Granuloma count in small intestine at day 28 post infection. Data from two independent experiments are reported each performed with n=3 mice/group. Bars represent mean and circles correspond to individual mice. Statistical analysis was performed using unpaired t test or Mann-Whitney test. Statistical analysis in A between untreated and IFN-γ treated mice was separately performed at each time point. *$p \leq 0.05$, **$p \leq 0.01$.

4.5 IFN-γ promotes the overall systemic GATA-3+ T cell response

Interestingly, early exposure to IFN-γ, by promoting the expansion of circulating Th2/1 cells **(Fig. 4.4.1)**, led to the more pronounced accumulation of total GATA-3+ Teff cells in blood over the course of H.p. infection compared to untreated infection controls **(Fig. 4.5A)**. Given the established role of IFN-γ as Th1 promoter and suppressor of Th2 responses as mentioned earlier, the dramatic expansion of GATA-3+ T cells post IFN-γ exposure was highly confounding. The increased circulating GATA-3+ T cells in blood were also reflected by a remarkable rise in percentage as well as absolute numbers of total GATA-3+ T cells in spleen of IFN-γ treated mice at the chronic stage of infection **(Fig. 4.5B)**. The small intestinal accumulation of GATA-3+ T cells however remained unaffected post IFN-γ exposure (data not shown). Of note, the accumulation of splenic GATA-3+ T cells in IFN-γ treated mice was essentially contributed by strongly expanded IFN-γ producing GATA-3+ Th2/1 cells **(Fig. 4.5C)**. Overall, the findings highlight an obscured role of IFN-γ as a promoter of GATA-3+ responses by specifically driving expansion of Th2/1 cells.

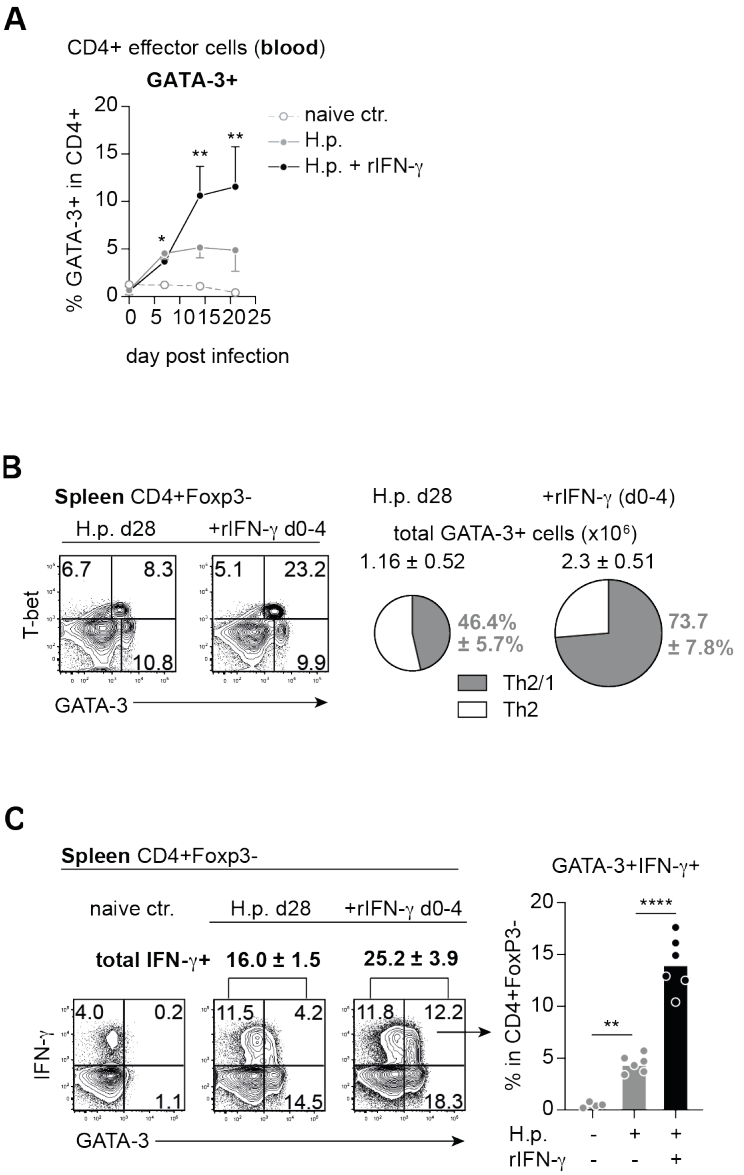


Fig. 4.5. IFN-γ promotes the overall systemic GATA-3+ T cell response.
A Kinetics of GATA-3+ cells in CD4+ T cells in blood over the course of infection. Data from two independent experiments are shown as mean ± SD (n naïve=3, infected ctr/rIFN-γ treated=6 mice). **B** Representative flow cytometry plots depicting T-bet vs. GATA-3 expression in CD4+FoxP3- T cells in spleen. Pie charts report the percentage of GATA-3+T-bet+ Th2/1 cells in CD4+GATA-3+ T cells isolated from the spleen at day 28 post infection. The size of the pie charts is adjusted according to the absolute cell count of GATA-3+ cells as mentioned. Data derive from two independent experiments (n=6 mice/group). **C** Left: Representative flow cytometry plots depicting IFN-γ vs. GATA-3 expression of PMA/Ionomycin stimulated splenocytes at day 28 post infection. Right: Bar graph depicts the frequencies of GATA-3+IFN-γ+ cells in spleen-derived CD4+FoxP3- T cells. Data from two independent experiments are shown (n naïve=4; infected ctr/rIFN-γ treated=6 mice). Bars represent mean and circles correspond to individual mice. Statistical analysis in A was performed between untreated and IFN-γ treated mice at each time point using unpaired t test. In C, statistical significance was determined using one-way ANOVA with Tukey's multiple comparisons test. *$p \leq 0.05$, **$p \leq 0.01$, ****$p \leq 0.0001$.

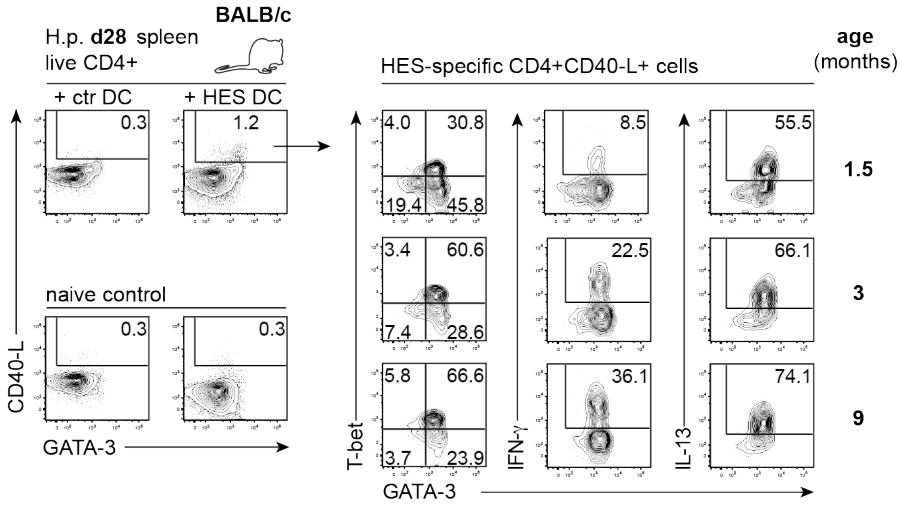
4.6 Differential IFN-γ availability dramatically affects the parasite specific (PS) responses.

Next to the overall Teff responses, we further assessed the effect of age-associated and experimentally altered IFN-γ availability on the phenotype of parasite specific CD4+ T cells generated in response to H.p. infection. The parasite specific CD4+ T cells were identified as follows: Spleen or mLN-derived cells were cultured with dendritic cells (DC) pre-loaded with H.p. excretory/secretory products (HES). Following an overnight co-culture, parasite specific CD4+ T cells upregulated CD40-ligand (CD40-L) in response to TCR activation by HES-primed DCs. CD40-L+ CD4+ T cells were further assessed for GATA-3/IL-13 and T-bet/IFN-γ expression in young adult vs. mature, IFN-γ restricted or IFN-γ exposed mice.

4.6.1 PS IFN-γ producing cells rise in an age-dependent manner.

Assessing the parasite specific responses in differentially aged mice showed that PS cells (CD4+CD40-L+) were enriched in GATA-3 expression in mice of all age groups. While PS GATA-3 as well as IL-13 expression remained unaffected with age, CD40-L+ cells co-expressing T-bet were increased in an age-dependent manner **(Fig. 4.6.1A, B)**. Along similar lines, IFN-γ production from PS cells was dramatically increased with age, where 9-month-old mice displayed a four-fold rise in PS IFN-γ producing cells compared to 1.5-month-old mice. Hence, age-dependent rise in steady state IFN-γ competence correlated with skewing of CD40-L+ PS cells towards acquisition of a Th2/1 phenotype and increased IFN-γ production without altering the expression of Th2-associated parameters.

A



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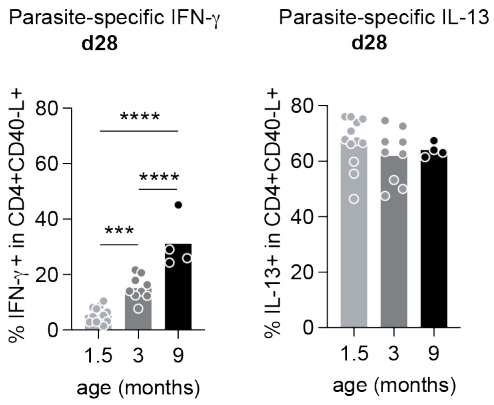
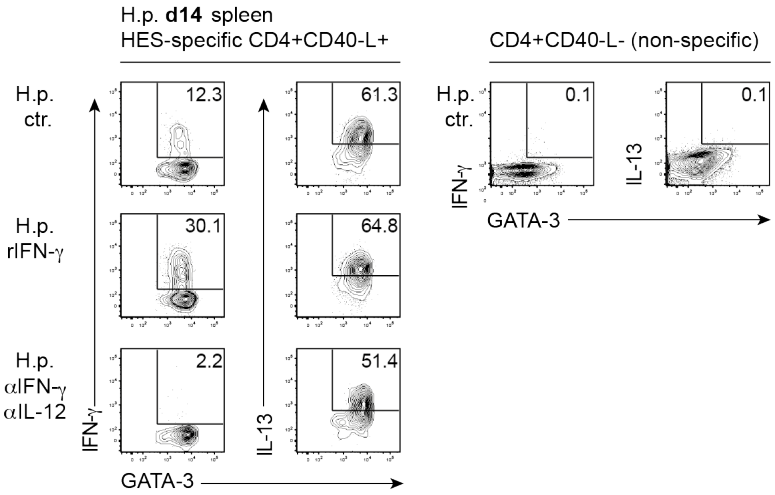


Fig. 4.6.1. PS IFN-γ producing cells rise in an age-dependent manner.
The phenotype of parasite specific CD4+ T cells was determined by stimulating spleen cells with BmDCs primed with H.p. excretory/secretory products (HES). **A** Flow cytometry plots depicting selective upregulation of CD40-L by CD4+ T cells derived from chronically infected BALB/c mice upon co-culture with HES-primed BmDCs (left). CD4+ T cells of naïve control mice (bottom) did not respond to HES-DC stimulation. Plots on the right depict T-bet vs. GATA-3 expression (left), IFN-γ expression (mid) and IL-13 expression (right) in CD40-L+ cells. CD40-L negative cells did not comprise cytokine expressing cells (data not shown). All plots were generated by merging the data of three mice per age group. **B** Graphs report the frequencies of IFN-γ and IL-13 producing cells determined in CD40-L+ cells of 1.5 months (n=12), 3 months (n=9) and 9 months (n=4) old mice as determined in four independent experiments. Bars represent mean and circles correspond to individual mice. Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparisons test. ***$p \leq 0.001$, ****$p \leq 0.0001$.

4.6.2 Experimental manipulation of early IFN-γ availability alters the production of PS IFN-γ.

Similar to mature mice, young adult mice transiently exposed to IFN-γ displayed a highly significant rise in PS IFN-γ+ cells, whereas experimental blocking of early type 1 cytokines led to a near-complete ablation of PS IFN-γ production (**Fig. 4.6.2A-B**). Of note, IFN-γ exposure as well as blocking of type 1 signals led to a modest, but significant decline in IL-13 production by parasite-specific cells, as opposed to the unaltered phenotype of PS IL-13 producing cells in differentially aged mice. Overall, the findings suggest that the phenotype of PS cells in H.p. infected mice is highly amenable to the age-associated or even the transient manipulation of IFN-γ availability during the priming phase of infection. IFN-γ poised natural or artificially induced milieu in H.p. infection supports the acquisition of Th2/1 phenotype by PS cells.

A



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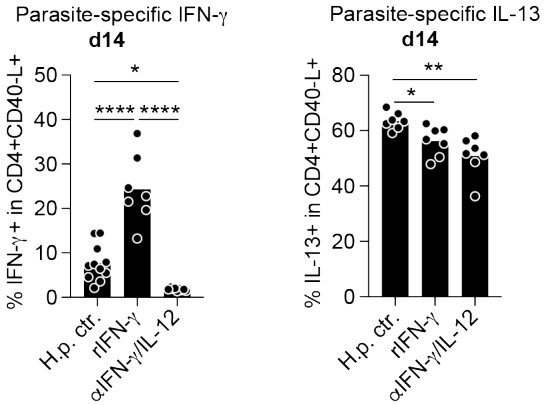

Fig. 4.6.2. Experimental manipulation of early IFN-γ availability alters the production of PS IFN-γ

The parasite specific CD4+ T cells was identified as mentioned earlier. **A** Plots report the percentages of IFN-γ (1st column) and IL-13 producing cells (2nd column) determined in CD40-L+ cells (left) and CD40-L- cells (right). **B** Graphs depict the frequencies of IFN-γ and IL-13 producing cells determined in CD40-L+ cells of untreated infection controls (n=7-11), rIFN-γ supplemented (n=7) and αIL-12/αIFN-γ treated mice (n=7) as determined in two to three independent experiments. Bars represent mean and circles correspond to individual mice. Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparisons test. *$p \leq 0.05$, **$p \leq 0.01$, ****$p \leq 0.0001$.

4.7 Increased mucosal IFN-γ availability promotes the fitness of developing larvae.

As the fecundity of adult female worms correlated with IFN-γ production by intestinal T cells and the small intestinal accumulation of IFN-γ competent Th2/1 cells, we hypothesized that the inflammatory milieu experienced by the developing tissue-standing larvae may account for differences in reproductive potential of the eventually matured adult worms. To address this possibility, BALB/c mice (1.5 months old) were infected with H.p. and treated with rIFN-γ as earlier followed by the dissection at day 6 post infection.

4.7.1 IFN-γ treatment leads to an early rise in IFN-γ competent Th2/1 cells in small intestine.

Early IFN-γ exposure led to expansion of small intestinal Th2/1 cells, identified as T-bet+GATA-3+, T-bet+IL-4+ or IFN-γ+GATA-3+ cells at the early stage of infection **(Fig. 4.7.1A-C)**. IFN-γ treated mice further displayed reduced early accumulation of small

intestinal Th2 cells. However, the total GATA-3+ T cells populating the small intestine in IFN-γ treated mice remained comparable to the untreated controls, as expanded Th2/1 cells compensated the reduced frequencies of Th2 cells **(Fig. 4.7.1A)**. In line with GATA-3+ Teff response, IL-4 producing T cells remained unimpaired in IFN-γ treated mice **(Fig. 4.7.1B)**. However, IFN-γ treated mice displayed an increased proportion of IFN-γ producing cells in small intestine essentially due to the expansion of IFN-γ producing Th2/1 cells **(Fig. 4.7.1C)**. Hence, early supplementation with IFN-γ in H.p. infection resulted in an IFN-γ poised environment in small intestine resulted by the accumulation of IFN-γ producing Th2/1 cells.

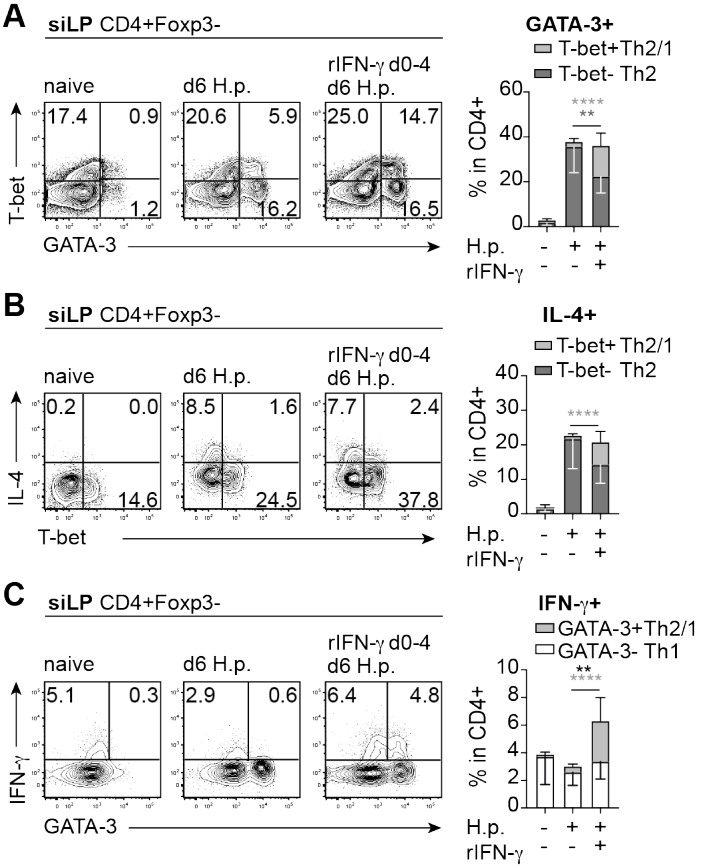

Fig. 4.7.1. IFN-γ treatment leads to an early rise in IFN-γ competent Th2/1 cells in small intestine.

BALB/c mice (1.5 mo) infected with H.p. were treated with rIFN-γ (2.5 μg twice a day) during the first five days of infection followed by dissection at day 6 post infection. Flow cytometry plots (left) and bar graphs (right) depict T-bet vs. GATA-3 expression **(A)**, IL-4 vs. T-bet expression **(B)** and IFN-γ vs. GATA-3 expression **(C)** in siLP-derived CD4+FoxP3- T cells. Stacked bars report the percentage of total GATA-3+, IL-4+ and IFN-γ+ cells, deciphering between T-bet+/- and GATA-3+/- cells as indicated. Cells were stimulated with PMA/ionomycin. Data from three independent experiments (n naïve=6; n infected=9-10 mice/group) are reported as mean ± SD. Statistical analysis was performed using one-way ANOVA and Tukey's multiple comparisons test or Kruskal-Wallis test and Dunn's multiple comparisons test. The statistical significance comparing each sub-population is shown by asterisks in the corresponding colors. Black asterisks in C indicate significantly different overall frequencies of IFN-γ+ producing cells comparing infection controls and IFN-γ-treated mice. $^{**}p \leq 0.01$, $^{****}p \leq 0.0001$.

4.7.2 Higher mucosal IFN-γ competence correlates with increased capacity of L4 migration in IFN-γ treated mice.

To assess if the early small intestinal Th2/1 bias in IFN-γ treated mice translated to differences in the fitness of developing L4 larvae, we adopted a procedure to harvest L4 larvae from the small intestinal tissue (Ey et al., 1981). Freshly excised proximal small intestinal explants were secured at the ends and cultured for four hours to allow the larval egress towards abluminal side. The inverted migratory behavior allowed to determine the percentage of egressed larvae from the tissue explants retrieved from IFN-γ treated vs. untreated mice **(Fig. 4.7.2A)**. Significantly higher percentage of L4 larvae were able to migrate and fully exit the tissue explants derived from IFN-γ treated mice compared to the untreated controls **(Fig. 4.7.2B)**. The data hence suggest that the L4 larvae maturating in small intestine populated by more IFN-γ competent Th2/1 cells exhibit higher fitness manifested in increased migration capacity as compared to the larvae developing in small intestine of untreated controls.

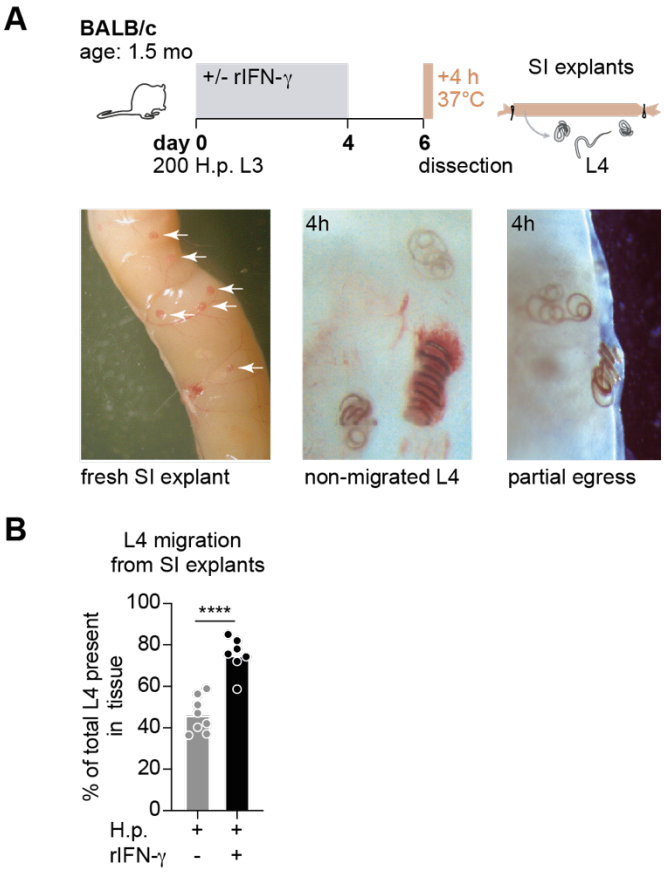

Fig. 4.7.2. Higher mucosal IFN-γ competence correlates with increased capacity of L4 migration in IFN-γ treated mice.

A Experimental scheme: Freshly isolated proximal small intestinal explants were secured at both ends and cultured at 37°C. The number of L4 larvae migrating from the tissue was determined at the end of 4 h. Pictures show a freshly prepared small intestinal explant with embedded L4 (white arrows); L4 remaining in the tissue after 4h of culture; and partially egressed L4 larvae. **B** Graph depicts L4 migrated from the tissue within 4h, expressed as the percentage of total larvae in the tissue explant. Data from two independent experiments are pooled (n=7-8 mice/group). Bars represent mean and circles correspond to individual mice. Statistical significance was determined using unpaired t test. ****$p \leq 0.0001$.

4.8 Susceptible C57BL/6 mice are characterized by higher steady state Th1 accumulation and extensive generation of Th2/1 cells.

Inbred mice lines display inherent differences in resistance to H.p. infection where BALB/c mice are more resistant than the fully susceptible C57BL/6 mice (Filbey et al., 2014). C57BL/6 mice are in fact considered 'Th1 prone' in displaying increased pro-inflammatory gene expression and M1 polarization associated with higher resistance to *Leishmania* infection, as opposed to BALB/c mice which display Th2 response and M2 polarization leading to disease exacerbation (Restrepo et al., 2021). We hence asked if C57BL/6 mice accumulate IFN-γ competent cells more rapidly at steady state and generate more pronounced Th2/1 hybrid responses post H.p. infection.

4.8.1 More rapid accumulation of Th1 cells is seen at steady state in C57BL/6 mice.

Comparing the uninfected C57BL/6 and BALB/c mice (mean age of both strains: 1.9 +/- 0.5 months), the frequencies of Th1 cells, the professional IFN-γ producing cells, were elevated in C57BL/6 compared to BALB/c mice in lymphoid organs mLN and spleen as well as in small intestine **(Fig. 4.8.1)**. Hence, in line with their reported resistance phenotype to *H. polygyrus*, young adult C57BL/6 mice are skewed towards a higher type 1 activity compared to the age matched BALB/c mice.

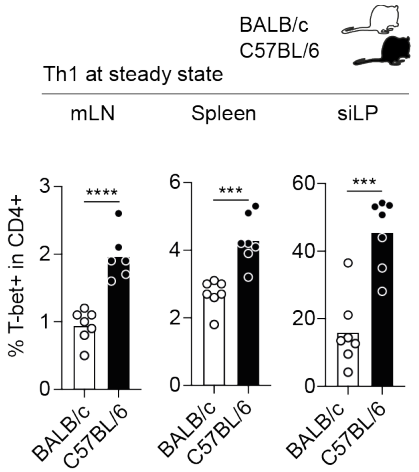

Fig. 4.8.1. More rapid accumulation of Th1 cells is seen at steady state in C57BL/6 mice.

A Bar graphs report the frequencies of T-bet+ cells in CD4+ T cells in mLN, spleen and small intestine of naïve young adult BALB/c and C57BL/6 mice aged 1.5 months. Data derive from three independent experiments (n=6-7 mice/ group). Bars represent mean and circles correspond to individual mice. Statistical significance was determined using unpaired t test or Mann-Whitney test. $^{**}p \leq 0.01$, $^{***}p \leq 0.001$, $^{****}p \leq 0.0001$.

4.8.2 IFN-γ competent C57BL/6 mice are populated by more Th2/1 cells post H.p. infection compared to BALB/c mice.

In line with more rapid accumulation of Th1 cells at steady state, C57BL/6 mice harbored higher proportions of small intestinal IFN-γ producing cells, deciphered between Th1 or CD4- cells in naïve uninfected mice **(Fig. 4.8.2A)**. H.p. infected C57BL/6 mice generated more Th2/1 cells evidently seen in spleen as well as small intestine at the chronic stage of infection **(Fig. 4.8.3B-D)**. Classical Th2 cells were comparable between the two strains. More Th2/1 cells in C57BL/6 compared to BALB/c mice contributed to increased proportions of overall IFN-γ producing cells, next to IFN-γ producing Th1 and CD4- cells in H.p. infected mice **(Fig. 4.8.3A)**. Overall, the findings suggest that higher IFN-γ competence translates to more Th2/1 cells at chronic stage of infection in C57BL/6 compared to BALB/c mice.

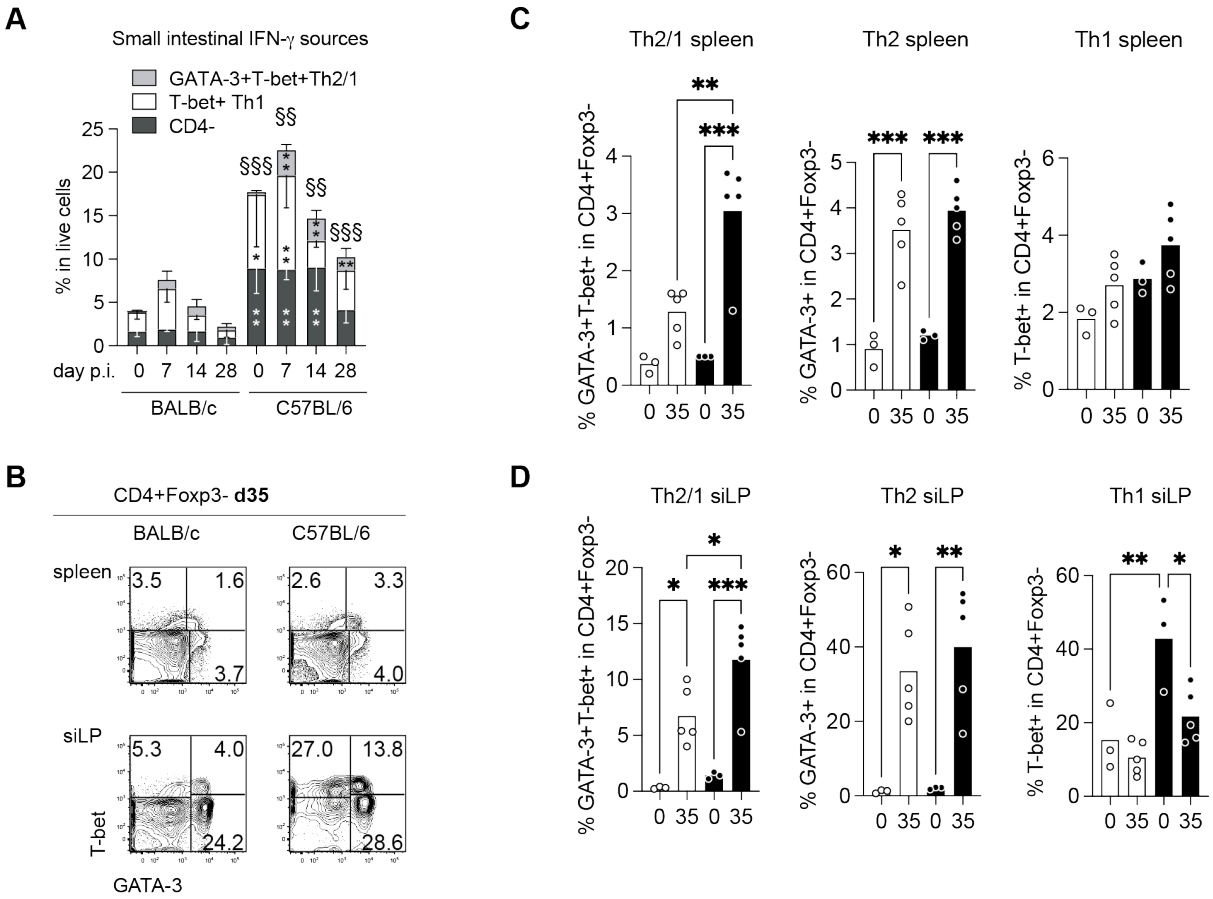

Fig. 4.8.2. IFN-γ competent C57BL/6 mice are populated by more Th2/1 cells post H.p. infection compared to BALB/c mice.

A Bar graphs report small intestinal IFN-γ responses by CD4-, Th1 and Th2/1 cells shown from one dataset representative for 2-3 experiments as mean ± SD (n=3-6 per infection time point). Naïve controls (n=5 and 8) are pooled from four individual experiments. IFN-γ production was assessed in response to stimulation with PMA/Ionomycin. **B** Flow cytometry plots depicting T-bet vs. GATA-3 expression in CD4+Foxp3- T cells in spleen and small intestine at day 35 post infection. Bar graphs report the frequencies of Th2/1, Th2 and Th1 cells in **C** spleen and **D** small intestine. Data from one out of two experiments with similar outcome are shown (n naïve=3, infected=5 mice). Bars represent mean and circles correspond to individual mice. Statistical analysis was performed using one-way ANOVA and Tukey's multiple comparisons test or Kruskal-Wallis test and Dunn's multiple comparisons test. Significant differences between the mouse lines at a given time point concerning the total and cell type-specific IFN-γ signals are indicated by (§) and (*), respectively. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.

4.8.3 C57BL/6 mice harboring more Th2/1 cells display poor parasite control.

In line with the increased generation of Th2/1 cells, C57BL/6 mice displayed poor parasite control **(Fig. 4.8.3)**. Fecal egg shedding was elevated in C57BL/6 compared to BALB/c mice throughout the course of infection **(Fig. 4.8.3A)**. Furthermore, while most individuals expelled the worms by day 35 in case of BALB/c mice, C57BL/6 mice displayed significantly higher worm burden **(Fig. 4.8.3B)**. Hence, higher Th2/1 bias in H.p. infected C57BL/6 compared to BALB/c mice coincides with poor resistance.

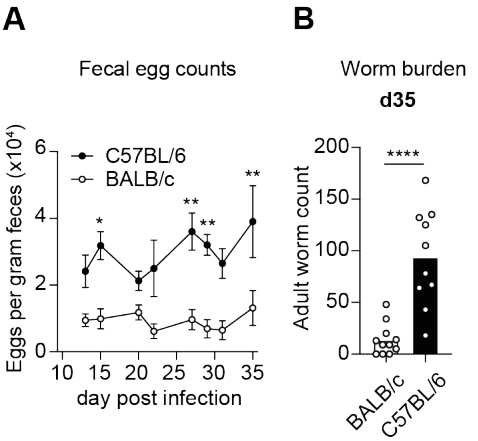

Fig. 4.8.3. C57BL/6 mice harboring more Th2/1 cells display poor parasite control. **A** Kinetics of fecal egg shedding in H.p. infected BALB/c and C57BL/6 mice over the course of five weeks. Data derive from one out of two experiments with similar results and are reported as mean ± SD (n=5 mice/group). **B** Adult worm counts at day 35 post infection. Data from three independent experiments (n=10-11 mice/group) are combined. Bars represent mean and circles correspond to individual mice. Statistical analysis was performed using unpaired t test or Mann-Whitney test. *$p \leq 0.05$, **$p \leq 0.01$, ****$p \leq 0.0001$.

4.8.4 Early Th2/1 bias in C57BL/6 mice is associated with increased L4 migration capacity.

To assess if the increased egg shedding in C57BL/6 compared to BALB/c mice corresponded with the development of fitter larvae, we compared the small intestinal responses and the larval migratory behavior in the two strains at day 6 post infection. Young adult C57BL/6 mice harbored more small intestinal Th2/1 cells, identified as T-bet expressing and IFN-γ producing GATA-3+ T cells compared to age-matched BALB/c mice at the early stage of infection **(Fig. 4.8.4A-B)**. The type 1 milieu in C57BL/6 mice was further accentuated by poor classical Th2 accumulation and generation of more Th1 cells compared to BALB/c mice. The higher small intestinal Th2/1 bias in C57BL/6 mice correlated with more L4 larvae managing to fully exit the tissue explants cultured *ex vivo* **(Fig. 4.8.4C)**. Hence, early local Th2/1 bias in C57BL/6 compared to BALB/c mice is associated with the development of fitter L4 larvae.

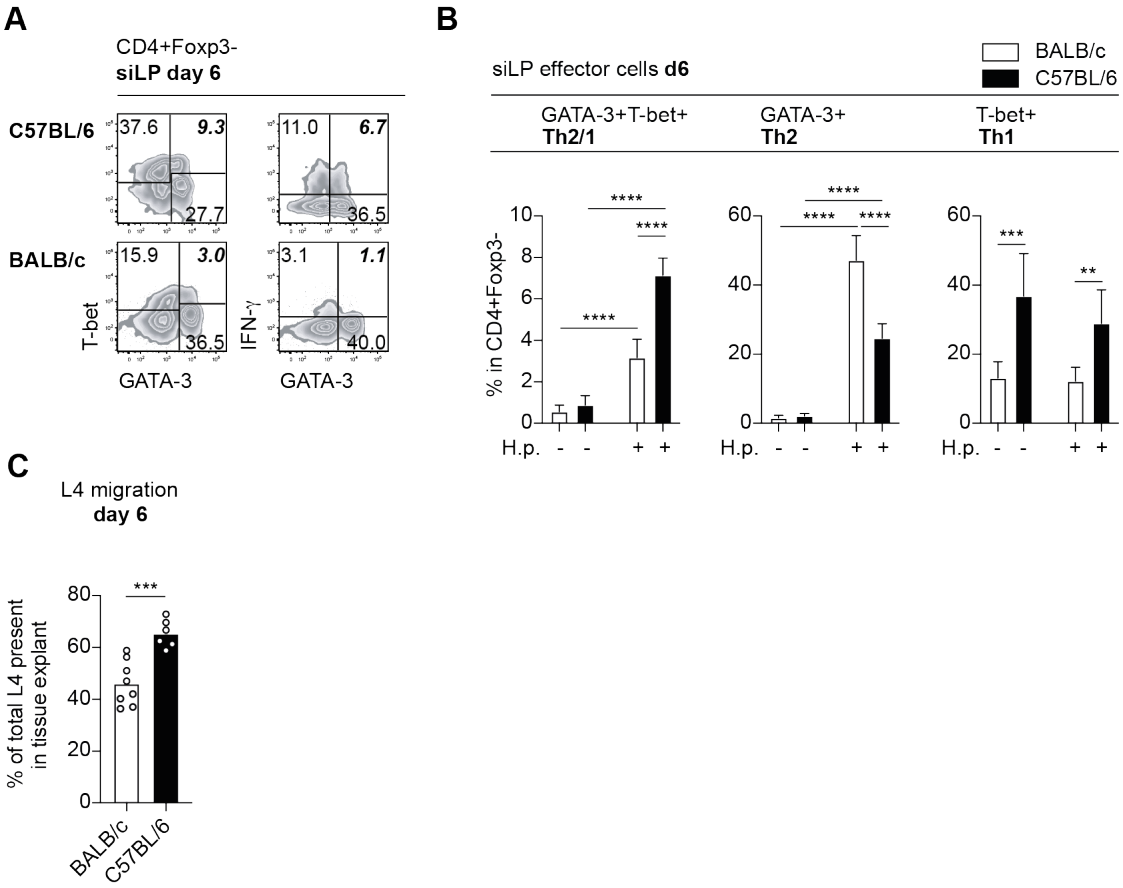

Fig. 4.8.4. Early Th2/1 bias in C57BL/6 mice is associated with increased L4 migration capacity.
A Flow cytometry plots depicting T-bet vs. GATA-3 expression (left) and IFN-γ vs GATA-3 expression in CD4+FoxP3- T cells in small intestine at day 6 post infection. **B** Bar graphs report the frequencies of Th2/1, Th2 and Th1 cells in small intestine in BALB/c and C57BL/6 mice at day 6 post infection. Data from two (C57BL/6) or three (BALB/c) experiments are reported as mean + SD (n BALB/c naïve=7, C57BL/6 naïve=4; BALB/c infected=10, C57BL/6 infected=6 mice). **C** Quantification of L4 migrated from small intestinal tissue explants within 4h, expressed as percentage of the total larvae present in tissue. Data from two experiments each performed with four BALB/c and three C57BL/6 mice per group are combined. Bars represent mean and circles correspond to individual mice. Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparisons test or Kruskal-Wallis test with Dunn's multiple comparisons test in B and unpaired t test or Mann-Whitney test in c. ***$p \leq 0.001$, ****$p \leq 0.0001$.

4.8.5. C57BL/6 mice display higher Th2/1 bias compared to resistant SJL mice.

The bias in favor of Th2/1 cells in C57BL/6 mice was also observed when comparing the Teff responses to SJL mice which display very high resistance and clear the infection within 2-3 weeks (Filbey et al., 2014) **(Fig. 4.8.5)**. Akin to BALB/c mice, poor IFN-γ competence is seen in SJL mice (**Fig. 4.8.5A**) coinciding with impaired accumulation of small intestinal Th2/1 cells at early stage of infection (**Fig. 4.8.5B**). The poor Th2/1 responses correlated with higher M2 polarization in the peritoneal cavity compared to susceptible C57BL/6 mice **(Fig. 4.8.5C)**. Hence, the extent of Th2/1 accumulation is a correlate for susceptibility across mice lines of different genotypes.

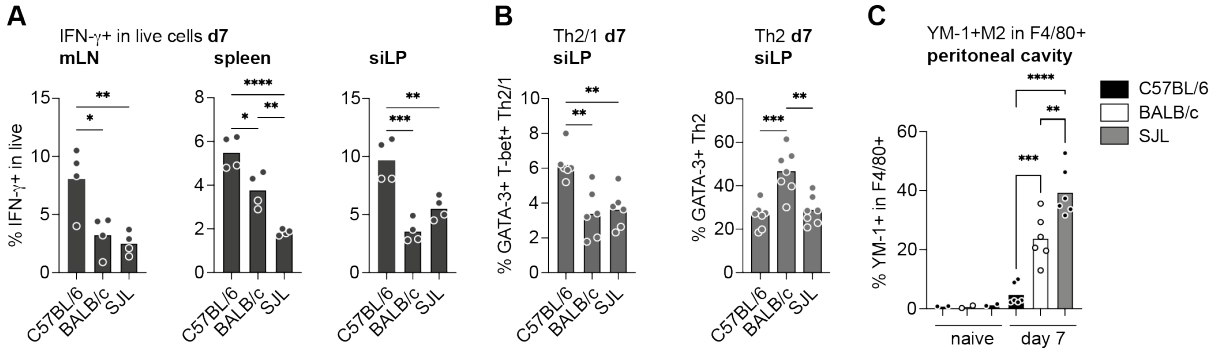

Fig. 4.8.5. C57BL/6 mice display higher Th2/1 bias compared to resistant SJL mice. **A** Graphs report the frequencies of IFN-γ producing cells in response to PMA/ionomycin in mLN, spleen and small intestine at day 7 post infection. Data derive from one out of two experiments with similar results (n=4 mice/strain). **B** Frequencies of Th2/1 and Th2 cells in small intestine. **C** Frequencies of Ym-1 expressing M2 macrophages in C57BL/6 vs. BALB/c vs. SJL mice. Data from two experiments are pooled in B and C (n=8 mice/strain). Bars represent mean and circles correspond to individual mice. Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparisons test or Kruskal-Wallis test with Dunn's multiple comparisons test. *$p \leq 0.05$, **$p \leq 0.01$,***$p \leq 0.001$, ****$p \leq 0.0001$.

4.8.6 Age-dependent rise in susceptibility is seen in both resistant and susceptible strains.

To determine if the age-associated rise in susceptibility is not an exclusive feature of BALB/c mice, we aimed to compare the Teff responses and resistant phenotype in young adult (3 months old) and mature (6 months old) BALB/c and C57BL/6 mice. The bias in favor of Th2/1 cells, reported by lower Th2 to Th2/1 ratios, was increased in an age-dependent manner in BALB/c mice **(Fig. 4.8.6A)**. While the differentiation of splenic Th2/1 cells was comparable in young adult vs. mature C57BL/6 mice, a significant drop in the ratios of Th2 to Th2/1 cells was cogently observed in both mLN as well as small intestine in mature compared to young adult C57BL/6 mice two weeks post infection **(Fig. 4.8.6A)**. Furthermore, the Th2/1 poised small intestinal milieu in mature compared to young adult mice corresponded with increased fecundity of female worms observed to a significant degree in BALB/c and in trend in C57BL/6 mice **(Fig. 4.8.6A)**. Importantly, similar bias for Th2/1 cells in mature BALB/c and young adult C57BL/6 mice correlated with similar egg production by female worms, thereby corroborating the reliability of fecundity analysis across host genotypes and age groups **(Fig. 4.8.6B)**. In brief, our findings suggest that age-dependent rise in Th2/1 bias and correspondingly increased parasite fitness is seen in resistant BALB/c as well as susceptible C57BL/6 mice. However, the more divergent differences seen at the younger age tend to level out in older mice.

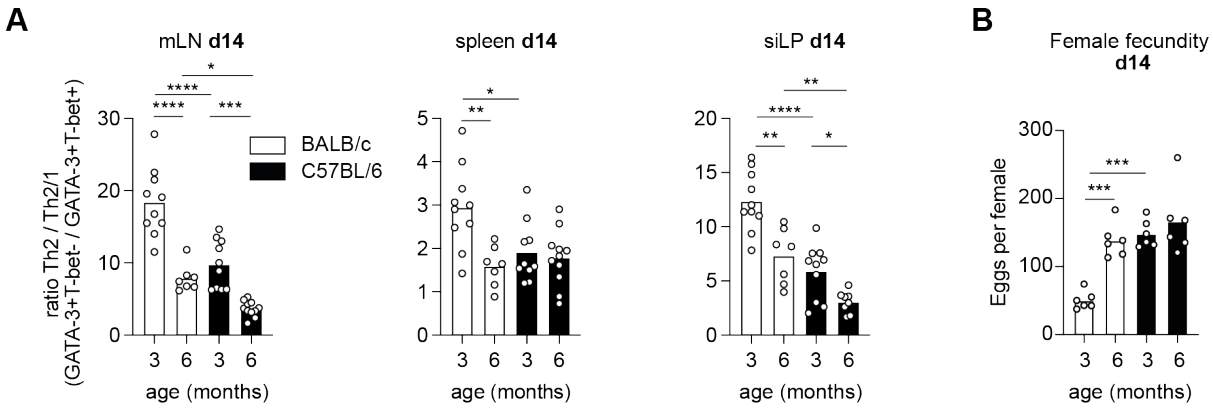

Fig. 4.8.6. Age-dependent rise in susceptibility is seen in both resistant and susceptible strains.
A Graphs report the ratio of Th2 to Th2/1 cells in mLN, spleen and small intestine at day 14 post infection. Data from 2-3 individual experiments each performed with 3-5 mice/group are pooled. **B** Mean fecundity of individual female worms within 24 h of culture. Data derive from two independent experiments each performed with n=3 mice/group. Bars represent mean and circles correspond to individual mice. Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparisons test or Kruskal-Wallis test with Dunn's multiple comparisons test. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$.

4.9 The susceptible phenotype of C57BL/6 mice is contingent to the early IFN-γ availability.

While the susceptible phenotype of C57BL/6 mice strongly correlated with the higher steady state Th1 accumulation and generation of significantly more Th2/1 cells post H.p. infection, whether the poor resistance was indeed resulted by increased IFN-γ availability was uncertain. We hence asked if limiting early endogenous IFN-γ availability converted the immunological phenotype of C57BL/6 mice to that of the BALB/c mice displaying more effective response to the infection. To that end, young adult C57BL/6 mice (3 months old) infected with H.p. were treated with *in vivo* neutralizing antibodies against IL-12 and IFN-γ as earlier followed by dissection at day 14 post infection **(Fig. 4.9.1A)**.

4.9.1 Early restriction of IFN-γ availability leads to poor Th2/1 response.

As expected, restricting early IFN-γ availability correlated with reduced circulating Th2/1 cells in blood, while classical Th2 cells, although increased at day 7 in blocked mice, reached similar levels as untreated controls by two weeks post infection **(Fig. 4.9.1A)**. The Teff phenotype in blood was mirrored in small intestine, characterized by dramatically reduced accumulation of Th2/1 cells **(Fig. 4.9.1B)**. As shown earlier, accumulation of classical Th2 cells remained unaffected upon blocking IFN-γ **(Fig. 4.9.1B)**. Restricting early IFN-γ availability further led to a trend of reduced Th1 cells in small intestine **(Fig. 4.9.1B)**. Overall, restricting early IFN-γ availability led to significantly impaired mucosal accumulation of Th2/1 cells in C57BL/6 mice, confirming the findings in BALB/c mice.

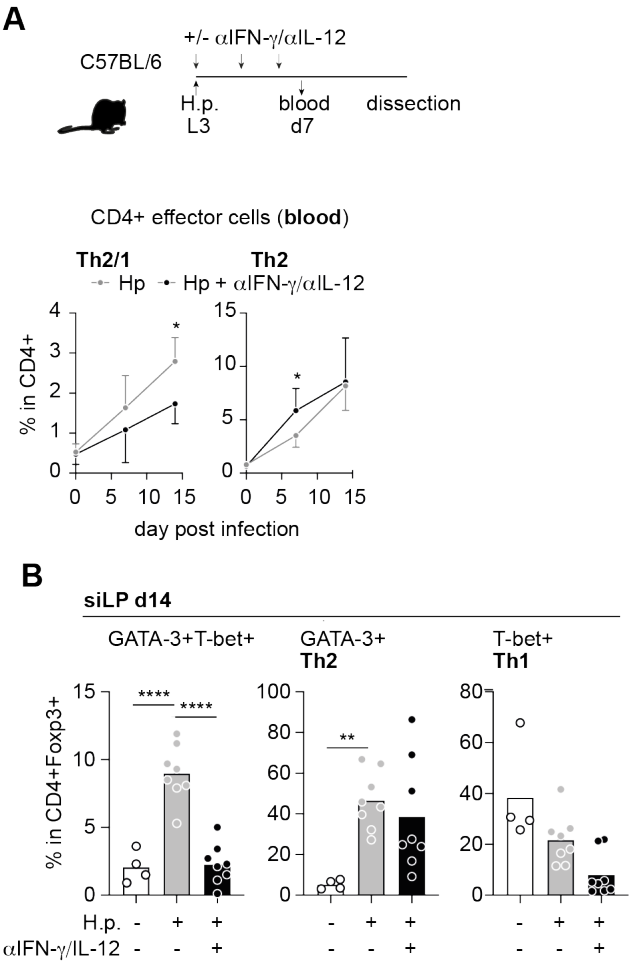

Fig. 4.9.1. Early restriction of IFN-γ availability leads to poor Th2/1 response.
A Experimental scheme: C57BL/6 mice (3.5 ± 2 months) infected with H.p. were treated with αIL-12 and αIFN-γ as described earlier followed by dissection at day 14 post infection. Graphs report the kinetics Th2/1 hybrid and Th2 cells in circulating CD4+ T cells over the course of infection. Data from two independent experiments are shown as mean ± SD (n naïve=6, n infected=4-7 mice/group). **B** Bar graphs depict the frequencies of Th2/1, Th2 and Th1 cells in CD4+FoxP3- T cells derived from small intestine. Data derive from two independent experiments (n naïve=4; H.p. ctr=8; αIFN-γ/αIL-12=8 mice). Bars represent mean and circles correspond to individual mice. Statistical analysis was performed between infected groups using unpaired t test or Mann-Whitney test in A. Naïve and infected groups were compared using one-way ANOVA with Tukey's multiple comparisons test or Kruskal-Wallis test and Dunn's multiple comparisons test in B. *$p \leq 0.05$, **$p \leq 0.01$, ****$p \leq 0.0001$.

4.9.2 Early blocking of IFN-γ leads to lowered mucosal IFN-γ competence.

Assessing the cytokine profile of Teff cells in small intestine, restricting early IFN-γ availability led to reduced proportions of IFN-γ producing T cells **(Fig. 4.9.2)**. The proportions of both IFN-γ+ Th1 (GATA-3-) cells and Th2/1 (GATA-3+) cells were halved in IFN-γ/IL-12 blocked mice. In line with the unaffected small intestinal accumulation of classical Th2 cells (**Fig. 4.9.1**), IL-4 producing T cells remained comparable between IFN-γ restricted mice and untreated controls **(Fig. 4.9.2)**. In brief, early blocking of IFN-γ significantly impaired the endogenous IFN-γ competence of Th1 as well as Th2/1 cells populating the small intestine in C57BL/6 mice.

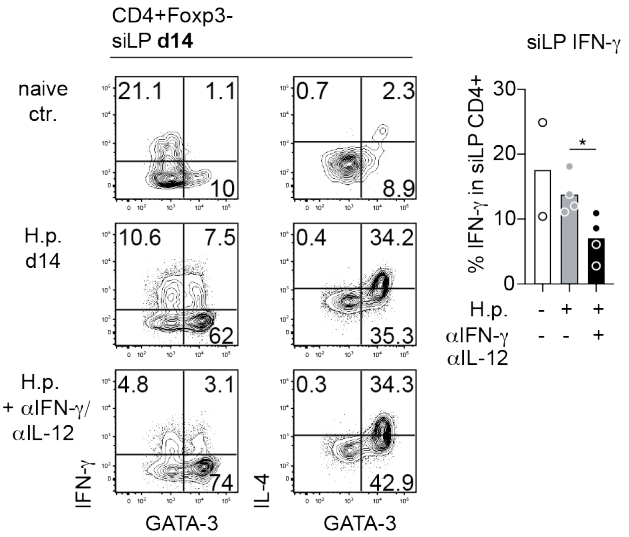

Fig. 4.9.2. Early blocking of IFN-γ leads to lowered mucosal IFN-γ competence.
Contour plots reporting IFN-γ vs. IL-4 expression in CD4+FoxP3- T cells post PMA/Ionomycin stimulation. Bar graphs depict IFN-γ producing cells in small intestinal CD4+ T cells. One out of two experiments with similar outcome is shown (n naïve=2; H.p. ctr=4; αIFN-γ/αIL-12=4 mice). Cells were stimulated with PMA/ionomycin. Bars represent mean and circles correspond to individual mice. Statistical significance between two infected groups is determined using Mann-Whitney test. *$p \leq 0.05$.

4.9.3 Diminished PS IFN-γ production seen upon early blocking of IFN-γ.

Assessing the parasite specific cytokine profile in IFN-γ/IL-12 blocked mice vs. untreated C57BL/6 infection controls, IFN-γ release in response to stimulation with parasite-derived products was dramatically reduced in mice with restricted IFN-γ availability in spleen as well as peritoneal cavity **(Fig. 4.9.3)**. The frequencies of CD40-L expressing IL-4 producing cells however, remained comparable between IFN-γ/IL-12 blocked mice and untreated controls. Hence, restricting early IFN-γ availability leads to diminished levels of PS IFN-γ producing cells.

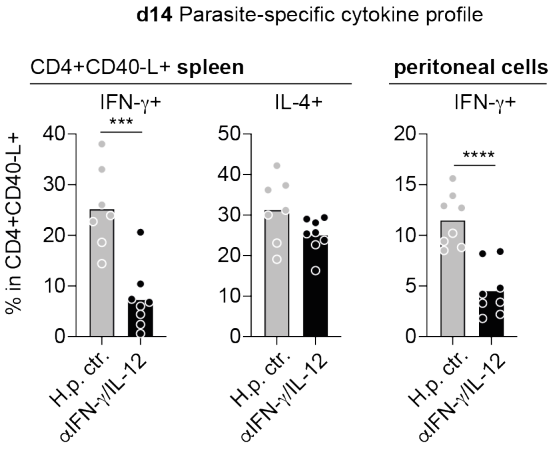

Fig. 4.9.3. Diminished PS IFN-γ production seen upon early blocking of IFN-γ
Bar graphs depict IFN-γ and IL-4 producing cells in parasite-specific CD4+ T cells in spleen and peritoneal cavity. Data derive from two independent experiments (n H.p. ctr=7-8; αIFN-γ/αIL-12=8 mice). Bars represent mean and circles correspond to individual mice. Statistical analysis was performed using unpaired t test or Mann-Whitney test. ***$p \leq 0.001$, ****$p \leq 0.0001$.

4.9.4 Pronounced innate type 2 responses are seen in IFN-γ restricted mice.

The M2 polarization in the peritoneal cavity, determined by the frequencies of RELM-α expressing macrophages, was increased in trend in IFN-γ restricted mice displaying poor Th2/1 bias **(Fig. 4.9.4A)**. Furthermore, the diminished circulating and mucosal Th2/1 cells in IFN-γ/IL12 blocked mice correlated with elevated systemic and tissue eosinophilia as determined by the higher percentage of Siglec-F expressing eosinophils in blood and small intestine **(Fig. 4.9.4B)**. Hence, poor IFN-γ availability at the priming phase of infection correlated with elevated innate type 2 responses in IFN-γ/IL-12 blocked mice.

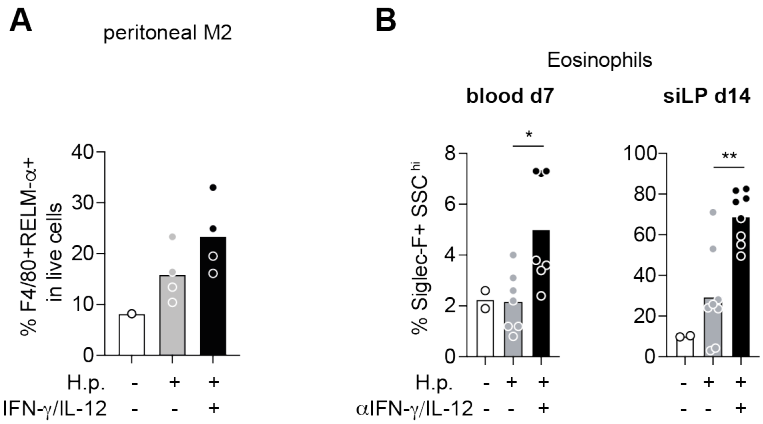

Fig. 4.9.4. Pronounced innate type 2 responses are seen in IFN-γ restricted mice.
A Bar graphs depict the frequencies of F4/80+RELM-α+ M2 macrophages in live peritoneal cells. Data derive from one experiment (n naïve=1; H.p. ctr=4; αIFN-γ/αIL-12= 4 mice). **B** Frequencies of Siglec-F+ SSC^{hi} eosinophils in blood and small intestine at indicated time points reported as bar graphs. Data from two independent experiments are pooled (n naïve=2; H.p. ctr=7-8; αIFN-γ/αIL-12=8 mice). Bars represent mean and circles correspond to individual mice. Statistical analysis was performed using unpaired t test or Mann-Whitney test between infected groups in B. *$p \leq 0.05$, **$p \leq 0.01$.

4.9.5 More efficient parasite control is seen in IFN-γ restricted mice.

Limiting early IFN-γ availability did not accelerate the parasite expulsion in C57BL/6 mice as determined by comparable adult worm burden in blocked mice vs. untreated controls two weeks post infection **(Fig. 4.9.5A)**. However, the impaired accumulation of Th2/1 cells in IFN-γ restricted mice correlated with increased parasite fitness, evidenced by lower fecal egg counts and reduced fecundity of individual female worms **(Fig. 4.9.5B-C)**. Hence, increased susceptibility in C57BL/6 mice is indeed associated with the early accumulation of IFN-γ competent cells which promote the generation of robust Th2/1 responses in infected mice.

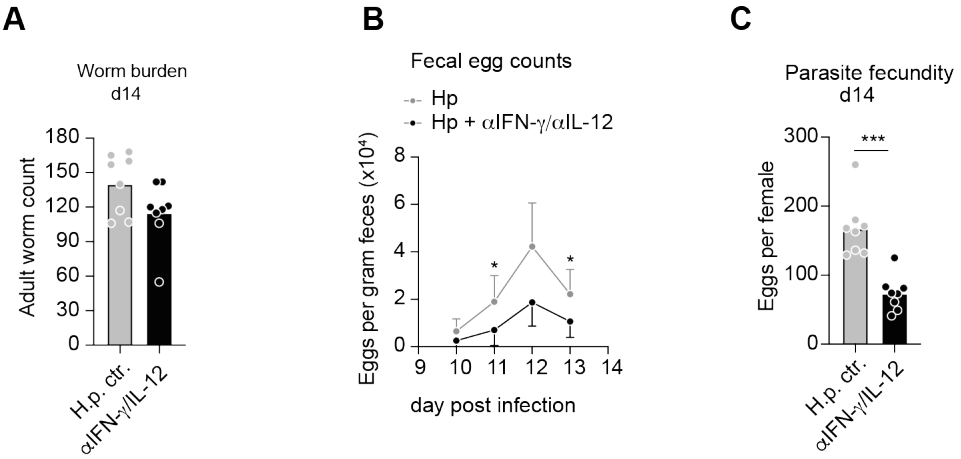

Fig. 4.9.5. More efficient parasite control is seen in IFN-γ restricted mice.
A Adult worm burden at day 14 post infection. Data from two independent experiments are pooled (n H.p. ctr=8; αIFN-γ/αIL-12=8 mice). **B** Fecal egg counts determined from day 10 – 13 post infection shown as mean ± SD. Data from two independent experiments are pooled (n=8 mice per group) at each time point except day 12 where one experiment with n=4 mice/group is shown. **C** Data points depict the mean egg production of 8 individual female worms per mouse within 24h of culture. Data from two independent experiments are pooled. (n H.p. ctr=8; αIFN-γ/αIL-12=8 mice). Bars report the mean, circles represent individual mice. Statistical analysis was performed using unpaired t test or Mann-Whitney test. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$.

4.9.6 Ameliorated small intestinal pathology in IFN-γ restricted mice.

Development of small intestinal granulomas is associated with the graded resistance to the H.p. infection. Resistant mice develop extensive macroscopic granulomas while granulomatous response is poorer in susceptible lines (Filbey et al., 2014). Reduced small intestinal IFN-γ competence in blocked mice alongside unimpaired IL-4 production licensed the formation of significantly increased numbers of small intestinal granulomas at the site of larval invasion **(Fig. 4.9.6A-B)**. Hence, limiting early IFN-γ availability in C57BL/6 mice leads to the increased Th2 associated small intestinal pathology akin to the response typically observed in resistant BALB/c mice.

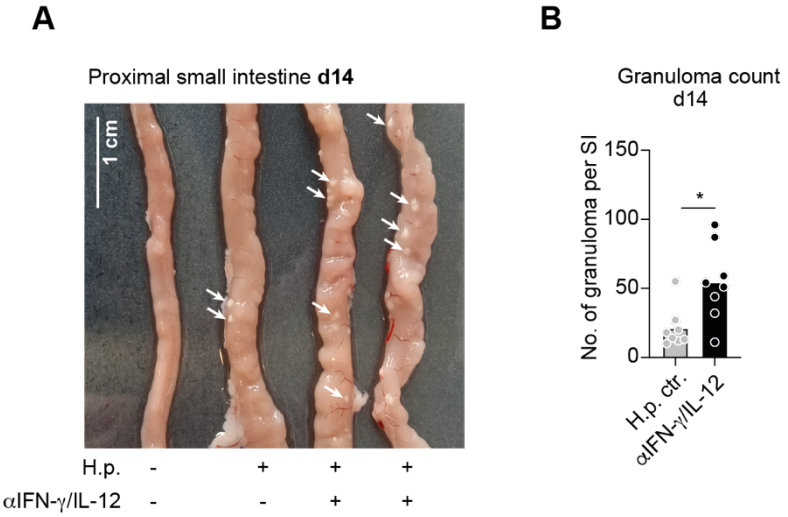

Fig. 4.9.6. Ameliorated small intestinal pathology in IFN-γ restricted mice.
A Representative picture depicting proximal small intestines of one naïve and one H.p. infected control mouse alongside two mice that had received IFN-γ and IL-12 blocking antibodies. Arrows point to the granuloma. **B** Bar graph reports the small intestinal granuloma

count in untreated control and IFN-γ/IL-12 blocked mice. Data from two independent experiments are pooled (n H.p. ctr=8; αIFN-γ/αIL-12=8 mice). Bars represent mean and circles correspond to individual mice. Statistical significance was determined using Mann-Whitney test. *$p \leq 0.05$.

5. CONCLUSION AND PERSPECTIVES

5.1 Conclusion

Our studies report an age-associated rise in homeostatic IFN-γ availability in genetically resistant mice which translates to increased bias in favor of T-bet+GATA-3+ Th2/1 cells post infection with GI nematode *H. polygyrus*. GATA-3+ Teff response skewed towards IFN-γ producing Th2/1 cells is further associated with exacerbated control of the nematode infection. The findings further add to the current understanding of age-dependent rise in susceptibility against GI nematodes by reporting that the phenotype of poor resistance reportedly correlated with very advanced age is seen much earlier in life before mice approach senescence. The findings further demonstrate that in a resistant mouse line displaying robust type 2 immunity, endogenous IFN-γ is not dispensable for the infection outcome, as even the transient episodes of restricted IFN-γ availability are sufficient to improve parasite control without tangibly affecting the classical Th2 response. Poor resistance in artificial settings of elevated IFN-γ availability akin to as observed in unmanipulated mature mice further authenticated IFN-γ in exacerbating parasite control. Our data also highlight a method to evaluate larval fitness by estimating the migratory potential of tissue-standing larvae. Comparison of resistance profile and local Th2/1 bias in mice of difference age groups across inbred lines corroborated the robustness of the negative correlation between Th2/1 accumulation and resistance against the parasite. Finally, our findings substantiate the implication of IFN-γ in driving the susceptible phenotype of C57BL/6 mice by reporting higher resistance in the otherwise susceptible mice upon restricting early IFN-γ availability.

5.2 Perspectives

As Th2/1 cells persist in the chronic phase of infection when most worms have already cleared the worms, it is likely that the functional relevance of Th2/1 cells exists beyond modulating the parasite control. Th2/1 cells are earlier reported to ameliorate type 1 as well as type 2 immunopathology by presenting attenuated features of either responses (Peine et al., 2013). Furthermore, IFN-γ itself has been reported as a restraining factor against IL-17 mediated immunopathology in *S. mansoni* infected mice immunized with parasite egg antigens (Rutitzky & Stadecker, 2011). Along similar lines, we observed reduced granuloma formation and relatively healthy gut in settings of higher Th2/1 bias in IFN-γ exposed mice. Hence, Th2/1 cells, next to affecting the resistance phenotype, might be additionally involved in the optimal operation of tissue repair mechanisms in the nematode infested small intestine and act as a safeguard against an overt detrimental type 2 bias.

Another possibility is that Th2/1 cells might behave as a source of immediate IFN-γ release to control translocated microbes from the gut lumen. The breaching of intestinal barrier by larvae migrating into the small intestine can lead to a leaky gut which might permit the translocation of microbes residing in the lumen into the lamina propria and further into circulation. Hence, the induction of IFN-γ producing Th2/1 cells at the systemic sites might be a way to mitigate the harm by the translocated microbes. To investigate this possibility, we plan to quantify the microbial DNA by qPCR in spleen and mLN of the H.p. infected mice harboring differential Th2/1 proportions.

Additionally, we observe that IFN-γ applied during T cell priming leads to overt accumulation of systemic type 2 effector cells. Further work is hence needed to address the mechanism underlying the non-classical type 2 promoting effect of IFN-γ.

- **Impaired homing to the small intestine:** IFN-γ treatment may result in poor homing of type 2 effector cells to the site of infection, thereby leading to accumulation of GATA-3+ T cells in blood and spleen. Further work should hence address the impact of systemic IFN-γ treatment on gut homing marker expression. Current work performed in the group also shows that Th2/1 accumulate in the liver next to spleen and blood

possibly related to IFN-γ driven expression of ligands for CXCR3 expressed by Th2/1 cells.

- **Increased proliferation of GATA-3+ Teff cells:** IFN-γ may induce excessive proliferation of GATA-3+ Teff cells thereby leading to their increased numbers. However, our preliminary data on Ki67 expression in GATA-3+ T cells in IFN-γ treated mice do not support this hypothesis.
- **Preferential survival of IL-4 producing cells:** IFN-γ driven accumulation of GATA-3+ T cells may also result due to preferentially increased survival of IL-4 competent T cells. IFN-γ is known to drive increased numbers of IL-4+ CD4+ T cells due to either their selective survival or the preferential death of non-IL-4 producing cells (Bocek et al., 2004). Hence, it is possible that IFN-γ selectively induces pro-survival programs in GATA-3+ T cells, possibly by modulating intrinsic apoptotic machinery or inhibiting activation induced cell death, hence leading to their over-representation in BALB/c mice.
- **Preferential cell death of non-IL-4 producing cells:** Alternatively, IFN-γ may initiate cell death in non-IL-4 producing cells, thereby freeing up empty niches for the generation of more GATA-3+ T cells. The pro- or anti-survival effects may further be conveyed directly by IFN-γ, or indirectly by IFN-γ-driven modulation of cytokines implicated in cell survival including IL-2, IL-4, IL-7 and IL-15. The experiments to address whether IFN-γ promotes cell survival in our model and to identify the underlying mechanistic basis are currently in progress.

Of note, while classical Th2 responses are unimpaired in long run in IFN-γ treated vs. untreated mice, IFN-γ has a cogent suppressive effect on the early induction of classical Th2 cells. Previous studies have shown that IFN-γ negatively regulates antigen processing and presentation by APCs (O'Neil et al., 1999; Ozawa et al., 1996; Rongcun et al., 1998). The capacity to induce proliferative responses in CD4+ T cells is also reduced in dendritic cells exposed to IFN-γ (Rongcun et al., 1998). Furthermore, IFN-γ is reported to restrict the expression of the surface marker CD1a on DCs, which is recognized by NK1+ T cells to release high levels of Th2 polarizing cytokine IL-4 (Bendelac et al., 1996; Rongcun et al., 1998). Hence, defective machinery of APCs, poor induction of proliferative responses and reduced CD1a expression by DCs are potential mechanisms underlying poor classical Th2 induction in IFN-γ treated mice, which require further investigation.

Finally, as the natural infections are not very reflective of lab-based mice inoculated with a standard larval dose under monitored conditions and are further confounded by numerous factors as reported earlier, further work aims to address the IFN-γ-Th2/1-susceptiblity axis in *Ascaris* infected pigs which provide an excellent model of *in natura* infections. *Ascaris* infected pigs display elevated type 1 activity in the form of increased IL-12, STAT-4, IL-18 and IFN-γ expression and further harbor IFN-γ producing cells in the *Ascaris*-responsive T cell pool (H. Dawson et al., 2009; H. D. Dawson et al., 2005; Ebner et al., 2017). Experiments to evaluate the significance of IFN-γ+IL-4+ Th2/1 cells in modulating the susceptible phenotype of *Ascaris* infected pigs are currently under investigation.

6. DISCUSSION

The current work demonstrates that the age-dependent rise in homeostatic IFN-γ competence skewed the phenotype of H.p. induced GATA-3+ Teff cells in favor of T-bet co-expressing Th2/1 cells and simultaneously undermined the control of nematode infection. Working with genetically resistant BALB/c mice, we observed that immune maturation with age is associated with higher type 1 activity at steady state manifested in the form of more non-specific IFN-γ producing lymphocytes and generation of more T-bet enriched Teff/mem like cells populating mucosal and lymphatic organs. Age-dependent enrichment of IFN-γ producing cells in uninfected mice ensued the generation of more systemic and mucosal Th2/1 cells in the acute as well as chronic phase of H.p. infection. The bias in favor of Th2/1 cells in mature mice allowed the maturation of more fecund worms, thereby leading to poor parasite control in the otherwise resistant strain.
In addition to age-Th2/1-susceptibility axis, the resistance phenotype of inbred mice was also traced to the differences in endogenous type 1 activity. Fully susceptible C57BL/6 mice accumulated Th1 cells more rapidly at steady state and harbored more Th2/1 cells post H.p. infection compared to the resistant BALB/c mice. Of note, the age-dependent modulation of Th2/1 cells was not confined to BALB/c mice. Susceptible C57BL/6 mice displayed a greater bias in favor of Th2/1 cells and allowed the maturation of female worms tending towards increased reproduction potential with the rise in age. The replicability of the age-dependent differences in Teff response in resistant as well as susceptible lines suggest greater implications of age-Th2/1-susceptbility axis in nematode infection studies.
Restricting endogenous IFN-γ availability in the priming phase of Th response resulted in higher resistance and induction of GATA-3+ Teff response characterized by fewer accumulation of Th2/1 cells in BALB/c as well as C57BL/6 mice. Diminished Th2/1 cells correlated with further improved resistance in BALB/c mice and augmented the features associated with resistant strains in otherwise susceptible C57BL/6 mice. Conversely, supplementing early IFN-γ availability led to impaired parasite control in resistant BALB/c mice and promoted the expansion and small intestinal accumulation of Th2/1 cells as seen in mature mice.
Of note, age-dependent as well as artificial modulation of IFN-γ availability led to very tangible effects on the phenotype of parasite specific responses as determined by the generation of HES-responsive GATA-3+ T cells enriched in T-bet co-expressing cells in settings of elevated IFN-γ availability and correspondingly diminished PS Th2/1 cells as well as parasite specific IFN-γ production in IFN-γ/IL-12 blocked mice. The higher fecundity of worms maturated in the milieu enriched in Th2/1 cells was traced to the development of fitter L4 larvae, as determined by their higher migratory potential of the tissue-standing larvae in C57BL/6 or IFN-γ treated BALB/c mice.

6.1 Age-dependent bias in favor of IFN-γ and poor resistance in mature mice

Earlier studies in C57BL/6 mice have reported spontaneous generation and age-dependent accumulation of $CD44^{hi}CD62L^{lo}$ Foxp3− MP CD4+ T cells at steady state in C57BL/6 mice (Kawabe et al., 2017). A fraction of MP Th1 cells identified by upregulated T-bet expression rapidly releases IFN-γ in an innate fashion post IL-12 stimulus by APCs (Kawabe et al., 2017, 2020). Expanding on these findings, the current study reported homeostatic age-dependent rise in IFN-γ producing Th1 cells in BALB/c mice genetically predisposed towards the induction of robust type 2 responses in in type 2 as well as type 1 infections (Filbey et al., 2014; Heinzel et al., 1989; C. Hsieh et al., 1993).
The MP Th1 cells are further reported be protective in type 1 response mandating *T. gondii* infection by rapidly releasing IFN-γ in an innate fashion and hence supporting the induction of adaptive type 1 immunity (Kawabe et al., 2017). Along similar lines, the findings in current studies illustrate that age-dependent rise in IFN-γ producing Th1 cells

is associated with an impaired development of effective type 2 protective immunity leading to poor parasite control in mature compared to young adult nematode infected BALB/c mice.
Previous studies on age-dependent resistance in parasite infection compared young cohorts with the mice which approached senescence (age ≥ 1.5 years). In a model of Leishmaniasis, susceptible BALB/c mice developed a Th1 profile and displayed higher resistance upon approaching senescence (Ehrchen et al., 2004). Along similar lines, innate type 2 features including alternative activation of macrophages and mucus production are impaired in aged compared to young mice in the context of nematode infections (Morimoto et al., 2015; Sugawara et al., 2011). The bias in favor of IFN-γ at the expense of Th2 cytokines resulted in poor protective humoral and innate type 2 responses culminating in an age-associated switch to susceptibility in senescent mice infected with *T. muris*, *N. brasiliensis* and inoculated with Schistosoma eggs (Humphreys & Grencis, 2002; Smith et al., 2001). Expanding on these findings, our studies demonstrate that age-associated rise in Th1 profile occurs much earlier in life before mice enter senescent state. Furthermore, as senescence is characterized by a general impairment of immune system (Crooke et al., 2019), the increased susceptibility in aged mice is possibly more attributed to the poor type 2 immune activation (Humphreys & Grencis, 2002; Smith et al., 2001; Sugawara et al., 2011) as compared to the simultaneous rise in Th1 bias. However, mice in the age group of 5-10 months old in our studies are not as immunologically far-removed from younger cohorts, which curtails the additional senescence associated features which may affect resistance phenotype next to endogenous IFN-γ availability.

6.2 Cross-inhibition of effective anti-nematode immunity by type 1 cytokines

In addition to our studies, numerous reports have highlighted the suppressive effects of type 1 candidates on the anti-nematode Th2 immunity. Clearance of intestinal nematode *Trichinella spiralis* is accelerated in IFN-$\gamma^{-/-}$ BALB/c mice. *In vivo* neutralization of IFN-γ in susceptible AKR mice leads to quicker expulsion of intestinal nematode *Trichuris muris* (Else et al., 1994; Urban et al., 2000). Neutralization of endogenous IFN-γ in B cell depleted C57BL/6 mice leads to a rescued clearance of *T. muris* worms (Sahputra et al., 2019). IL-12 enhances parasite survival and fitness by inhibiting Th2 cytokine expression, IgE and innate type 2 effector responses in primary *N. brasiliensis* infection (Finkelman et al., 1994). IL-18 promotes delayed parasite clearance associated with reduced mastocytosis and Th2 cytokine production in *T. spiralis* infected mice (Helmby & Grencis, 2002).
Of note, IFN-γ is released by the small intestinal granulomas formed during larval invasion in H.p. infected mice (Gentile et al., 2020; Nusse et al., 2018). Furthermore, IL-12 and IL-18 are reported to synergistically promote IFN-γ production by NK and Th1 cells (Nakanishi, 2018). IL-18 released by inflammasome activation is indeed shown to promote IFN-γ in the context of whipworm infections (Alhallaf et al., 2018). Expression of IL-12 and IL-18 in nematode infected mice (Alhallaf et al., 2018; Everts et al., 2016) may well contribute to the priming of steady state MP Th1 cells, CD8+ T cells or NK cells for IFN-γ release. Hence, the priming of more numerous IFN-γ competent cells in mature compared to young adult mice may promote the generation more vigorous Th2/1 responses eventually resulting in poor parasite control.

6.3 IFN-γ availability and Th2/1 bias in inbred mice with differential resistance

The association of poor resistance with elevated type 1 activity is also seen upon comparing inbred mice lines possessing genetic predisposition to differential susceptibility in H.p. infection. Susceptible C57BL/6 mice harboring more steady state IFN-γ producing cells, accumulate more Th2/1 cells coinciding with poor M2 activation compared to resistant BALB/c and SJL mice. However, SJL mice are characterized by a rapid and robust antibody responses post H.p. infection which possibly suggest a critical role of humoral immunity in regulation of their resistance phenotype next to T cell dependent immunity (Lawrence & Pritchard, 1994). Importantly, the simultaneous comparison of age dependent Teff profile in the BALB/c vs. C57BL/6 mice suggested a progressive rise in Th2/1 bias from young adult to mature BALB/c

mice. Mature BALB/c mice displayed comparable Th2/1 dominance as young adult C57BL/6 mice suggesting that contrasting differences to susceptible mice are leveled out with the rise in age of BALB/c mice. The bias in favor of Th2/1 cells further increased and was highest in mature C57BL/6 mice. The expected resistance phenotype largely mirrored the GATA-3+ Teff profile in the differently aged mice of both strains, thereby suggesting that the IFN-γ availability either genetically determined or phenotypically altered with age is a robust regulator of GATA-3 Teff phenotype and the resistance to nematode infection.

6.4 Determination of larval fitness by L4 migration assay

While the parasite expulsion remained largely unimpaired upon manipulation of Th2/1 responses in BALB/c mice, female fecundity was a reliable correlate of Th2/1 responses in all the experimental regimens. The increased parasite egg production from the earliest time of parasite reproduction prompted the hypothesis of development of fitter tissue-standing larvae which eventually maturate into more fecund worms. The protocol typically used to harvest L4 larvae which harnessed the inverted larval migration capacity towards abluminal side (Ey et al., 1981) was employed to assess differences in larval fitness as determined by their migratory capacity. Indeed, the larvae developing in IFN-γ exposed mice exhibited higher migratory potential. Importantly, the small intestinal milieu in IFN-γ exposed mice was characterized by the higher accumulation of Th2/1 cells at the expense of classical Th2 cells, suggesting that the local bias in favor of IFN-γ producing Th2/1 cells allows the maturation of fitter L4 larvae. Furthermore, the IFN-γ exposed mice harboring higher small intestinal Th2/1 accumulation developed significantly poor granulomatous response further suggesting that the larvae developing in sufficiency of IFN-γ and higher local Th2/1 bias are fitter possibly due to poor recruitment of type 2 inflammatory cells involved in granuloma formation.

6.5 Experimental manipulation of early IFN-γ availability

Corroborating the findings in young adult vs. mature mice, blocking early IFN-γ/IL-12 availability led to selective reduction of Th2/1 cells in both the strains. The depletion of Th2/1 cells led to dramatically reduced PS IFN-γ pool in C57BL/6 mice and almost nullified the parasite specific IFN-γ production in BALB/c mice, suggesting that IFN-γ competence of parasite specific cells is imprinted in the early stage of infection. Interference with the early IFN-γ availability leads to further augmentation of type 2 response in both the strains.

Conversely, supplementation with IFN-γ resulted in an enrichment of T-bet co-producing cells in PS GATA-3+ cells and a nearly three-fold rise in PS IFN-γ producing cells. Importantly, early exposure to IFN-γ converted the phenotype of young adult BALB/c mice to that observed in mature individuals. Akin to mature mice, IFN-γ treated mice displayed an increased IFN-γ bias in T cells responding to parasite products, higher accumulation of circulating and small intestinal Th2/1 cells and poor parasite control manifested by higher fecundity of female worms. The reproducibility of the Th2/1 biased GATA-3+ Teff response and impaired resistance between unmanipulated mature and IFN-γ treated young mice further corroborates that the age-dependent rise in IFN-γ is not merely an parallel phenomenon but indeed a contributing factor involved in poor resistance observed in mature compared to young adult BALB/c mice.

6.6 Type 2 promoting effect of IFN-γ in nematode infection.

Interestingly, IFN-γ exposure by intraperitoneal treatments or the natural age-dependent rise in IFN-γ does not impair Th2 responses in the long run in H.p. infected BALB/c mice. Although the immunologically mature or IFN-γ treated young adult mice indeed display an impaired accumulation of classical Th2 cells in the small intestine in the first week of infection, the Th2 cells populating the small intestine of chronically infected mice are comparable between young adult vs. mature or untreated vs. IFN-γ treated mice. Hence, IFN-γ while leads to an early delay, does not stably suppress Th2 responses in the context of H.p. infection. In fact, IFN-γ treated mice display a robust expansion of Th2/1 cells thereby resulting in significantly higher

accumulation of systemic GATA-3+ T cells compared to the younger individuals. The compatibility of IFN-γ signaling with Th2 responses are also previously reported (Bocek et al., 2004). The authors show that addition of IFN-γ neutralizing antibodies in the priming cultures of CD4+ T cells resulted in significantly reduced IL-4 producing cells. Similarly, IL-4 production by CD4+ T cells derived from IFN-$\gamma^{-/-}$ mice was suboptimal and strikingly increased upon exogenous addition of IFN-γ. While these findings together with ours highlight a non-classical role of IFN-γ as a promoter of Th2 response, our findings report the IFN-γ driven expansion of GATA-3+ T cells in the context of naturally induced immune response to nematode infection which has not been previously described.

6.7 Role of IFN-γ in ameliorating small intestinal immunopathology

Besides the resistance phenotype, the extent of IFN-γ availability significantly impacted the small intestinal pathology manifested in the formation of IL-4Rα-dependent granulomas (Jankovic et al., 1999). Stronger and pronounced development of small intestinal granulomas is a hallmark of resistant strains which correlates with the expulsion phenotype (Filbey et al., 2014). Fully susceptible C57BL/6 mice display fewer small intestinal granulomas compared to more resistant BALB/c mice (Filbey et al., 2014). Our studies report a significant impairment in small intestinal granulomatous response following exposure to IFN-γ in BALB/c mice, whereas IFN-γ deprived milieu was associated with more numerous granulomas in C57BL/6 mice. Along similar lines, a recent study reported that the conditional ablation of IFN-γ responsiveness in enteric glial cells correlated with poor resolution of small intestinal granulomas in H.p. infection (Progatzky et al., 2021). Furthermore, small intestinal type 1 signature and IFN-γ production around tissue-standing larvae was associated with the remodeling of intestinal stem cell niche and NK cell recruitment further leading to tissue repair (Gentile et al., 2020; Nusse et al., 2018). Hence, IFN-γ signaling by enteric cells is apparently critical in preventing tissue damage and promoting repair mechanisms, which possibly explains ameliorated small intestinal granulomatous response in settings of higher accumulation of IFN-γ competent Th2/1 cells in small intestine.

6.8 Implications of our findings on epidemiological systems

Akin to murine immune system, human counterparts of homeostatic MP T cells are identified which act as an innate source of IFN-γ and expand in an age-dependent manner (Jacomet et al., 2015; Song et al., 2005). While murine MP Th1 cells are shown to expand after birth and approach a plateau after six months of age (Kawabe et al., 2017), the age-associated kinetics of human MP cells are not detailed beyond the suggestions of positive correlations between abundance of memory phenotype CD4+ cells and the age of the individuals (Song et al., 2005). Interestingly, allelic variations at or near the IFN-γ gene locus has been linked with the resistance in nematode infected sheep and buffalos (Coltman et al., 2001; Ezenwa et al., 2010; Sayers et al., 2005), suggesting that IFN-γ has an influence on the outcome of infection in naturally parasitized populations.

However, contrary to the genetically resistant inbred mice, the resistance in humans and other *in natura* models develops slowly and remains incomplete throughout the life of an individual (Colombo & Grencis, 2020). The modelling of age dependent resistance in helminth infections in nature is further confounded by numerous factors including previous exposure to the helminth under study, infection history with other pathogens, variable dose of infection, differences in intestinal microbiota, variations in geographical and socio-economic factors among others.

Most epidemiological studies surveying the resistance phenotype in natural helminth infections reported a rise in resistance with age. Juvenile rhesus monkeys infected with *S. mansoni* display an increased resistance to the parasite compared to the adult animals (Fallon et al., 2003). Similarly, resistance to parasitic nematode *A. vasorum* increases in an age-dependent manner from juvenile to adult red foxes (Webster et al., 2017). Subjects in endemic areas infected with human whipworm *Trichuris trichura* displayed a convex infection intensity profile where parasite egg shedding increased from four-seven years old children and

subsequently declined with the rise in age (Faulkner et al., 2002). Of note, the age-associated rise in infection in the ascending half of infection intensity kinetics was associated with increased parasite specific IFN-γ production which is possibly implicated in the development of chronic infection phenotype in older individuals. Furthermore, resistance to infection with intestinal nematode *Ascaris lumbricoides* in hyperendemic regions was increased with age of the individuals associated with a corresponding rise in Th2 cytokine production (Turner et al., 2003).

On the contrary, the infections with hookworms are associated with an age-dependent rise in infection prevalence and intensity. The prevalence of hookworm *Necator americanus* increased in an age-dependent manner and plateaued in subject of 41 years of age or higher (Gandhi et al., 2001). Hence, the age-infection intensity profile in epidemiological surveys is confounded by the helminth species under study.

Interestingly, *Ascaris* infected pigs display elevated levels of IL-12, STAT4 and IL-18 thereby indicating a suitable environment for the induction of IFN-γ expression by T cells and NK cells (H. Dawson et al., 2009; H. D. Dawson et al., 2005). Previous study from our group showed that parasite specific T cells pool in *Ascaris* infected pigs comprises IFN-γ producing cells (Ebner et al., 2017), suggesting a tangible influence of Th2/1 hybrid cells in context of natural GI nematode infections. Along those lines, assessment of IL-4/IFN-γ expression by parasite specific CD4+ T cells in *Ascaris* infected pigs are currently under investigation in our group.

Hence, the maturation of immune system has an ambiguous if not an opposing effect on susceptibility to natural helminth infections. However, considering the heterogeneity between lab-based and *in natura* models, the contrasting implications of age on susceptibility to helminth infections in the two settings are inevitable. The inbred mice in our studies are in a controlled environment with standard diet, no reproduction, and no prior exposure to pathogens which highly contrasts with epidemiological models characterized by cross-sectional variations in genetic conditions, humoral responses, dietary habits, and history of preceding infections. Furthermore, the antibody response significantly differs between laboratory and epidemiological models for a given helminth. In BALB/c mice, IgE production in response to Schistosoma antigens is negatively associated with age (Smith et al., 2001), whereas in a model of human schistosomiasis, IgE levels increase with age and correlate with increased resistance to re-infections (Hagan et al., 1991). Furthermore, the mice in our studies were inoculated with a high dose of parasite larvae, whereas infection doses are rather low and variable in a nature-dwelling populations (Gregory et al., 1992). Low dose trickle infections are shown to elicit a state of partial resistance due to acquisition of immunity over time (Glover et al., 2019) which may further explain the increased resistance in naturally infected older individuals.

The immune system and microbiota of laboratory animals maintained in clean SPF conditions were found to significantly differ from mice in barrier-free environment (Hamilton et al., 2020; Leung et al., 2018). Wild house mice exhibit enriched microbiota and a higher type 1 signature in displaying increased proportions of IFN-γ competent Teff/mem cells and more active NK cells which possibly correlates with the development of inadequate protective immunity observed in wild rodents. Indeed, rewilding of laboratory mice led to poor resistance and a mixed Th1/Th2 profile in a model of whipworm infection (Leung et al., 2018). To narrow the gaps between lab-based and epidemiological models, trickle infections and rewilding of the 'clean' mice are potential promising approaches that would further allow better mimicking of parasite infections in nature.

Importantly, despite the disparities observed in lab-based vs. wild infection models, it is conceivable that resultant IFN-γ availability regulated by the combination of genetic background and previous infection history next to the age of the individual impacts the resistance in natural systems akin to as observed in rather straightforward settings in laboratory mice.

7. ZUSAMMENFASSUNG

Rolle von IFN-γ bei der Immunität und Kontrolle gastrointestinaler Nematodeninfektionen

Infektionen durch Magen-Darm-Nematoden erfordern eine effektive Typ-2 Immunantwort für die Beseitigung des Parasiten. Entsprechend der Kreuzregulation der T-Helferzelldifferenzierung durch molekulare Faktoren, welche die Th1-Differenzierung unterstützen, kann eine starke Typ-1-Immunaktivität als Ergebnis von Immunseneszenz, spezifischer genetischer Prädisposition oder der Ko-Infektionen mit intrazellulären Pathogenen die Ausbildung von Th2-Antworten behindern, was zu einer geringen Resistenz gegen Nematodeninfektionen führen kann. Vorherige *in vitro* und *in vivo* Studien haben gezeigt, dass sich die Differenzierungswege in Th2- beziehungsweise Th1- Zellen nicht gegenseitig ausschließen, da T-Helferzellen unter gleichzeitiger Einwirkung der Th1- beziehungsweise Th2-polarisierenden Zytokine IFN-γ/IL-12 und IL-4 einen Th2/1-Hybridphänotyp annehmen. Dieser Differenzierungsstatus ist durch die Koexpression von GATA-3 und T-bet gekennzeichnet und vorherige Arbeiten haben gezeigt, dass die Differenzierung von Th2/1-Hybridzellen auch als natürliche Reaktion auf Infektionen mit Darmnematoden vorkommt (Affinass et al., 2018; Bock et al., 2017; Peine et al., 2013).
Die vorliegende Arbeit zeigt, dass es in gesunden Labormäusen der gegen Nematodeninfektionen hoch resistenten BALB/c-Linie mit steigendem Alter zu einer Zunahme der IFN-γ-Kompetenz kommt. Als Konsequenz dieses Th1-Bias ist die Kontrolle einer Nematodeninfektionen beeinträchtigt, wenn BALB/c-Mäuse im Alter von 5-10 Monaten anstatt von 2-3 Monaten mit *H. polygyrus* infiziert werden, was aus der erhöhten Eiproduktion der Würmer in älteren Mäusen ersichtlich wird. Die verminderte Resistenz der geht einher mit der Anreicherung von Th2/1-Hybridzellen in der Mukosa und mit einem erhöhten Anteil von IFN-γ produzierenden, parasiten-spezifischen T-Helferzellen.
Dieser Zusammenhang konnte durch die Blockade von IFN-γ in der frühen Phase der Immunantwort weiter belegt werden. Dieser Ansatz führte zu einer drastischen Reduktion von systemischen und mukosalen Th2/1-Hybridantworten und dem nahezu vollständigen Ausbleiben der IFN-γ Produktion durch parasiten-spezifische T-Zellen, wodurch die Resistenz erhöhte wurde. Wurde jedoch die Verfügbarkeit von IFN-γ in der frühen Infektionsphase durch Gabe des Zytokins erhöht, so resultierte dies in der starken Expansion von Th2/1 Hybridzellen, einer signifikanten Zunahme von IFN-γ produzierenden Zellen in der parasiten-spezifischen T-Zellpopulation und der verminderten Kontrolle der Parasiteninfektion. Zudem zeigte sich, dass die erhöhte Verfügbarkeit von IFN-γ die Differenzierung von klassischen GATA-3+ Th2-Zellen langfristig nicht inhibiert, sondern dass es nach IFN-γ-Behandlung von BALB/c Mäusen zu einer gesteigerten systemischen Akkumulation von GATA-3+ Zellen kommt. Weiter konnte die nach IFN-γ Gabe erhöhte Eiproduktion durch die Parasiten mit einer erhöhten Fitness des sich im Darm entwickelnden vierten Larvenstadiums nach IFN-γ Gabe in Zusammenhang gebracht werden.
Abweichend von der verzögerten, altersbedingten Zunahme der IFN-γ-Kompetenz von BALB/c Mäusen zeigten Mäuse der C57BL/6-Linie mit einer genetisch veranlagten hohen Empfänglichkeit für die *H. polygyrus*-Infektion bereits als junge Tiere eine Anreicherung von Th1-Zellen in Abwesenheit von Infektionen. Nach Wurminfektion resultierte die stärkere Typ-1 Aktivität der C57BL/6 Linie in der stärkeren Anreicherung von Th2/1-Zellen im infizierten Dünndarm und in der geringeren Resistenz im Vergleich zur BALB/c Linie. Diese deutlichen Unterschiede zwischen den beiden Mauslinien zeigten sich insbesondere bei jungen Tieren. In älteren Tieren glichen sich jedoch die Reaktionen auf die Infektion durch die Zunahme der IFN-γ Kompetenz und die verstärkte Bildung von Th2/1 Zellen in älteren BALB/c Mäusen an. Die Beschränkung der IFN-γ Verfügbarkeit in C57BL/6 Mäusen führte zu einer erhöhten Resistenz, was die Bedeutung von IFN-γ für die unterschiedliche Empfänglichkeit von Mauslinien unterstreicht.

Zusammenfassend zeigt diese Studie also eine altersabhängige Abnahme der Typ-2 abhängigen Immunität gegenüber Nematodeninfektionen durch die spontane Zunahme von IFN-γ kompetenten Zellen.

8. SUMMARY

Infections by gastrointestinal nematodes mandate an effective type 2 response for the parasite clearance. In line with the cross-regulatory nature of Th1 lineage specifying factors on the differentiation of Th2 cells seen at the molecular level, strong type 1 activity resulted by inflammation associated with senescence, specific genetic predisposition and co-infections with intracellular pathogens exacerbate the Th2 responses resulting in poor resistance in nematode infections. Despite reported cross-regulation, the Th1 and Th2 pathways are not mutually exclusive as polarization with IFN-γ, IL-12 and IL-4 results in the generation of T-bet and GATA-3 co-expressing Th2/1 cells *in vitro*. Th2/1 cells are also induced naturally in nematode infections (Affinass et al., 2018; Bock et al., 2017; Peine et al., 2013).

In the current study we demonstrate that the IFN-γ competence progressively rises with the age of uninfected BALB/c mice displaying high resistance to nematode infection. The elevated type 1 bias in 5-10 months old compared to 2-3 months old mice resulted in poor parasite control manifested by higher female fecundity in the mature cohort infected with *H. polygyrus*. The poor resistance was accompanied by stronger generation and mucosal accumulation of Th2/1 hybrid cells and elevated proportions of parasite specific IFN-γ producing cells.

Substantiating the above findings, restriction of IFN-γ availability in the priming phase of nematode infection led to improved resistance coinciding with sharp reduction in systemic and mucosal Th2/1 cells and a near complete absence of parasite specific IFN-γ producing cells. Conversely, supplementation of early IFN-γ availability led to impaired parasite control associated with robust expansion of Th2/1 cells and a significant rise in IFN-γ producing cells in parasite specific T cell pool. Importantly, elevated IFN-γ availability did not inhibit classical Th2 cells in the long run and rather promoted an accumulation of systemic GATA-3+ Teff cells in IFN-γ treated BALB/c mice. The increased parasite egg production upon IFN-γ treatment was traced back to the increased fitness of the L4 larvae maturating in the gut of IFN-γ treated mice.

In line with the findings in differently aged mice, C57BL/6 mice genetically predisposed to higher susceptibility in *H. polygyrus* infection more rapidly accumulate Th1 cells at steady state compared to resistant BALB/c mice. The elevated type 1 activity in C57BL/6 mice translated to greater accumulation of small intestinal Th2/1 cells post infection and poor resistance compared to BALB/c mice. However, the stark differences seen between the strains at younger age leveled out in older mice due to increased IFN-γ competence and increased bias in favor of Th2/1 cells in mature BALB/c mice. Restricting IFN-γ availability in C57BL/6 mice led to increased resistance thereby substantiating the significance of IFN-γ in differential susceptibility across inbred lines. Overall, our findings report an age-dependent reduction in anti-nematode type 2 immunity resulted by steady state accumulation of IFN-γ competent effector cells in the vertebrate host.

9. BIBLIOGRAPHY

Affinass, N., Zhang, H., Löhning, M., Hartmann, S., & Rausch, S. (2018). Manipulation of the balance between Th2 and Th2/1 hybrid cells affects parasite nematode fitness in mice. *European Journal of Immunology*, *48*(12), 1958–1964. https://doi.org/10.1002/eji.201847639

Afkarian, M., Sedy, J. R., Yang, J., Jacobson, N. G., Cereb, N., Yang, S. Y., Murphy, T. L., & Murphy, K. M. (2002). T-bet is a STAT1-induced regulator of IL-12R expression in naïve CD4+ T cells. *Nature Immunology*, *3*(6), 549–557. https://doi.org/10.1038/ni794

Ahmed, N., French, T., Rausch, S., Kühl, A., Hemminger, K., Dunay, I. R., Steinfelder, S., & Hartmann, S. (2017). Toxoplasma Co-infection Prevents Th2 Differentiation and Leads to a Helminth-Specific Th1 Response. *Frontiers in Cellular and Infection Microbiology*, *7*, 341. https://doi.org/10.3389/fcimb.2017.00341

Alhallaf, R., Agha, Z., Miller, C. M., Robertson, A. A. B., Sotillo, J., Croese, J., Cooper, M. A., Masters, S. L., Kupz, A., Smith, N. C., Loukas, A., & Giacomin, P. R. (2018). The NLRP3 Inflammasome Suppresses Protective Immunity to Gastrointestinal Helminth Infection. *Cell Reports*, *23*(4), 1085–1098. https://doi.org/10.1016/j.celrep.2018.03.097

Anthony, R. M., Urban, J. F., Alem, F., Hamed, H. A., Rozo, C. T., Boucher, J.-L., Van Rooijen, N., & Gause, W. C. (2006). Memory TH2 cells induce alternatively activated macrophages to mediate protection against nematode parasites. *Nature Medicine*, *12*(8), 955–960. https://doi.org/10.1038/nm1451

Aujla, S. J., Chan, Y. R., Zheng, M., Fei, M., Askew, D. J., Pociask, D. A., Reinhart, T. A., McAllister, F., Edeal, J., Gaus, K., Husain, S., Kreindler, J. L., Dubin, P. J., Pilewski, J. M., Myerburg, M. M., Mason, C. A., Iwakura, Y., & Kolls, J. K. (2008). IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. *Nature Medicine*, *14*(3), 275–281. https://doi.org/10.1038/nm1710

Bancroft, A. J., Else, K. J., Sypek, J. P., & Grencis, R. K. (1997). Interleukin-12 promotes a chronic intestinal nematode infection. *European Journal of Immunology*, *27*(4), 866–870. https://doi.org/10.1002/eji.1830270410

Behnke, J. M., Menge, D. M., & Noyes, H. (2009). *Heligmosomoides bakeri*: A model for exploring the biology and genetics of resistance to chronic gastrointestinal nematode infections. *Parasitology*, *136*(12), 1565–1580. https://doi.org/10.1017/S0031182009006003

Bendelac, A., Hunziker, R. D., & Lantz, O. (1996). Increased interleukin 4 and immunoglobulin E production in transgenic mice overexpressing NK1 T cells. *Journal of Experimental Medicine*, *184*(4), 1285–1293. https://doi.org/10.1084/jem.184.4.1285

Bettelli, E., Carrier, Y., Gao, W., Korn, T., Strom, T. B., Oukka, M., Weiner, H. L., & Kuchroo, V. K. (2006). Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature*, *441*(7090), 235–238. https://doi.org/10.1038/nature04753

Bluestone, J. A., Mackay, C. R., O'Shea, J. J., & Stockinger, B. (2009). The functional plasticity of T cell subsets. *Nature Reviews Immunology*, *9*(11), 811–816. https://doi.org/10.1038/nri2654

Bocek, P., Foucras, G., & Paul, W. E. (2004). Interferon γ Enhances Both In Vitro and In Vivo Priming of CD4+ T Cells for IL-4 Production. *Journal of Experimental Medicine*, *199*(12), 1619–1630. https://doi.org/10.1084/jem.20032014

Bock, C. N., Babu, S., Breloer, M., Rajamanickam, A., Boothra, Y., Brunn, M.-L., Kühl, A. A., Merle, R., Löhning, M., Hartmann, S., & Rausch, S. (2017). Th2/1 Hybrid Cells Occurring in Murine and Human Strongyloidiasis Share Effector Functions of Th1 Cells. *Frontiers in Cellular and Infection Microbiology*, *7*, 261. https://doi.org/10.3389/fcimb.2017.00261

Breitfeld, D., Ohl, L., Kremmer, E., Ellwart, J., Sallusto, F., Lipp, M., & Förster, R. (2000). Follicular B Helper T Cells Express Cxc Chemokine Receptor 5, Localize to B Cell Follicles, and Support Immunoglobulin Production. *Journal of Experimental Medicine*, *192*(11), 1545–1552. https://doi.org/10.1084/jem.192.11.1545

Brucklacher-Waldert, V., Carr, E. J., Linterman, M. A., & Veldhoen, M. (2014). Cellular Plasticity of CD4+ T Cells in the Intestine. *Frontiers in Immunology*, *5*. https://doi.org/10.3389/fimmu.2014.00488

Califano, D., Sweeney, K. J., Le, H., VanValkenburgh, J., Yager, E., O'Connor, W., Kennedy, J. S., Jones, D. M., & Avram, D. (2014). Diverting T helper cell trafficking through increased plasticity attenuates autoimmune encephalomyelitis. *Journal of Clinical Investigation*, *124*(1), 174–187. https://doi.org/10.1172/JCI70103

Cautivo, K. M., Matatia, P. R., Lizama, C. O., Mroz, N. M., Dahlgren, M. W., Yu, X., Sbierski-Kind, J., Taruselli, M. T., Brooks, J. F., Wade-Vallance, A., Caryotakis, S. E., Chang, A. A., Liang, H.-E., Zikherman, J., Locksley, R. M., & Molofsky, A. B. (2022). Interferon gamma constrains type 2 lymphocyte niche boundaries during mixed inflammation. *Immunity*, *55*(2), 254-271.e7. https://doi.org/10.1016/j.immuni.2021.12.014

Chen, W., Jin, W., Hardegen, N., Lei, K., Li, L., Marinos, N., McGrady, G., & Wahl, S. M. (2003). Conversion of Peripheral CD4+CD25− Naive T Cells to CD4+CD25+ Regulatory T Cells by TGF-β Induction of Transcription Factor Foxp3. *Journal of Experimental Medicine*, *198*(12), 1875–1886. https://doi.org/10.1084/jem.20030152

Coffman, R. L., Seymour, B. W. P., Hudak, S., Jackson, J., & Rennick, D. (1989). Antibody to Interleukin-5 Inhibits Helminth-Induced Eosinophilia in Mice. *Science*, *245*(4915), 308–310. https://doi.org/10.1126/science.2787531

Colombo, S. A. P., & Grencis, R. K. (2020). Immunity to Soil-Transmitted Helminths: Evidence From the Field and Laboratory Models. *Frontiers in Immunology*, *11*, 1286. https://doi.org/10.3389/fimmu.2020.01286

Coltman, D. W., Wilson, K., Pilkington, J. G., Stear, M. J., & Pemberton, J. M. (2001). A microsatellite polymorphism in the gamma interferon gene is associated with resistance to gastrointestinal nematodes in a naturally-parasitized population of Soay sheep. *Parasitology*, *122*(5), 571–582. https://doi.org/10.1017/S0031182001007570

Coomes, S. M., Pelly, V. S., Kannan, Y., Okoye, I. S., Czieso, S., Entwistle, L. J., Perez-Lloret, J., Nikolov, N., Potocnik, A. J., Biró, J., Langhorne, J., & Wilson, M. S. (2015).

IFNγ and IL-12 Restrict Th2 Responses during Helminth/Plasmodium Co-Infection and Promote IFNγ from Th2 Cells. *PLoS Pathogens*, *11*(7), e1004994. https://doi.org/10.1371/journal.ppat.1004994

Cote-Sierra, J., Foucras, G., Guo, L., Chiodetti, L., Young, H. A., Hu-Li, J., Zhu, J., & Paul, W. E. (2004). Interleukin 2 plays a central role in Th2 differentiation. *Proceedings of the National Academy of Sciences*, *101*(11), 3880–3885. https://doi.org/10.1073/pnas.0400339101

Crooke, S. N., Ovsyannikova, I. G., Poland, G. A., & Kennedy, R. B. (2019). Immunosenescence: A systems-level overview of immune cell biology and strategies for improving vaccine responses. *Experimental Gerontology*, *124*, 110632. https://doi.org/10.1016/j.exger.2019.110632

Dawson, H. D., Beshah, E., Nishi, S., Solano-Aguilar, G., Morimoto, M., Zhao, A., Madden, K. B., Ledbetter, T. K., Dubey, J. P., Shea-Donohue, T., Lunney, J. K., & Urban, J. F. (2005). Localized Multigene Expression Patterns Support an Evolving Th1/Th2-Like Paradigm in Response to Infections with *Toxoplasma gondii* and *Ascaris suum*. *Infection and Immunity*, *73*(2), 1116–1128. https://doi.org/10.1128/IAI.73.2.1116-1128.2005

Dawson, H., Solano-Aguilar, G., Beal, M., Beshah, E., Vangimalla, V., Jones, E., Botero, S., & Urban, J. F. (2009). Localized Th1-, Th2-, T Regulatory Cell-, and Inflammation-Associated Hepatic and Pulmonary Immune Responses in *Ascaris suum* -Infected Swine Are Increased by Retinoic Acid. *Infection and Immunity*, *77*(6), 2576–2587. https://doi.org/10.1128/IAI.00827-07

Djuretic, I. M., Levanon, D., Negreanu, V., Groner, Y., Rao, A., & Ansel, K. M. (2007). Transcription factors T-bet and Runx3 cooperate to activate Ifng and silence Il4 in T helper type 1 cells. *Nature Immunology*, *8*(2), 145–153. https://doi.org/10.1038/ni1424

Doran, E., Cai, F., Holweg, C. T. J., Wong, K., Brumm, J., & Arron, J. R. (2017). Interleukin-13 in Asthma and Other Eosinophilic Disorders. *Frontiers in Medicine*, *4*, 139. https://doi.org/10.3389/fmed.2017.00139

Doucet, C. (1998). IL-4 and IL-13 specifically increase adhesion molecule and inflammatory cytokine expression in human lung fibroblasts. *International Immunology*, *10*(10), 1421–1433. https://doi.org/10.1093/intimm/10.10.1421

Ebner, F., Schwiertz, P., Steinfelder, S., Pieper, R., Zentek, J., Schütze, N., Baums, C. G., Alber, G., Geldhof, P., & Hartmann, S. (2017). Pathogen-Reactive T Helper Cell Analysis in the Pig. *Frontiers in Immunology*, *8*, 565. https://doi.org/10.3389/fimmu.2017.00565

Ehrchen, J., Sindrilaru, A., Grabbe, S., Schönlau, F., Schlesiger, C., Sorg, C., Scharffetter-Kochanek, K., & Sunderkötter, C. (2004). Senescent BALB/c Mice Are Able To Develop Resistance to *Leishmania major* Infection. *Infection and Immunity*, *72*(9), 5106–5114. https://doi.org/10.1128/IAI.72.9.5106-5114.2004

Else, K. J., Finkelman, F. D., Maliszewski, C. R., & Grencis, R. K. (1994). Cytokine-mediated regulation of chronic intestinal helminth infection. *Journal of Experimental Medicine*, *179*(1), 347–351. https://doi.org/10.1084/jem.179.1.347

Else, K. J., & Grencis, R. K. (1996). Antibody-independent effector mechanisms in resistance to the intestinal nematode parasite Trichuris muris. *Infection and Immunity*, *64*(8), 2950–2954. https://doi.org/10.1128/iai.64.8.2950-2954.1996

Everts, B., Tussiwand, R., Dreesen, L., Fairfax, K. C., Huang, S. C.-C., Smith, A. M., O'Neill, C. M., Lam, W. Y., Edelson, B. T., Urban, J. F., Murphy, K. M., & Pearce, E. J. (2016). Migratory CD103+ dendritic cells suppress helminth-driven type 2 immunity through constitutive expression of IL-12. *Journal of Experimental Medicine*, *213*(1), 35–51. https://doi.org/10.1084/jem.20150235

Ey, P. L., Prowse, S. J., & Jenkin, C. R. (1981). Heligmosomoides polygyrus: Simple recovery of post-infective larvae from mouse intestines. *Experimental Parasitology*, *52*(1), 69–76. https://doi.org/10.1016/0014-4894(81)90062-X

Ezenwa, V. O., Etienne, R. S., Luikart, G., Beja-Pereira, A., & Jolles, A. E. (2010). Hidden consequences of living in a wormy world: Nematode-induced immune suppression facilitates tuberculosis invasion in African buffalo. *The American Naturalist*, *176*(5), 613–624. https://doi.org/10.1086/656496

Fallon, P. G., Gibbons, J., Vervenne, R. A., Richardson, E. J., Fulford, A. J. C., Kiarie, S., Sturrock, R. F., Coulson, P. S., Deelder, A. M., Langermans, J. A. M., Thomas, A. W., & Dunne, D. W. (2003). Juvenile Rhesus Monkeys Have Lower Type 2 Cytokine Responses than Adults after Primary Infection with *Schistosoma mansoni*. *The Journal of Infectious Diseases*, *187*(6), 939–945. https://doi.org/10.1086/368130

Fallon, P. G., Ballantyne, S. J., Mangan, N. E., Barlow, J. L., Dasvarma, A., Hewett, D. R., McIlgorm, A., Jolin, H. E., & McKenzie, A. N. J. (2006). Identification of an interleukin (IL)-25–dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. *Journal of Experimental Medicine*, *203*(4), 1105–1116. https://doi.org/10.1084/jem.20051615

Faulkner, H., Turner, J., Kamgno, J., Pion, S. D., Boussinesq, M., & Bradley, J. E. (2002). Age- and Infection Intensity–Dependent Cytokine and Antibody Production in Human Trichuriasis: The Importance of IgE. *The Journal of Infectious Diseases*, *185*(5), 665–672. https://doi.org/10.1086/339005

Filbey, K. J., Grainger, J. R., Smith, K. A., Boon, L., Rooijen, N., Harcus, Y., Jenkins, S., Hewitson, J. P., & Maizels, R. M. (2014). Innate and adaptive type 2 immune cell responses in genetically controlled resistance to intestinal helminth infection. *Immunology & Cell Biology*, *92*(5), 436–448. https://doi.org/10.1038/icb.2013.109

Finkelman, F. D., Madden, K. B., Cheever, A. W., Katona, I. M., Morris, S. C., Gately, M. K., Hubbard, B. R., Gause, W. C., & Urban, J. F. (1994). Effects of interleukin 12 on immune responses and host protection in mice infected with intestinal nematode parasites. *Journal of Experimental Medicine*, *179*(5), 1563–1572. https://doi.org/10.1084/jem.179.5.1563

Fontenot, J. D., Gavin, M. A., & Rudensky, A. Y. (2003). Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nature Immunology*, *4*(4), 330–336. https://doi.org/10.1038/ni904

Gagliani, N., Vesely, M. C. A., Iseppon, A., Brockmann, L., Xu, H., Palm, N. W., de Zoete, M. R., Licona-Limón, P., Paiva, R. S., Ching, T., Weaver, C., Zi, X., Pan, X., Fan, R., Garmire, L. X., Cotton, M. J., Drier, Y., Bernstein, B., Geginat, J., … Flavell, R. A.

(2015). Th17 cells transdifferentiate into regulatory T cells during resolution of inflammation. *Nature*, *523*(7559), 221–225. https://doi.org/10.1038/nature14452

Gandhi, N. S., Jizhang, C., Khoshnood, K., Fuying, X., Shanwen, L., Yaoruo, L., Bin, Z., Haechou, X., Chongjin, T., Yan, W., Wensen, W., Dungxing, H., Chong, C., Shuhua, X., Hawdon, J. M., & Hotez, P. J. (2001). Epidemiology of Necator Americanus Hookworm Infections in Xiulongkan Village, Hainan Province, China: High Prevalence and Intensity Among Middle-Aged and Elderly Residents. *Journal of Parasitology*, *87*(4), 739–743. https://doi.org/10.1645/0022-3395(2001)087[0739:EONAHI]2.0.CO;2

Gentile, M. E., Li, Y., Robertson, A., Shah, K., Fontes, G., Kaufmann, E., Polese, B., Khan, N., Parisien, M., Munter, H. M., Mandl, J. N., Diatchenko, L., Divangahi, M., & King, I. L. (2020). NK cell recruitment limits tissue damage during an enteric helminth infection. *Mucosal Immunology*, *13*(2), 357–370. https://doi.org/10.1038/s41385-019-0231-8

Gerbe, F., Sidot, E., Smyth, D. J., Ohmoto, M., Matsumoto, I., Dardalhon, V., Cesses, P., Garnier, L., Pouzolles, M., Brulin, B., Bruschi, M., Harcus, Y., Zimmermann, V. S., Taylor, N., Maizels, R. M., & Jay, P. (2016). Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. *Nature*, *529*(7585), 226–230. https://doi.org/10.1038/nature16527

Glover, M., Colombo, S. A. P., Thornton, D. J., & Grencis, R. K. (2019). Trickle infection and immunity to Trichuris muris. *PLOS Pathogens*, *15*(11), e1007926. https://doi.org/10.1371/journal.ppat.1007926

Gorelik, L., Fields, P. E., & Flavell, R. A. (2000). Cutting Edge: TGF-β Inhibits Th Type 2 Development Through Inhibition of GATA-3 Expression. *The Journal of Immunology*, *165*(9), 4773–4777. https://doi.org/10.4049/jimmunol.165.9.4773

Gregory, R. D., Montgomery, S. S. J., & Montgomery, W. I. (1992). Population Biology of Heligmosomoides polygyrus (Nematoda) in the Wood Mouse. *The Journal of Animal Ecology*, *61*(3), 749. https://doi.org/10.2307/5628

Güler, M. L., Jacobson, N. G., Gubler, U., & Murphy, K. M. (1997). T cell genetic background determines maintenance of IL-12 signaling: Effects on BALB/c and B10.D2 T helper cell type 1 phenotype development. *Journal of Immunology (Baltimore, Md.: 1950)*, *159*(4), 1767–1774.

Hagan, P., Blumenthal, U. J., Dunn, D., Simpson, A. J. G., & Wilkins, H. A. (1991). Human IgE, IgG4 and resistance to reinfection with Schistosoma haematobium. *Nature*, *349*(6306), 243–245. https://doi.org/10.1038/349243a0

Halim, T. Y. F., Hwang, Y. Y., Scanlon, S. T., Zaghouani, H., Garbi, N., Fallon, P. G., & McKenzie, A. N. J. (2016). Group 2 innate lymphoid cells license dendritic cells to potentiate memory TH2 cell responses. *Nature Immunology*, *17*(1), 57–64. https://doi.org/10.1038/ni.3294

Hamilton, S. E., Badovinac, V. P., Beura, L. K., Pierson, M., Jameson, S. C., Masopust, D., & Griffith, T. S. (2020). New Insights into the Immune System Using Dirty Mice. *The Journal of Immunology*, *205*(1), 3–11. https://doi.org/10.4049/jimmunol.2000171

Harrington, L. E., Hatton, R. D., Mangan, P. R., Turner, H., Murphy, T. L., Murphy, K. M., & Weaver, C. T. (2005). Interleukin 17–producing CD4+ effector T cells develop via a

lineage distinct from the T helper type 1 and 2 lineages. *Nature Immunology*, *6*(11), 1123–1132. https://doi.org/10.1038/ni1254

Hashimoto, K., Uchikawa, R., Tegoshi, T., Takeda, K., Yamada, M., & Arizono, N. (2009). Depleted intestinal goblet cells and severe pathological changes in SCID mice infected with *Heligmosomoides polygyrus*. *Parasite Immunology*, *31*(8), 457–465. https://doi.org/10.1111/j.1365-3024.2009.01123.x

Heinzel, F. P., Sadick, M. D., Holaday, B. J., Coffman, R. L., & Locksley, R. M. (1989). Reciprocal expression of interferon gamma or interleukin 4 during the resolution or progression of murine leishmaniasis. Evidence for expansion of distinct helper T cell subsets. *Journal of Experimental Medicine*, *169*(1), 59–72. https://doi.org/10.1084/jem.169.1.59

Helmby, H., & Grencis, R. K. (2003). IFN-γ-Independent Effects of IL-12 During Intestinal Nematode Infection. *The Journal of Immunology*, *171*(7), 3691–3696. https://doi.org/10.4049/jimmunol.171.7.3691

Helmby, H., & Grencis, R. K. (2002). IL-18 Regulates Intestinal Mastocytosis and Th2 Cytokine Production Independently of IFN-γ During *Trichinella spiralis* Infection. *The Journal of Immunology*, *169*(5), 2553–2560. https://doi.org/10.4049/jimmunol.169.5.2553

Herbert, D. R., Yang, J.-Q., Hogan, S. P., Groschwitz, K., Khodoun, M., Munitz, A., Orekov, T., Perkins, C., Wang, Q., Brombacher, F., Urban, J. F., Rothenberg, M. E., & Finkelman, F. D. (2009). Intestinal epithelial cell secretion of RELM-beta protects against gastrointestinal worm infection. *The Journal of Experimental Medicine*, *206*(13), 2947–2957. https://doi.org/10.1084/jem.20091268

Hernandez, A. D., & Sukhdeo, M. V. (1995). Host grooming and the transmission strategy of Heligmosomoides polygyrus. *The Journal of Parasitology*, *81*(6), 865–869.

Hirota, K., Turner, J.-E., Villa, M., Duarte, J. H., Demengeot, J., Steinmetz, O. M., & Stockinger, B. (2013). Plasticity of TH17 cells in Peyer's patches is responsible for the induction of T cell–dependent IgA responses. *Nature Immunology*, *14*(4), 372–379. https://doi.org/10.1038/ni.2552

Hovhannisyan, Z., Treatman, J., Littman, D. R., & Mayer, L. (2011). Characterization of Interleukin-17–Producing Regulatory T Cells in Inflamed Intestinal Mucosa From Patients With Inflammatory Bowel Diseases. *Gastroenterology*, *140*(3), 957–965. https://doi.org/10.1053/j.gastro.2010.12.002

Hsieh, C., Macatonia, S., Tripp, C., Wolf, S., O'Garra, A., & Murphy, K. (1993). Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. *Science*, *260*(5107), 547–549. https://doi.org/10.1126/science.8097338

Hsieh, C. S., Macatonia, S. E., O'Garra, A., & Murphy, K. M. (1995). T cell genetic background determines default T helper phenotype development in vitro. *Journal of Experimental Medicine*, *181*(2), 713–721. https://doi.org/10.1084/jem.181.2.713

Humphreys, N. E., & Grencis, R. K. (2002). Effects of Ageing on the Immunoregulation of Parasitic Infection. *Infection and Immunity*, *70*(9), 5148–5157. https://doi.org/10.1128/IAI.70.9.5148-5157.2002

Humphreys, N. E., Xu, D., Hepworth, M. R., Liew, F. Y., & Grencis, R. K. (2008). IL-33, a Potent Inducer of Adaptive Immunity to Intestinal Nematodes. *The Journal of Immunology*, *180*(4), 2443–2449. https://doi.org/10.4049/jimmunol.180.4.2443

Hwang, E. S. (2005). T Helper Cell Fate Specified by Kinase-Mediated Interaction of T-bet with GATA-3. *Science*, *307*(5708), 430–433. https://doi.org/10.1126/science.1103336

Inclan-Rico, J. M., & Siracusa, M. C. (2018). First Responders: Innate Immunity to Helminths. *Trends in Parasitology*, *34*(10), 861–880. https://doi.org/10.1016/j.pt.2018.08.007

Ito, T., Wang, Y.-H., Duramad, O., Hori, T., Delespesse, G. J., Watanabe, N., Qin, F. X.-F., Yao, Z., Cao, W., & Liu, Y.-J. (2005). TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *Journal of Experimental Medicine*, *202*(9), 1213–1223. https://doi.org/10.1084/jem.20051135

Ivanov, I. I., McKenzie, B. S., Zhou, L., Tadokoro, C. E., Lepelley, A., Lafaille, J. J., Cua, D. J., & Littman, D. R. (2006). The Orphan Nuclear Receptor RORγt Directs the Differentiation Program of Proinflammatory IL-17+ T Helper Cells. *Cell*, *126*(6), 1121–1133. https://doi.org/10.1016/j.cell.2006.07.035

Jacobson, R. H., & Reed, N. D. (1974). The Immune Response of Congenitally Athymic (Nude) Mice to the Intestinal Nematode Nippostrongylus brasiliensis. *Experimental Biology and Medicine*, *147*(3), 667–670. https://doi.org/10.3181/00379727-147-38412

Jacomet, F., Cayssials, E., Basbous, S., Levescot, A., Piccirilli, N., Desmier, D., Robin, A., Barra, A., Giraud, C., Guilhot, F., Roy, L., Herbelin, A., & Gombert, J.-M. (2015). Evidence for eomesodermin-expressing innate-like CD8 $^{+}$ KIR/NKG2A $^{+}$ T cells in human adults and cord blood samples: Innate immunity. *European Journal of Immunology*, *45*(7), 1926–1933. https://doi.org/10.1002/eji.201545539

Jankovic, D., Kullberg, M. C., Noben-Trauth, N., Caspar, P., Ward, J. M., Cheever, A. W., Paul, W. E., & Sher, A. (1999). Schistosome-infected IL-4 receptor knockout (KO) mice, in contrast to IL-4 KO mice, fail to develop granulomatous pathology while maintaining the same lymphokine expression profile. *Journal of Immunology (Baltimore, Md.: 1950)*, *163*(1), 337–342.

Jenkins, M. K., Khoruts, A., Ingulli, E., Mueller, D. L., McSorley, S. J., Reinhardt, R. L., Itano, A., & Pape, K. A. (2001). In Vivo Activation of Antigen-Specific CD4 T Cells. *Annual Review of Immunology*, *19*(1), 23–45. https://doi.org/10.1146/annurev.immunol.19.1.23

Joffre, O., Santolaria, T., Calise, D., Saati, T. A., Hudrisier, D., Romagnoli, P., & van Meerwijk, J. P. M. (2008). Prevention of acute and chronic allograft rejection with CD4+CD25+Foxp3+ regulatory T lymphocytes. *Nature Medicine*, *14*(1), 88–92. https://doi.org/10.1038/nm1688

Johnston, C. J. C., Robertson, E., Harcus, Y., Grainger, J. R., Coakley, G., Smyth, D. J., McSorley, H. J., & Maizels, R. (2015). Cultivation of Heligmosomoides Polygyrus: An Immunomodulatory Nematode Parasite and its Secreted Products. *Journal of Visualized Experiments*, *98*, 52412. https://doi.org/10.3791/52412

Kaplan, M. H., Sun, Y.-L., Hoey, T., & Grusby, M. J. (1996). Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice. *Nature*, *382*(6587), 174–177. https://doi.org/10.1038/382174a0

Kawabe, T., Jankovic, D., Kawabe, S., Huang, Y., Lee, P.-H., Yamane, H., Zhu, J., Sher, A., Germain, R. N., & Paul, W. E. (2017). Memory-phenotype CD4 $^{+}$ T cells spontaneously generated under steady-state conditions exert innate T $_{H}$ 1-like effector function. *Science Immunology*, *2*(12), eaam9304. https://doi.org/10.1126/sciimmunol.aam9304

Kawabe, T., Yi, J., Kawajiri, A., Hilligan, K., Fang, D., Ishii, N., Yamane, H., Zhu, J., Jankovic, D., Kim, K. S., Trinchieri, G., & Sher, A. (2020). Requirements for the differentiation of innate T-bethigh memory-phenotype CD4+ T lymphocytes under steady state. *Nature Communications*, *11*(1), 3366. https://doi.org/10.1038/s41467-020-17136-1

Klein, L., Kyewski, B., Allen, P. M., & Hogquist, K. A. (2014). Positive and negative selection of the T cell repertoire: What thymocytes see (and don't see). *Nature Reviews Immunology*, *14*(6), 377–391. https://doi.org/10.1038/nri3667

Kopf, M., Gros, G. L., Bachmann, M., Lamers, M. C., Bluethmann, H., & Köhler, G. (1993). Disruption of the murine IL-4 gene blocks Th2 cytokine responses. *Nature*, *362*(6417), 245–248. https://doi.org/10.1038/362245a0

Korn, T., Bettelli, E., Oukka, M., & Kuchroo, V. K. (2009). IL-17 and Th17 Cells. *Annual Review of Immunology*, *27*(1), 485–517. https://doi.org/10.1146/annurev.immunol.021908.132710

Lawrence, C. E., & Pritchard, D. I. (1994). Immune response profiles in responsive and non-responsive mouse strains infected with Heligmosomoides polygyrus. *International Journal for Parasitology*, *24*(4), 487–494. https://doi.org/10.1016/0020-7519(94)90139-2

Lee, Y. K., Turner, H., Maynard, C. L., Oliver, J. R., Chen, D., Elson, C. O., & Weaver, C. T. (2009). Late Developmental Plasticity in the T Helper 17 Lineage. *Immunity*, *30*(1), 92–107. https://doi.org/10.1016/j.immuni.2008.11.005

Lemos, M. P., Fan, L., Lo, D., & Laufer, T. M. (2003). CD8α $^{+}$ and CD11b $^{+}$ Dendritic Cell-Restricted MHC Class II Controls Th1 CD4 $^{+}$ T Cell Immunity. *The Journal of Immunology*, *171*(10), 5077–5084. https://doi.org/10.4049/jimmunol.171.10.5077

Leung, J. M., Budischak, S. A., Chung The, H., Hansen, C., Bowcutt, R., Neill, R., Shellman, M., Loke, P., & Graham, A. L. (2018). Rapid environmental effects on gut nematode susceptibility in rewilded mice. *PLOS Biology*, *16*(3), e2004108. https://doi.org/10.1371/journal.pbio.2004108

Liang, S. C., Tan, X.-Y., Luxenberg, D. P., Karim, R., Dunussi-Joannopoulos, K., Collins, M., & Fouser, L. A. (2006). Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *Journal of Experimental Medicine*, *203*(10), 2271–2279. https://doi.org/10.1084/jem.20061308

Liesenfeld, O., Dunay, I. R., & Erb, K. J. (2004). Infection with *Toxoplasma gondii* Reduces Established and Developing Th2 Responses Induced by *Nippostrongylus brasiliensis* Infection. *Infection and Immunity*, *72*(7), 3812–3822. https://doi.org/10.1128/IAI.72.7.3812-3822.2004

Lighvani, A. A., Frucht, D. M., Jankovic, D., Yamane, H., Aliberti, J., Hissong, B. D., Nguyen, B. V., Gadina, M., Sher, A., Paul, W. E., & O'Shea, J. J. (2001). T-bet is rapidly induced by interferon- in lymphoid and myeloid cells. *Proceedings of the National*

Academy of Sciences, *98*(26), 15137–15142. https://doi.org/10.1073/pnas.261570598

Liu, Y.-J. (2006). Thymic stromal lymphopoietin: Master switch for allergic inflammation. *Journal of Experimental Medicine*, *203*(2), 269–273. https://doi.org/10.1084/jem.20051745

Luckheeram, R. V., Zhou, R., Verma, A. D., & Xia, B. (2012). CD4 $^{+}$ T Cells: Differentiation and Functions. *Clinical and Developmental Immunology*, *2012*, 1–12. https://doi.org/10.1155/2012/925135

Mangan, P. R., Harrington, L. E., O'Quinn, D. B., Helms, W. S., Bullard, D. C., Elson, C. O., Hatton, R. D., Wahl, S. M., Schoeb, T. R., & Weaver, C. T. (2006). Transforming growth factor-β induces development of the TH17 lineage. *Nature*, *441*(7090), 231–234. https://doi.org/10.1038/nature04754

Marillier, R. G., Michels, C., Smith, E. M., Fick, L. C. E., Leeto, M., Dewals, B., Horsnell, W. G. C., & Brombacher, F. (2008). IL-4/IL-13 independent goblet cell hyperplasia in experimental helminth infections. *BMC Immunology*, *9*, 11. https://doi.org/10.1186/1471-2172-9-11

Marone, G., Granata, F., Pucino, V., Pecoraro, A., Heffler, E., Loffredo, S., Scadding, G. W., & Varricchi, G. (2019). The Intriguing Role of Interleukin 13 in the Pathophysiology of Asthma. *Frontiers in Pharmacology*, *10*, 1387. https://doi.org/10.3389/fphar.2019.01387

McKnight, A. J., Zimmer, G. J., Fogelman, I., Wolf, S. F., & Abbas, A. K. (1994). Effects of IL-12 on helper T cell-dependent immune responses in vivo. *Journal of Immunology (Baltimore, Md.: 1950)*, *152*(5), 2172–2179.

Mocci, S., & Coffman, R. L. (1995). Induction of a Th2 population from a polarized Leishmania-specific Th1 population by in vitro culture with IL-4. *Journal of Immunology (Baltimore, Md.: 1950)*, *154*(8), 3779–3787.

Morimoto, M., Saito, C., Muto, C., Akamatsu, Y., Chiba, T., Abe, T., Azuma, N., & Suto, Y. (2015). Impairment of host resistance to helminthes with age in murine small intestine. *Parasite Immunology*, *37*(4), 171–179. https://doi.org/10.1111/pim.12170

Morrison, P. J., Bending, D., Fouser, L. A., Wright, J. F., Stockinger, B., Cooke, A., & Kullberg, M. C. (2013). Th17-cell plasticity in Helicobacter hepaticus–induced intestinal inflammation. *Mucosal Immunology*, *6*(6), 1143–1156. https://doi.org/10.1038/mi.2013.11

Mosmann, T. R., & Coffman, R. L. (1989). TH1 and TH2 Cells: Different Patterns of Lymphokine Secretion Lead to Different Functional Properties. *Annual Review of Immunology*, *7*(1), 145–173. https://doi.org/10.1146/annurev.iy.07.040189.001045

Murray, H. W., Rubin, B. Y., Carriero, S. M., Harris, A. M., & Jaffee, E. A. (1985). Human mononuclear phagocyte antiprotozoal mechanisms: Oxygen-dependent vs oxygen-independent activity against intracellular Toxoplasma gondii. *Journal of Immunology (Baltimore, Md.: 1950)*, *134*(3), 1982–1988.

Nakanishi, K. (2018). Unique Action of Interleukin-18 on T Cells and Other Immune Cells. *Frontiers in Immunology*, *9*, 763. https://doi.org/10.3389/fimmu.2018.00763

Noval Rivas, M., Burton, O. T., Wise, P., Charbonnier, L.-M., Georgiev, P., Oettgen, H. C., Rachid, R., & Chatila, T. A. (2015). Regulatory T Cell Reprogramming toward a Th2-Cell-like Lineage Impairs Oral Tolerance and Promotes Food Allergy. *Immunity*, *42*(3), 512–523. https://doi.org/10.1016/j.immuni.2015.02.004

Nurieva, R. I., Chung, Y., Hwang, D., Yang, X. O., Kang, H. S., Ma, L., Wang, Y., Watowich, S. S., Jetten, A. M., Tian, Q., & Dong, C. (2008). Generation of T Follicular Helper Cells Is Mediated by Interleukin-21 but Independent of T Helper 1, 2, or 17 Cell Lineages. *Immunity*, *29*(1), 138–149. https://doi.org/10.1016/j.immuni.2008.05.009

Nurieva, R. I., Chung, Y., Martinez, G. J., Yang, X. O., Tanaka, S., Matskevitch, T. D., Wang, Y.-H., & Dong, C. (2009). Bcl6 Mediates the Development of T Follicular Helper Cells. *Science*, *325*(5943), 1001–1005. https://doi.org/10.1126/science.1176676

Nusse, Y. M., Savage, A. K., Marangoni, P., Rosendahl-Huber, A. K. M., Landman, T. A., de Sauvage, F. J., Locksley, R. M., & Klein, O. D. (2018). Parasitic helminths induce fetal-like reversion in the intestinal stem cell niche. *Nature*, *559*(7712), 109–113. https://doi.org/10.1038/s41586-018-0257-1

Oliphant, C. J., Hwang, Y. Y., Walker, J. A., Salimi, M., Wong, S. H., Brewer, J. M., Englezakis, A., Barlow, J. L., Hams, E., Scanlon, S. T., Ogg, G. S., Fallon, P. G., & McKenzie, A. N. J. (2014). MHCII-Mediated Dialog between Group 2 Innate Lymphoid Cells and CD4+ T Cells Potentiates Type 2 Immunity and Promotes Parasitic Helminth Expulsion. *Immunity*, *41*(2), 283–295. https://doi.org/10.1016/j.immuni.2014.06.016

O'Neil, D., Swanton, C., Jones, A., Medd, P. G., Rayment, N., & Chain, B. (1999). IFN-gamma down-regulates MHC expression and antigen processing in a human B cell line. *Journal of Immunology (Baltimore, Md.: 1950)*, *162*(2), 791–798.

Oosterwegel, M. A., Mandelbrot, D. A., Boyd, S. D., Lorsbach, R. B., Jarrett, D. Y., Abbas, A. K., & Sharpe, A. H. (1999). The role of CTLA-4 in regulating Th2 differentiation. *Journal of Immunology (Baltimore, Md.: 1950)*, *163*(5), 2634–2639.

Oriss, T. B., McCarthy, S. A., Morel, B. F., Campana, M. A., & Morel, P. A. (1997). Crossregulation between T helper cell (Th)1 and Th2: Inhibition of Th2 proliferation by IFN-gamma involves interference with IL-1. *Journal of Immunology (Baltimore, Md.: 1950)*, *158*(8), 3666–3672.

Oswald, I. P., Caspar, P., Jankovic, D., Wynn, T. A., Pearce, E. J., & Sher, A. (1994). IL-12 inhibits Th2 cytokine responses induced by eggs of Schistosoma mansoni. *Journal of Immunology (Baltimore, Md.: 1950)*, *153*(4), 1707–1713.

Ozawa, H., Aiba, S., Nakagawa, S., & Tagami, H. (1996). Interferon-γ and interleukin-10 inhibit antigen presentation by Langerhans cells for T helper type 1 cells by suppressing their CD80 (B7-1) expression. *European Journal of Immunology*, *26*(3), 648–652. https://doi.org/10.1002/eji.1830260321

Pai, S.-Y., Truitt, M. L., & Ho, I.-C. (2004). GATA-3 deficiency abrogates the development and maintenance of T helper type 2 cells. *Proceedings of the National Academy of Sciences*, *101*(7), 1993–1998. https://doi.org/10.1073/pnas.0308697100

Park, H., Li, Z., Yang, X. O., Chang, S. H., Nurieva, R., Wang, Y.-H., Wang, Y., Hood, L., Zhu, Z., Tian, Q., & Dong, C. (2005). A distinct lineage of CD4 T cells regulates

tissue inflammation by producing interleukin 17. *Nature Immunology*, *6*(11), 1133–1141. https://doi.org/10.1038/ni1261

Paul, W. E., & Seder, R. A. (1994). Lymphocyte responses and cytokines. *Cell*, *76*(2), 241–251. https://doi.org/10.1016/0092-8674(94)90332-8

Pei, C., Zhao, C., Wang, A.-J., Fan, A. X., Grinchuk, V., Smith, A., Sun, R., Xie, Y., Lu, N., Urban, J. F., Shea-Donohue, T., Zhao, A., & Yang, Z. (2016). Critical Role for Interleukin-25 in Host Protective Th2 Memory Response against Heligmosomoides polygyrus bakeri. *Infection and Immunity*, *84*(12), 3328–3337. https://doi.org/10.1128/IAI.00180-16

Peine, M., Rausch, S., Helmstetter, C., Fröhlich, A., Hegazy, A. N., Kühl, A. A., Grevelding, C. G., Höfer, T., Hartmann, S., & Löhning, M. (2013). Stable T-bet(+)GATA-3(+) Th1/Th2 hybrid cells arise in vivo, can develop directly from naive precursors, and limit immunopathologic inflammation. *PLoS Biology*, *11*(8), e1001633. https://doi.org/10.1371/journal.pbio.1001633

Prete, G. D. (1992). Human Th1 and Th2 lymphocytes: Their role in the pathophysiology of atopy. *Allergy*, *47*(5), 450–455. https://doi.org/10.1111/j.1398-9995.1992.tb00662.x

Progatzky, F., Shapiro, M., Chng, S. H., Garcia-Cassani, B., Classson, C. H., Sevgi, S., Laddach, A., Bon-Frauches, A. C., Lasrado, R., Rahim, M., Amaniti, E.-M., Boeing, S., Shah, K., Entwistle, L. J., Suárez-Bonnet, A., Wilson, M. S., Stockinger, B., & Pachnis, V. (2021). Regulation of intestinal immunity and tissue repair by enteric glia. *Nature*, *599*(7883), 125–130. https://doi.org/10.1038/s41586-021-04006-z

Quinnell, R. J., Pritchard, D. I., Raiko, A., Brown, A. P., & Shaw, M. (2004). Immune Responses in Human Necatoriasis: Association between Interleukin-5 Responses and Resistance to Reinfection. *The Journal of Infectious Diseases*, *190*(3), 430–438. https://doi.org/10.1086/422256

Restrepo, C. M., Llanes, A., Herrera, L., Ellis, E., Lleonart, R., & Fernández, P. L. (2021). Gene expression patterns associated with Leishmania panamensis infection in macrophages from BALB/c and C57BL/6 mice. *PLOS Neglected Tropical Diseases*, *15*(2), e0009225. https://doi.org/10.1371/journal.pntd.0009225

Reynolds, L. A., Filbey, K. J., & Maizels, R. M. (2012). Immunity to the model intestinal helminth parasite Heligmosomoides polygyrus. *Seminars in Immunopathology*, *34*(6), 829–846. https://doi.org/10.1007/s00281-012-0347-3

Rongcun, Y., Maes, H., Corsi, M., Dellner, F., Wen, T., & Kiessling, R. (1998). INTERFERON γ IMPAIRS THE ABILITY OF MONOCYTE-DERIVED DENDRITIC CELLS TO PRESENT TUMOUR-SPECIFIC AND ALLO-SPECIFIC ANTIGENS AND REDUCES THEIR EXPRESSION OF CD1A, CD80 AND CD4. *Cytokine*, *10*(10), 747–755. https://doi.org/10.1006/cyto.1998.0357

Rudolph, M. G., Stanfield, R. L., & Wilson, I. A. (2006). HOW TCRS BIND MHCS, PEPTIDES, AND CORECEPTORS. *Annual Review of Immunology*, *24*(1), 419–466. https://doi.org/10.1146/annurev.immunol.23.021704.115658

Ruterbusch, M., Pruner, K. B., Shehata, L., & Pepper, M. (2020). In Vivo CD4 $^{+}$ T Cell Differentiation and Function: Revisiting the Th1/Th2 Paradigm. *Annual Review of Immunology*, *38*(1), 705–725. https://doi.org/10.1146/annurev-immunol-103019-085803

Rutitzky, L. I., & Stadecker, M. J. (2011). Exacerbated egg-induced immunopathology in murine Schistosoma mansoni infection is primarily mediated by IL-17 and restrained by IFN-γ. *European Journal of Immunology*, *41*(9), 2677–2687. https://doi.org/10.1002/eji.201041327

Sahputra, R., Ruckerl, D., Couper, K. N., Muller, W., & Else, K. J. (2019). The Essential Role Played by B Cells in Supporting Protective Immunity Against Trichuris muris Infection Is by Controlling the Th1/Th2 Balance in the Mesenteric Lymph Nodes and Depends on Host Genetic Background. *Frontiers in Immunology*, *10*, 2842. https://doi.org/10.3389/fimmu.2019.02842

Sayers, G., Good, B., Hanrahan, J. P., Ryan, M., & Sweeney, T. (2005). Intron 1 of the interferon γ gene: Its role in nematode resistance in Suffolk and Texel sheep breeds. *Research in Veterinary Science*, *79*(3), 191–196. https://doi.org/10.1016/j.rvsc.2004.12.002

Sharpe, C., Thornton, D. J., & Grencis, R. K. (2018). A sticky end for gastrointestinal helminths; the role of the mucus barrier. *Parasite Immunology*, *40*(4), e12517. https://doi.org/10.1111/pim.12517

Shimoda, K., van Deursent, J., Sangster, M. Y., Sarawar, S. R., Carson, R. T., Tripp, R. A., Chu, C., Quelle, F. W., Nosaka, T., Vignali, D. A. A., Doherty, P. C., Grosveld, G., Paul, W. E., & Ihle, J. N. (1996). Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted State6 gene. *Nature*, *380*(6575), 630–633. https://doi.org/10.1038/380630a0

Smith, P., Dunne, D. W., & Fallon, P. G. (2001). Defective in vivo induction of functional type 2 cytokine responses in aged mice. *European Journal of Immunology*, *31*(5), 1495–1502. https://doi.org/10.1002/1521-4141(200105)31:5<1495::AID-IMMU1495>3.0.CO;2-8

Sokol, C. L., Chu, N.-Q., Yu, S., Nish, S. A., Laufer, T. M., & Medzhitov, R. (2009). Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. *Nature Immunology*, *10*(7), 713–720. https://doi.org/10.1038/ni.1738

Song, K., Rabin, R. L., Hill, B. J., De Rosa, S. C., Perfetto, S. P., Zhang, H. H., Foley, J. F., Reiner, J. S., Liu, J., Mattapallil, J. J., Douek, D. C., Roederer, M., & Farber, J. M. (2005). Characterization of subsets of CD4 $^{+}$ memory T cells reveals early branched pathways of T cell differentiation in humans. *Proceedings of the National Academy of Sciences*, *102*(22), 7916–7921. https://doi.org/10.1073/pnas.0409720102

Sorobetea, D., Svensson-Frej, M., & Grencis, R. (2018). Immunity to gastrointestinal nematode infections. *Mucosal Immunology*, *11*(2), 304–315. https://doi.org/10.1038/mi.2017.113

Sugawara, Y., Azuma, N., Onodera, S., Tsunoka, Y., & Morimoto, M. (2011). Th2 Immune Responses and Alternatively Activated Macrophages (AAMacs) in Helminth Infection in Aged Mice. *Journal of Veterinary Medical Science*, *73*(4), 511–516. https://doi.org/10.1292/jvms.10-0269

Szabo, S. J., Kim, S. T., Costa, G. L., Zhang, X., Fathman, C. G., & Glimcher, L. H. (2000). A Novel Transcription Factor, T-bet, Directs Th1 Lineage Commitment. *Cell*, *100*(6), 655–669. https://doi.org/10.1016/S0092-8674(00)80702-3

Szabo, S. J., Sullivan, B. M., Stemmann, C., Satoskar, A. R., Sleckman, B. P., & Glimcher, L. H. (2002). Distinct Effects of T-bet in T_H 1 Lineage Commitment and IFN-γ Production in CD4 and CD8 T Cells. *Science*, *295*(5553), 338–342. https://doi.org/10.1126/science.1065543

Takeda, K., Tanaka, T., Shi, W., Matsumoto, M., Minami, M., Kashiwamura, S., Nakanishi, K., Yoshida, N., Kishimoto, T., & Akira, S. (1996). Essential role of Stat6 in IL-4 signalling. *Nature*, *380*(6575), 627–630. https://doi.org/10.1038/380627a0

Tanaka, S., Motomura, Y., Suzuki, Y., Yagi, R., Inoue, H., Miyatake, S., & Kubo, M. (2011). The enhancer HS2 critically regulates GATA-3-mediated Il4 transcription in TH2 cells. *Nature Immunology*, *12*(1), 77–85. https://doi.org/10.1038/ni.1966

Tao, X., Constant, S., Jorritsma, P., & Bottomly, K. (1997). Strength of TCR signal determines the costimulatory requirements for Th1 and Th2 CD4+ T cell differentiation. *Journal of Immunology (Baltimore, Md.: 1950)*, *159*(12), 5956–5963.

Thierfelder, W. E., van Deursen, J. M., Yamamoto, K., Tripp, R. A., Sarawar, S. R., Carson, R. T., Sangster, M. Y., Vignali, D. A. A., Doherty, P. C., Grosveld, G. C., & Ihle, J. N. (1996). Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells. *Nature*, *382*(6587), 171–174. https://doi.org/10.1038/382171a0

Trinchieri, G., & Sher, A. (2007). Cooperation of Toll-like receptor signals in innate immune defence. *Nature Reviews Immunology*, *7*(3), 179–190. https://doi.org/10.1038/nri2038

Tsuji, M., Komatsu, N., Kawamoto, S., Suzuki, K., Kanagawa, O., Honjo, T., Hori, S., & Fagarasan, S. (2009). Preferential Generation of Follicular B Helper T Cells from Foxp3 $^+$ T Cells in Gut Peyer's Patches. *Science*, *323*(5920), 1488–1492. https://doi.org/10.1126/science.1169152

Turner, J. D., Faulkner, H., Kamgno, J., Cormont, F., Van Snick, J., Else, K. J., Grencis, R. K., Behnke, J. M., Boussinesq, M., & Bradley, J. E. (2003). Th2 Cytokines Are Associated with Reduced Worm Burdens in a Human Intestinal Helminth Infection. *The Journal of Infectious Diseases*, *188*(11), 1768–1775. https://doi.org/10.1086/379370

Urban, J. F., Katona, I. M., & Finkelman, F. D. (1991). Heligmosomoides polygyrus: CD4+ but not CD8+ T cells regulate the IgE response and protective immunity in mice. *Experimental Parasitology*, *73*(4), 500–511. https://doi.org/10.1016/0014-4894(91)90074-7

Urban, J. F., Noben-Trauth, N., Donaldson, D. D., Madden, K. B., Morris, S. C., Collins, M., & Finkelman, F. D. (1998). IL-13, IL-4Rα, and Stat6 Are Required for the Expulsion of the Gastrointestinal Nematode Parasite Nippostrongylus brasiliensis. *Immunity*, *8*(2), 255–264. https://doi.org/10.1016/S1074-7613(00)80477-X

Urban, J. F., Schopf, L., Morris, S. C., Orekhova, T., Madden, K. B., Betts, C. J., Gamble, H. R., Byrd, C., Donaldson, D., Else, K., & Finkelman, F. D. (2000). Stat6 Signaling Promotes Protective Immunity Against *Trichinella spiralis* Through a Mast Cell- and T Cell-Dependent Mechanism. *The Journal of Immunology*, *164*(4), 2046–2052. https://doi.org/10.4049/jimmunol.164.4.2046

Usui, T., Nishikomori, R., Kitani, A., & Strober, W. (2003). GATA-3 Suppresses Th1 Development by Downregulation of Stat4 and Not through Effects on IL-12Rβ2 Chain or T-bet. *Immunity*, *18*(3), 415–428. https://doi.org/10.1016/S1074-7613(03)00057-8

Usui, T., Preiss, J. C., Kanno, Y., Yao, Z. J., Bream, J. H., O'Shea, J. J., & Strober, W. (2006). T-bet regulates Th1 responses through essential effects on GATA-3 function rather than on IFNG gene acetylation and transcription. *Journal of Experimental Medicine*, *203*(3), 755–766. https://doi.org/10.1084/jem.20052165

Veldhoen, M., Hocking, R. J., Atkins, C. J., Locksley, R. M., & Stockinger, B. (2006). TGFβ in the Context of an Inflammatory Cytokine Milieu Supports De Novo Differentiation of IL-17-Producing T Cells. *Immunity*, *24*(2), 179–189. https://doi.org/10.1016/j.immuni.2006.01.001

Vinuesa, C. G., Tangye, S. G., Moser, B., & Mackay, C. R. (2005). Follicular B helper T cells in antibody responses and autoimmunity. *Nature Reviews Immunology*, *5*(11), 853–865. https://doi.org/10.1038/nri1714

Vogelzang, A., McGuire, H. M., Yu, D., Sprent, J., Mackay, C. R., & King, C. (2008). A Fundamental Role for Interleukin-21 in the Generation of T Follicular Helper Cells. *Immunity*, *29*(1), 127–137. https://doi.org/10.1016/j.immuni.2008.06.001

von Moltke, J., Ji, M., Liang, H.-E., & Locksley, R. M. (2016). Tuft-cell-derived IL-25 regulates an intestinal ILC2–epithelial response circuit. *Nature*, *529*(7585), 221–225. https://doi.org/10.1038/nature16161

Wakelin, D., & Selby, G. R. (1974). Thymus-dependency of the immune response of mice to a primary infection with the nematode Trichuris muris. *International Journal for Parasitology*, *4*(6), 657–661. https://doi.org/10.1016/0020-7519(74)90030-7

Wan, Y. Y., & Flavell, R. A. (2007). Regulatory T-cell functions are subverted and converted owing to attenuated Foxp3 expression. *Nature*, *445*(7129), 766–770. https://doi.org/10.1038/nature05479

Weaver, C. T., Harrington, L. E., Mangan, P. R., Gavrieli, M., & Murphy, K. M. (2006). Th17: An Effector CD4 T Cell Lineage with Regulatory T Cell Ties. *Immunity*, *24*(6), 677–688. https://doi.org/10.1016/j.immuni.2006.06.002

Webster, P., Monrad, J., Kapel, C. M. O., Kristensen, A. T., Jensen, A. L., & Thamsborg, S. M. (2017). The effect of host age and inoculation dose on infection dynamics of Angiostrongylus vasorum in red foxes (Vulpes vulpes). *Parasites & Vectors*, *10*(1), 4. https://doi.org/10.1186/s13071-016-1940-4

Wei, G., Abraham, B. J., Yagi, R., Jothi, R., Cui, K., Sharma, S., Narlikar, L., Northrup, D. L., Tang, Q., Paul, W. E., Zhu, J., & Zhao, K. (2011). Genome-wide Analyses of Transcription Factor GATA3-Mediated Gene Regulation in Distinct T Cell Types. *Immunity*, *35*(2), 299–311. https://doi.org/10.1016/j.immuni.2011.08.007

Williams, L. M., & Rudensky, A. Y. (2007). Maintenance of the Foxp3-dependent developmental program in mature regulatory T cells requires continued expression of Foxp3. *Nature Immunology*, *8*(3), 277–284. https://doi.org/10.1038/ni1437

Wojciechowski, W., Harris, D. P., Sprague, F., Mousseau, B., Makris, M., Kusser, K., Honjo, T., Mohrs, K., Mohrs, M., Randall, T., & Lund, F. E. (2009). Cytokine-Producing

Effector B Cells Regulate Type 2 Immunity to H. polygyrus. *Immunity*, *30*(3), 421–433. https://doi.org/10.1016/j.immuni.2009.01.006

Wurtz, O. (2004). IL-4-mediated inhibition of IFN- production by CD4+ T cells proceeds by several developmentally regulated mechanisms. *International Immunology*, *16*(3), 501–508. https://doi.org/10.1093/intimm/dxh050

Wynn, T. A. (2003). IL-13 Effector Functions. *Annual Review of Immunology*, *21*(1), 425–456. https://doi.org/10.1146/annurev.immunol.21.120601.141142

Xu, L., Kitani, A., Fuss, I., & Strober, W. (2007). Cutting Edge: Regulatory T Cells Induce CD4 $^{+}$ CD25 $^{-}$ Foxp3 $^{-}$ T Cells or Are Self-Induced to Become Th17 Cells in the Absence of Exogenous TGF-β. *The Journal of Immunology*, *178*(11), 6725–6729. https://doi.org/10.4049/jimmunol.178.11.6725

Yagi, H., Nomura, T., Nakamura, K., Yamazaki, S., Kitawaki, T., Hori, S., Maeda, M., Onodera, M., Uchiyama, T., Fujii, S., & Sakaguchi, S. (2004). Crucial role of FOXP3 in the development and function of human CD25+CD4+ regulatory T cells. *International Immunology*, *16*(11), 1643–1656. https://doi.org/10.1093/intimm/dxh165

Yagi, R., Junttila, I. S., Wei, G., Urban, J. F., Zhao, K., Paul, W. E., & Zhu, J. (2010). The Transcription Factor GATA3 Actively Represses RUNX3 Protein-Regulated Production of Interferon-γ. *Immunity*, *32*(4), 507–517. https://doi.org/10.1016/j.immuni.2010.04.004

Yamane, H., Zhu, J., & Paul, W. E. (2005). Independent roles for IL-2 and GATA-3 in stimulating naive CD4+ T cells to generate a Th2-inducing cytokine environment. *Journal of Experimental Medicine*, *202*(6), 793–804. https://doi.org/10.1084/jem.20051304

Yamashita, M., Ukai-Tadenuma, M., Kimura, M., Omori, M., Inami, M., Taniguchi, M., & Nakayama, T. (2002). Identification of a Conserved GATA3 Response Element Upstream Proximal from the Interleukin-13 Gene Locus. *Journal of Biological Chemistry*, *277*(44), 42399–42408. https://doi.org/10.1074/jbc.M205876200

Yang, X. O., Pappu, B. P., Nurieva, R., Akimzhanov, A., Kang, H. S., Chung, Y., Ma, L., Shah, B., Panopoulos, A. D., Schluns, K. S., Watowich, S. S., Tian, Q., Jetten, A. M., & Dong, C. (2008). T Helper 17 Lineage Differentiation Is Programmed by Orphan Nuclear Receptors RORα and RORγ. *Immunity*, *28*(1), 29–39. https://doi.org/10.1016/j.immuni.2007.11.016

Yang, Y., Ochando, J. C., Bromberg, J. S., & Ding, Y. (2007). Identification of a distant T-bet enhancer responsive to IL-12/Stat4 and IFNγ/Stat1 signals. *Blood*, *110*(7), 2494–2500. https://doi.org/10.1182/blood-2006-11-058271

Yasuda, K., & Nakanishi, K. (2018). Host responses to intestinal nematodes. *International Immunology*, *30*(3), 93–102. https://doi.org/10.1093/intimm/dxy002

Yoshimura, A., & Muto, G. (2010). TGF-β Function in Immune Suppression. In R. Ahmed & T. Honjo (Eds.), *Negative Co-Receptors and Ligands* (Vol. 350, pp. 127–147). Springer Berlin Heidelberg. https://doi.org/10.1007/82_2010_87

Yu, C.-R., Mahdi, R. M., Ebong, S., Vistica, B. P., Chen, J., Guo, Y., Gery, I., & Egwuagu, C. E. (2004). Cell Proliferation and STAT6 Pathways Are Negatively Regulated in T

Cells by STAT1 and Suppressors of Cytokine Signaling. *The Journal of Immunology*, *173*(2), 737–746. https://doi.org/10.4049/jimmunol.173.2.737

Zaph, C., Troy, A. E., Taylor, B. C., Berman-Booty, L. D., Guild, K. J., Du, Y., Yost, E. A., Gruber, A. D., May, M. J., Greten, F. R., Eckmann, L., Karin, M., & Artis, D. (2007). Epithelial-cell-intrinsic IKK-β expression regulates intestinal immune homeostasis. *Nature*, *446*(7135), 552–556. https://doi.org/10.1038/nature05590

Zhang, D.-H., Cohn, L., Ray, P., Bottomly, K., & Ray, A. (1997). Transcription Factor GATA-3 Is Differentially Expressed in Murine Th1 and Th2 Cells and Controls Th2-specific Expression of the Interleukin-5 Gene. *Journal of Biological Chemistry*, *272*(34), 21597–21603. https://doi.org/10.1074/jbc.272.34.21597

Zhang, Y., Apilado, R., Coleman, J., Ben-Sasson, S., Tsang, S., Hu-Li, J., Paul, W. E., & Huang, H. (2001). Interferon γ Stabilizes the T Helper Cell Type 1 Phenotype. *Journal of Experimental Medicine*, *194*(2), 165–172. https://doi.org/10.1084/jem.194.2.165

Zhao, A., Urban, J. F., Anthony, R. M., Sun, R., Stiltz, J., van Rooijen, N., Wynn, T. A., Gause, W. C., & Shea-Donohue, T. (2008). Th2 Cytokine-Induced Alterations in Intestinal Smooth Muscle Function Depend on Alternatively Activated Macrophages. *Gastroenterology*, *135*(1), 217-225.e1. https://doi.org/10.1053/j.gastro.2008.03.077

Zheng, W., & Flavell, R. A. (1997). The Transcription Factor GATA-3 Is Necessary and Sufficient for Th2 Cytokine Gene Expression in CD4 T Cells. *Cell*, *89*(4), 587–596. https://doi.org/10.1016/S0092-8674(00)80240-8

Zheng, Y., Danilenko, D. M., Valdez, P., Kasman, I., Eastham-Anderson, J., Wu, J., & Ouyang, W. (2007). Interleukin-22, a TH17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. *Nature*, *445*(7128), 648–651. https://doi.org/10.1038/nature05505

Zheng, Y., Valdez, P. A., Danilenko, D. M., Hu, Y., Sa, S. M., Gong, Q., Abbas, A. R., Modrusan, Z., Ghilardi, N., de Sauvage, F. J., & Ouyang, W. (2008). Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nature Medicine*, *14*(3), 282–289. https://doi.org/10.1038/nm1720

Zhou, B., Comeau, M. R., Smedt, T. D., Liggitt, H. D., Dahl, M. E., Lewis, D. B., Gyarmati, D., Aye, T., Campbell, D. J., & Ziegler, S. F. (2005). Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nature Immunology*, *6*(10), 1047–1053. https://doi.org/10.1038/ni1247

Zhou, L., Chong, M. M. W., & Littman, D. R. (2009). Plasticity of CD4+ T Cell Lineage Differentiation. *Immunity*, *30*(5), 646–655. https://doi.org/10.1016/j.immuni.2009.05.001

Zhou, L., Lopes, J. E., Chong, M. M. W., Ivanov, I. I., Min, R., Victora, G. D., Shen, Y., Du, J., Rubtsov, Y. P., Rudensky, A. Y., Ziegler, S. F., & Littman, D. R. (2008). TGF-β-induced Foxp3 inhibits TH17 cell differentiation by antagonizing RORγt function. *Nature*, *453*(7192), 236–240. https://doi.org/10.1038/nature06878

Zhou, X., Bailey-Bucktrout, S. L., Jeker, L. T., Penaranda, C., Martínez-Llordella, M., Ashby, M., Nakayama, M., Rosenthal, W., & Bluestone, J. A. (2009). Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells in vivo. *Nature Immunology*, *10*(9), 1000–1007. https://doi.org/10.1038/ni.1774

Zhu, J. (2018). T Helper Cell Differentiation, Heterogeneity, and Plasticity. *Cold Spring Harbor Perspectives in Biology*, *10*(10). https://doi.org/10.1101/cshperspect.a030338

Zhu, J., Cote-Sierra, J., Guo, L., & Paul, W. E. (2003). Stat5 Activation Plays a Critical Role in Th2 Differentiation. *Immunity*, *19*(5), 739–748. https://doi.org/10.1016/S1074-7613(03)00292-9

Zhu, J., Guo, L., Watson, C. J., Hu-Li, J., & Paul, W. E. (2001). Stat6 Is Necessary and Sufficient for IL-4's Role in Th2 Differentiation and Cell Expansion. *The Journal of Immunology*, *166*(12), 7276–7281. https://doi.org/10.4049/jimmunol.166.12.7276

Zhu, J., Jankovic, D., Oler, A. J., Wei, G., Sharma, S., Hu, G., Guo, L., Yagi, R., Yamane, H., Punkosdy, G., Feigenbaum, L., Zhao, K., & Paul, W. E. (2012). The Transcription Factor T-bet Is Induced by Multiple Pathways and Prevents an Endogenous Th2 Cell Program during Th1 Cell Responses. *Immunity*, *37*(4), 660–673. https://doi.org/10.1016/j.immuni.2012.09.007

Zhu, J., Min, B., Hu-Li, J., Watson, C. J., Grinberg, A., Wang, Q., Killeen, N., Urban, J. F., Guo, L., & Paul, W. E. (2004). Conditional deletion of Gata3 shows its essential function in TH1-TH2 responses. *Nature Immunology*, *5*(11), 1157–1165. https://doi.org/10.1038/ni1128

Zhu, J., & Paul, W. E. (2008). CD4 T cells: Fates, functions, and faults. *Blood*, *112*(5), 1557–1569. https://doi.org/10.1182/blood-2008-05-078154

10. LIST OF PUBLICATIONS

Publications:

- **Kapse, B.,** Zhang, H., Affinass, N., Ebner, F., Hartmann, S., & Rausch, S. (2022). Age-dependent rise in IFN-γ competence undermines effective type 2 responses to nematode infection. *Mucosal Immunology*. https://doi.org/10.1038/s41385-022-00519-6
- **Kapse, B.,** Adjah, J., Zhang, H., Hartmann, S., & Rausch, S. (2023) Differential resistance to enteric nematode infection is associated with the genotype- and age-dependent pace of Th2 cell recruitment to the infected gut (in preparation).

Digital conferences:

- **Kapse, B.,** Hartmann, S. & Rausch, S. (2021). Importance of Th2/1 hybrid cells in the susceptibility to nematode infection. *23rd Meeting on T cells, German Society for Immunology*
- **Kapse, B.,** Zhang, H., Löhning, M., Hartmann, S. & Rausch, S. (2021) IFN-γ as a regulator of type 2 immunity to gastrointestinal nematode infection. *Frontiers in comparative immunology series: T cell biology virtual conference, British Society for Immunology*
- **Kapse, B.,** Zhang, H., Löhning, M., Hartmann, S. & Rausch, S. (2021) IFN-γ simultaneously promotes mucosal Th2/1 hybrid cell responses and the systemic expansion of GATA-3+ T cells in intestinal nematode infection. *29th Annual Meeting of the German Society for Parasitology*

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I hereby declare that I have written my doctoral thesis titled ‘Role of IFN-γ in the immunity and control of gastrointestinal nematode infections’ myself without any unauthorized help or sources other than those mentioned. The work was financially supported by Research Training Group 2046 (GRK2046). In the context of this work, there are no conflict of interests due to contributions from third parties.

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