Establishment and characterization of a mouse model of chronic *Salmonella enterica* infection as a proposed animal model for human inflammatory bowel disease

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Nothing in life is to be feared,
it is only to be understood.
Now is the time to understand more,
so that we may fear less.

Maria Sklodowska Curie

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List of abbreviations

° C	degree Celsius
µg	Microgram
µl	Microliter
µm	Micrometer
AhR	Aryl hydrocarbon receptor
AMP	Anti-microbial peptide
ANCA	Anti-neutrophil cytoplasmic antibodies
APC	Antigen-presenting cell
CCL2	CC-chemokine ligand 2
CD	Cluster of differentiation
CD	Crohn's Disease
cfu	Colony forming unit
CO_2	Carbon dioxide
CXCL	C-X-C motif ligand
DNA	Deoxyrybonucleic acid
DAPI	4',6 diamidin-2-phenylindol
DC	Dendritic cell
DSS	Dextran sulphate sodium
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FCS	Fetal calf serum
GALT	Gut-associated lymphoid tissue
GF	Germ-free
GI	Gastrointestinal
h	Hour
HPF	High power field
H&E	Haematoxylin and eosin
HIER	Heat-induced epitope retrieval
HLA	Human leukocyte antigen
IBD	Inflammatory bowel disease
IHC	Immunohistochemistry

IEC	Intestinal epithelial cell
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IL-22R	IL-22-receptor
IL-102R	IL-10-receptor 2
IL-22RA1	IL-22-receptor-alpha
ILC	Innate lymphoid cell
iNOS	Inducible Nitric Oxide Synthase
KO	Knock-out
L	Liter
LP	Lamina propria
LPS	Lipopolysaccharide-binding protein
MAMP	Microorganism.associated molecular pattern
mg	Miligram
min	Minute
ml	Mililiter
MLN	Mesenteric lymph node
Mreg	Regulatory myeloid
MUC1	Mucin 1
MUC2	Mucin 2
MyD88	Myeloid differentiation factor 88
NGS	Next-generation sequencing
NK	Natural-killer
NLR	NOD-like receptor
nm	Nanometer
NOD2	Nucleotide oligomerization domain containing 2
OD	Optical density
p.i.	Post infection
PAMP	Pathogen-associated molecular patterns
PAS	Periodic acid-Schiff
PBS	Phosphate buffered saline
PIER	Protease.induced epitope retrieval
PPs	Peyer’s patches

PRR	Pattern recognition receptor
ROI	Region of interest
RORγt	Retinoic-acid related orphan receptor gamma t
RT	Room temperature
S.e.	*Salmonella enterica*
SD	Standard deviation
SE	*Salmonella* Enteritidis
SFB	Segmented filamentous bacteria
sIgA	secretory immunoglobulin A
SPI-1	*Salmonella* pathogenicity Island 1
STAT3	Signal transducer and activator of transcription 3
Strep.	streptomycin
TEZ	*Tierexperimentelles Zentrum* - Centre for Experimental Medicine
TGF	Transforming growth factor
Th	T helper
TJ	Tight junction
TLR	Toll-like-receptor
TMB	3,3′,5,5′-Tetramethylbenzidine
Treg	Regulatory T
TRIS	Tris(hydroxymethyl)-aminomethane
UC	Ulcerative colitis
w	Week
WT	Wild-type
XLD	Xylose lysine deoxycholate

1 Introduction

1.1 Immune system of the gastrointenstinal tract

The gastrointestinal (GI) tract is constantly exposed to a wide range of stimuli, i.e. environmental antigens, the commensal microbiota or pathogenic bacteria. A very important role of managing an appropriate immune response to these foreign antigens is fulfilled by the gut-associated lymphoid tissue (GALT). This independently functioning system is the most prominent collection of lymphoid tissues and consists of organized compartments, e.g. Peyer's patches (PPs) and mesenteric lymph nodes (MLNs), as well as diffusely scattered immune cells located in the gut mucosa (1, 2). The GI tract is a major site of immune modulation, considering the fact that approximately 70% of the entire immune system is located within the GALT, and almost 80% of IgA-producing plasma cells reside in the lamina propria (LP) of the gut (3). Thus, disturbance of the balance between tolerance and immunity can lead to serious chronic conditions, e.g. inflammatory bowel disease (IBD) or food allergies (4, 5).

1.1.1 Intestinal barrier

To manage the constant presence of potentially harmful antigens and to maintain the physiological organ function, the GI tract has developed various mechanisms to act as a physical and biochemical barrier and protect the gut homeostasis (6). The intestinal mucosa is the major site of immunological processes in the gut. It is composed of a single layer of epithelial cells, the underlying LP and the muscular mucosa (7). The intestinal epithelium plays a crucial role in the host defense against foreign antigens and pathogens by providing a mechanical barrier function. At the same time, the epithelial layer must allow absorption of dietary nutrients. Intestinal epithelial cells (IECs) comprise absorptive epithelial cells, goblet cells, which synthesize and release mucin, as well as Paneth cells, which secrete anti-microbial peptides (AMPs) (8). Enterocytes which represent the most prominent cell type of the epithelial monolayer produce proteins forming the glycocalix as well as anti-microbial agents, such as β-defensins. In some regions, enterocytes are interspersed with M cells, which are specialized in mediating the transport of luminal antigens across the epithelial barrier into the

underlying GALT. Restriction of the passage of even the smallest molecules into the mucosa is the main role of tight junctions

(TJs), which seal the intercellular spaces on the luminal surface (9–11). Another component of the physical barrier is the mucus layer covering the gut epithelium on the luminal side. The mucus layer contains mucin glycoproteins that are produced by intestinal secretory cells, the goblet cells. The mucus layer plays a crucial role in preventing pathogen infection and intestinal inflammation considering the large number of microorganisms residing in the gut. The most abundant component of the mucus is mucin 2 (MUC2), which by its large net-like polymers organizes the layer to serve as protective system against pathogenic bacteria (12). It has been shown that MUC2-deficient mice develop spontaneous colitis and have a predisposition for inflammation-induced colorectal cancer (13, 14). A variety of AMPs produced by Paneth cells serve as chemical barrier to protect the gut homeostasis. These small oligopeptides have a broad spectrum antimicrobial activity that contributes to the restriction of bacterial growth (15). Considering its extensiveness and complex function, any defect in or disintegration of the intestinal barrier can lead to disruption of the mucosal immune homeostasis and consequently to uncontrolled inflammation.

1.1.2 Regulation of gut homeostasis by the innate and adaptive immune system

The immune system has evolved various mechanisms to protect the host from invasion by pathogenic microorganisms. There are specialized innate and adaptive immune components present in the intestinal mucosa that contribute to the development of appropriate immune responses towards the numerous challenges. On the other hand, gut homeostasis depends on tolerance of the immune system towards microbial and environmental factors, such as food components. The innate immune system provides an immediate reaction to pathogenic invaders in the intestine. This includes the epithelial barrier and several types of antigen-presenting cells (APC). The APCs found in the gut mucosa encompass myeloid cells, such as dendritic cells (DCs), macrophages and regulatory myeloid (Mreg) cells. DCs promote immune tolerance against intestinal microbiota, as well as protective immune responses against pathogens. Thanks to the ability to recognize pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs), the innate immune system is involved in the activation of the DCs, which leads to development of T helper (Th)1/Th17 cells (16). Intestinal macrophages suppress the production of pro-inflammatory cytokines (e.g. interleukin-12 (IL-12) and TNF-α) by secreting large amounts of IL-10 in response to the commensal microbiota. Moreover, macrophage-derived IL-10 maintains expression of FoxP3 in regulatory T (Treg) cells. Thus, macrophages significantly contribute to prevention of intestinal inflammation in response to the

intestinal microbiota (17). Other important contributors in regulating intestinal inflammation are Mreg cells, which inhibit proliferation of T cells by a cell-cell-contact-dependent mechanism (18). It has been shown that disruption of the function of innate myeloid cells leads to excessive activation of innate immune responses and can result in the development of IBD (19). Next to innate myeloid cells, another cell population responsible for immune homeostasis is innate lymphoid cells (ILCs). They are considered significant contributors to the maintenance of intestinal barrier function, to the induction of inflammatory reactions as well as the regulation of tissue repair. Type-3 ILCs seem to play a special role due to production of IL-22 in the gut, which stimulates production of AMPs and mucus in epithelial cells (20). Although the precise underlying mechanism remains unclear, the role of aryl hydrocarbon receptor (AhR) has been reported as essential for maintenance of ILC3 homeostasis and their production of IL-22 (21, 22). AhR is a ligand-dependent transcription factor expressed by different types of immune cells (i.e. ILC3, Th17, Th22, Macrophages) (23). It can be activated by various natural and synthetic ligands, including catabolites derived from gut microbiota or nutrition, thus it significantly contributes to regulation of intestinal physiology (24).

The innate immune system not only provides an acute non-specific defense, but also plays a crucial role in the regulation of adaptive immune responses. Adaptive immunity in the gut depends on lymphocytes that play a key role in inducing specific and memory immune responses. The intestinal mucosa contains the greatest population of T cells in the body (25). T cell populations found in the gut are highly heterogeneous and upon stimulation, they become activated, secrete cytokines and influence many other cell types present in the local environment. There is a large population of $CD4^+$ T cells present in the LP that mostly comprises effector and memory T cells. For gut homeostasis, the number and activity of effector T cells, including Th1 and Th17 cells, have to be strictly regulated by various mechanisms. Increased numbers of these cells were found in the gut mucosa of IBD patients, emphasizing the importance of controlling their activity (26). On the other hand, appropriate Th1 and Th17 responses are crucial in the defense against invading pathogens. Th1 cells produce interferon (IFN)-γ essential for the activation of macrophages, while Th17 cytokines, such as IL-22, stimulate the production of AMPs and manage the integrity of the epithelial barrier (27). FoxP3+ Treg cells play a crucial role in the suppression of exaggerated immune responses driven by effector T cells. This cell subset controls the development of inflammation by producing anti-inflammatory cytokines, i.e. IL-10 and transforming growth factor (TGF)-β. Treg cells-derived IL-10 inhibits production of Th1 cytokines. Thus, IL-10-deficient mice develop spontaneous colitis with increased effector T cell activity (28). Moreover, Treg cells provide several mechanisms that inhibit APC function (29). Next to T cells, large numbers of plasma B cells are present in the normal intestinal LP and their frequency is the highest in the most proximal and distal parts of the gut. The

great majority of them are Immunoglobulin A (IgA)-producing plasma cells secreting the primary antibody isotype of the mucosal humoral immune system (30, 31).

1.1.3 Secretory IgA

Polymeric IgA (secretory IgA, sIgA) is the major immunoglobulin present at mucosal surfaces of mammals. The sIgA is produced by plasma cells in the LP and has a significantly different molecular structure than IgA antibodies found in the serum or tissues. This immunoglobulin serves as one of the primary factors preventing invasion of pathogens into the mucosa (32). The most commonly proposed mechanism by which sIgA blocks microbial pathogens and toxins from entering mucosal epithelial cells is called immune exclusion. The sIgA possesses a special ability to recognize polyvalent antigenic epitopes on the surface of bacteria or viruses and subsequently agglutinate the antigens in the intestinal lumen. Consequently, the possible invaders lose their potential to adhere to or penetrate the epithelium. (33). Moreover, sIgA plays a crucial role in presenting the contents of the microbiota to the immune system. Therefore, its contribution in maintaining homeostasis in the gut is essential. Immune complexes of sIgA and commensal bacteria are transferred from the lumen into the mucosa, and the bacterial components are selectively presented to tolerogenic DCs that produce anti-inflammatory IL-10. Thus, a tolerogenic immune response to the microbiota is induced (34).

1.1.4 Impact of the microbiota on immune homeostasis in the gut

Intestinal immune system develops and stabilizes within first years of life. Additionally, adequate host defense mechanisms need to be established as well. Therefore, initial bacterial colonization is required to stimulate the protective function of the gut immune system (35). It has been confirmed that a proper development as well as optimal function of the mucosal immune system depends on colonization with commensal microbiota. In healthy individuals, the intestinal microbiota includes over 1.000 species of bacteria, which contribute to the homeostasis in the gut. Presence of the abundant bacterial community is essential for proper digestion and absorption of nutrients, but also prevention of pathogenic infections (36). The immune system has evolved adaptations to protect the symbiotic relationship between the host and the microbiota and prevent continuous challenges caused by the close association of bacteria with the intestinal tissue. This mutualistic interaction is well-balanced in healthy organism but its disruption can result in the development of chronic conditions, e.g. IBD or metabolic disease
(37). Several recent studies have demonstrated that certain commensal bacteria modulate the host innate and adaptive immunity, thereby contributing to the maintenance of gut homeostasis. Germ-

free (GF) mouse models have become an important approach to demonstrate the critical role of the microbiota in the development and shaping of innate and adaptive immunity (38). Innate immune responses are essential for rapid elimination of pathogens, but also significantly contribute to the maintenance of mutualism with the gut microbiota. This relationship was first demonstrated in the myeloid differentiation factor 88 (MyD88)-deficient mouse model. MyD88 is a key factor in TLR (Toll-like-receptor) signaling and its deficiency results in diminished microbial sensing. Animals lacking MyD88 were found to have an altered composition of the microbiota resulting in predisposition to intestinal inflammation (39). There is evidence proving the crucial role of the commensal bacteria for the development of APCs. GF mice showed a decreased number of intestinal DCs, but colonization of these animals with *Escherichia coli* caused recruitment of DCs to the intestine (40). The microbiota also contributes to the regulation of anti-inflammatory functions of intestinal macrophages by controlling the production of IL-10 (41), which is mediated, among others, by AhR. It was shown, that AhR-deficient macrophages have an impaired ability to secrete IL-10 upon LPS stimulation, therefore the ligand-activated AhR is required for optimal IL-10 production in TLR-stimulated macrophages (42). Moreover, intestinal macrophages in GF mice appear to have impaired key functions, such as phagocytosis and chemotaxis (43, 44). The microbiota also influences the barrier function and integrity of the intestinal epithelium by regulating the expression of antimicrobial factors on IECs, which was demonstrated in GF mice. IECs in these mice showed decreased proliferation and reduced expression of genes for antimicrobial factors (45). The acquired immunity in the gut is also strongly affected by the microbiota. GF mice have a significantly reduced number of $CD4^+$ T cells in the LP as well as a disturbed balance between Th1 and Th2 responses in the intestine. However, this imbalance is reversible by reconstitution of the gut with conventional microbiota, confirming that these microorganisms regulate Th-cell-mediated immunity (46). Development and function of Treg cells in the gut also depend on the presence of intestinal bacteria. It was shown that the microbiota promotes development of a fully functional Treg population, whereas GF mice have significantly reduced numbers of these cells in the colonic LP (47). The gut resident intestinal microbiota is a major driving force for mucosal IgA production. There are studies proving that GF animals have a reduced number of IgA-secreting plasma cells. Moreover, these cells do not appear in neonates before they have been colonized with a commensal microbiota (48).

1.1.5 Interleukin-22

IL-22 is a recently discovered cytokine possessing multifaceted and unique biological properties. It belongs to the IL-10 family and is expressed by several cell types of the innate and adaptive immune

system, e.g. various $CD4^+$ T cells (Th17 and Th22 cells), type 3 ILCs, natural killer (NK) cells and DCs (49). IL-22 is involved in many processes, i.e. cell proliferation and survival, wound healing, tissue regeneration, host defense and inflammatory reactions (50). The IL-22-receptor (IL-22R) is a heterodimer, composed of the subunits IL-22-receptor-alpha (IL-22RA1) and IL-10-receptor 2 (IL-102R). While IL-10R2 is constitutively expressed in various cells throughout the body, IL-22RA1 expression is restricted almost exclusively to epithelial cells (51). Binding of IL-22 to the receptor allows regulation of epithelial cells, endothelial cells, fibroblasts and other stromal cells' responses by immune cells. This mechanism plays a crucial role at barrier surfaces, where epithelial cells are a critical component of immune responses (52). IL-22 signaling via the receptor complex leads to phosphorylation and activation of the transcription factor *Signal transducer and activator of transcription 3* (STAT3) (53). STAT 3 activation results in expression of tissue-specific genes, including both anti-inflammatory, as well as proinflammatory cytokines, e.g. IL-10, IL-6 and IL-1β (54). This significantly contributes to the maintenance of homeostasis as well as wound healing in the intestinal epithelium. Moreover, it has been shown that via this signaling pathway, IL-22 inhibits cell apoptosis and promotes cell proliferation and regeneration. For example, IL-22 induced hepatocyte cells survival and proliferation by activating STAT3 phosphorylation *in vitro* (55). Furthermore, IL-22 induces production of AMPs (56, 57) thanks to a cross talk between commensal bacteria and AhR. Microbiota can provide AhR ligands in order to stimulate IL-22 production by ILC3, which has been shown to be impaired by lack of AhR (58). It is difficult to assign a distinct tissue protecting or damaging function to IL-22. On the one hand, IL-22 was reported to play a protective role in colitis by regulating production of mucin by goblet cells (59). Additionally, IL-22 stimulates intestinal epithelial cells to express mucus proteins (e.g. MUC1), antimicrobial proteins (e.g. Reg3γ) and cell adhesion proteins (e.g. Claudin-1), which mediate the barrier function of the epithelial layer (60). On the other hand,

IL-22 was shown to be involved in the pathogenesis of psoriatic skin inflammation (61). Several studies have shown that patients with IBD have increased IL-22 levels in the serum and in disease-related lesions in the colon (62, 63). It has also been demonstrated that local gene expression of IL-22 in the colon of mice with colitis led to amelioration of inflammation (59). Moreover, administration of IL-22 to healthy mice resulted in neutralization of lipopolysaccharide-binding protein (LPS) and alleviation of inflammation, suggesting that IL-22 has a potential to act as an anti-inflammatory molecule in the treatment of IBD patients (64).

1.2 Influence of antibiotic therapy on gut microbiota and immunity

The gut microbiota plays a fundamental role in development and differentiation of the host's fully operant immune system. For over half a century, antibiotics have become a leading effective weapon against bacterial infections. However, a widespread and constantly increasing intake of antibiotics in modern societies has probably been the major factor leading to disruption of the commensal populations in many individuals. Given its role for a functional immune system, these changes result in multiple health problems e.g. increased susceptibility to infections, allergies, and possibly other diseases (65). Most of the commonly used antibiotics have a broad spectrum of action. Thus, they impact not only pathogenic bacteria, but also the physiological microbiota. This can lead to critical consequences, since a stable composition of the gut microbiota acts as a natural barrier against invading pathogens. Consequently, antibiotic-induced dysbiosis leads to reduced colonization resistance against pathogenic bacteria, for instance enteropathogenic strains like *Clostridium difficile* (*C. difficile*) or antibiotic-resistant Enterococcus. Moreover, loss of diversity and certain important taxa, as well as alterations in metabolic capacity are common issues caused by the shift in gut microbiota composition (66, 67). *C. difficile* infection typically occurs very rarely, but it has been demonstrated that alterations of the intestinal microbiota have a strong association with preceding antibiotic treatment (68). Another important concern is antibiotic therapy in children, considering that even short-term administration in the first two years of life may trigger long-lasting disturbance of the gut microbiota and therefore impaired mutualism with the host (69). There has been a growing recognition of significant associations between inflammatory and autoimmune diseases and the exposure to antibiotics during early life, which is obviously a crucial period for development, as well as formation of the immune system and tolerance (70). Hviid et al. showed that children exposed to antibiotics in early childhood had an increased risk of developing Crohn's Disease (CD) (71). Antibiotics have a significant impact on the intestinal barrier. It has been shown that treatment with metronidazole led to increased numbers of macrophages and NK cells in the LP. Moreover, a reduced expression of the most prominent component of the mucus layer, MUC2, was observed. Reduction of mucus layer integrity resulted in accelerated attachment of the enteric pathogen *Citrobacter rodentium* (*C. rodentium*) in mice (72). The microbiota impacts host immunity by activating microorganism-associated molecular pattern
(MAMP) recognition receptors, i.e. TLRs and NOD-like receptors (NLRs). Signaling via these receptors plays an important role in the maintenance of gut homeostasis. Consequently, reduction of the amount of MAMPs caused by microbiota depletion after antibiotic therapy can inhibit regulation of innate immune responses (73). Beyond antimicrobial defenses, antibiotics can impair the activity of immune cells. Mice treated with vancomycin or ampicillin showed depletion of Th17 cells in the

intestine (74). Furthermore, administration of a combination of ampicillin, gentamicin, metronidazole, neomycin, and vancomycin resulted in decreased frequency of IFN-γ-expressing T cells in the gut (75). Various clinical observations confirm that development and course of IBD is strongly related to dysbiosis in the intestinal microbiota. Most studies demonstrated reduced diversity of the gut microbiota, but also increased instability of microbiota composition was shown in patients with IBD (76). There are several examples demonstrating how alterations in the gut microbiota may be involved in the pathophysiology of IBD. Metabolites of the commensals support epithelial barrier function and the immune system of the host. A metagenomics analysis of gut microbiota from IBD patients revealed an increase in genes involved in the oxidative stress pathway, denoting it as an origin for intestinal inflammation (77). Some patients with ulcerative colitis (UC) demonstrate an increase of sulfate-reducing bacteria (SRB), which produce hydrogen sulfide that is toxic to the epithelial cells, thereby causing mucosal inflammation (78). Furthermore, it has been shown that in IBD patients, the number of bacteria in the mucus layer is elevated, implying defective mucosal barrier function (79). However, it is still unclear whether dysbiosis is a cause or a consequence of intestinal inflammation in IBD.

1.3 Inflammatory bowel disease (IBD)

IBD, including CD and UC, is a chronic-relapsing disorder of the GI tract, for which an increasing incidence is observed worldwide. Although these diseases have low mortality, the affected patients suffer greatly from diarrhea, weight loss, chronic pain and reoccurring bleeding incidents. Moreover, these patients have an increased risk of developing of colorectal cancer (80, 81). CD and UC are two main manifestation forms of IBD and can be distinguished by significant differences in clinical symptoms. While the tissue inflammation in UC is limited to the outer mucosa of the colon, in case of CD, inflammation can affect the entire GI tract, as well as all tissue layers. Moreover, in both cases, additional manifestations, mainly located in eyes, skin, liver and joints can also occur (82, 83).

The clinical symptoms of both diseases have been described extensively, however the pathophysiology of the inflammatory reaction have been only partially understood so far. Apart from genetic predisposition and environmental factors, an inappropriate immune response to the commensal flora is discussed as a major driving factor (84, 85). Approximately 1.4 millions of Americans are currently affected by IBD and a significant increase of incidence has been observed over the past years. Furthermore, several studies have shown, that immigrants originating from

regions with low occurrence of IBD, gain an increased risk of developing the disease after settling in countries with higher prevalence, especially among first-generation children (86, 87). Thus, there is some evidence indicating a critical role of environmental factors in development of the disease.

Next-generation sequencing (NGS) technologies allowed comprehensive investigation of genetic features of the host conditioning increased susceptibility to IBD. Several studies demonstrated, that some of IBD-associated genes are involved in maintenance of intestinal barrier, activation of mucosal immune response as well as detection of microorganisms (88). For instance, mutations of innate immune receptor nucleotide oligomerization domain containing 2 (NOD2) were the first ones associated with IBD. NOD2 is a general sensor of microbial cell wall component muramyl dipeptide present on all Gram-negative and Gram-positive bacteria and therefore essential for detection of these microorganisms (89). Furthermore, mutations of IL-23 receptor (IL-23R) are also commonly found in patients with CD and UC. These lead to impaired signal transmission by proinflammatory cytokine IL-23 and therefore disturbed activation of T cells and macrophages in the intestinal mucosa (90).

It seems that the combination of genetic and environmental factors triggers modifications in epithelial barrier function, which facilitate inflow of luminal antigens into the gut wall, followed by an excessive and inappropriate immune response, promoted by mucosal immune cells, such as T cells, macrophages and type 3 ILCs (84).

1.4 The immune response to *Salmonella enterica* infection

Salmonella enterica (*S. enterica*) is a facultative intracellular pathogen found in the intestinal tract of various host species. These Gram-negative bacteria are causative agents of infections with various symptoms, ranging from self-limiting gastroenteritis to typhoid fever (91). Salmonellae are usually consumed with contaminated water or food. This pathogen can use several different mechanisms to enter the intestinal mucosa. A main route of entry is through M cells, which transport antigens from the lumen to the LP. *Salmonella* can also actively invade enterocytes using its virulence-associated type-3 secretion system encoded by *Salmonella* pathogenicity Island 1 (SPI-1) (92). Finally, invasion occurs through resident DCs that extend protrusions into the gut lumen to sample luminal antigens. *Salmonella* penetration triggers disruption of the mucosal barrier, thus facilitating entry of additional bacteria via enterocytes (93). Initial interaction of the pathogen with epithelial cells induces the early immune response in PPs and MLNs, which sparks a massive inflammatory reaction featuring neutrophils, DCs, inflammatory monocytes and macrophages. Enterocytes and phagocytes are able

to detect various PAMPs, e.g. LPS and flagellin expressed by *Salmonella*, via TLRs. As a response to recognition of PAMPs, DCs increase the expression of MHC II and costimulatory molecules, which allows efficient antigen presentation to naïve $CD4^+$ T cells. This way, DCs contribute to a necessary induction of adaptive immunity (94). There are several studies that demonstrate contribution of different innate cells to the restriction of early *Salmonella* infection. It has been shown that HIV patients with decreased numbers of neutrophils are more susceptible to systemic *Salmonella* infection (95). In PPs and MLNs of *Salmonella*-infected mice, CC-chemokine ligand 2 (CCL2), C-X-C motif ligand 2 (CXCL2), and CXCL9 which are involved in recruiting myeloid cells were found to be upregulated. Inflammatory monocytes are immediately accumulated in those tissue structures and produce anti-microbial factors, such as inducible Nitric Oxide Synthase (iNOS), TNF-α, and IL-1β (96). Development of a robust protective immune reaction against *S. enterica* involves both T and B cells (97, 98). $CD4^+$ T cells play a crucial role in clearing primary infection. Moreover, they are essential to establish acquired resistance to secondary infection (99). Specific T cells become activated in PPs and MLNs and rapidly acquire Th1 effector functions. They are a prominent source of IFN-γ that further stimulates infected macrophages (100). Bacterial clearance requires development of Th1 cells, which is confirmed by the fact that mice lacking IL-12, T-bet, IFN-γ or IFN-γR are unable to resolve *Salmonella* infection (101–103). On the other hand, there are indicators suggesting an important protective role of Th17 cells, which produce IL-17 and IL-22 in the intestinal mucosa in response to *Salmonella* invasion (104). However, Schulz et al. presented results indicating dispensable role of Th17 cells in clearance of *Salmonella* Enteritidis infection, as long as an effective Th1 response is provided. Their study in IL-12 deficient mice showed an association of IL-22, but not IL-17 activity with response to systemic *Salmonella* infection in the absence of IL-12 (Schulz et al. 2008). An effective immune response against *Salmonella* infection also relies on B lymphocyte activity and production of specific antibodies. Even though *Salmonellae* are usually tightly associated to phagocytic cells, there is a short period of time after apoptosis of those infected cells, where opsonization of bacteria with antibodies can occur, thus preventing spread of the infection (102). Antibodies seem to play a vital role in the presentation of *Salmonella* antigens to $CD4^+$ T
cells and consequently in the development of a Th1 response (105). Beyond a systemic antibody response, induced production of immunoglobulins takes place at the mucosal site. sIgA, with its capacity to agglutinate the pathogens in the lumen, is a prominent factor in protecting the intestinal barrier against pathogen invasion. It was shown that oral administration of specific IgA antibodies protected mice from *S.* Typhimurium infection in the gut (106). Moreover, it was shown that *S. enterica* triggers a sIgA response directed primarily against the O-antigen component of LPS (107).

1.5 Mouse models of intestinal inflammation and the streptomycin model of *Salmonella* infection

There is a wide range of animal models, mostly mouse models, designed to investigate intestinal inflammation. Despite some disadvantages, mice share multiple specific intestinal genes with humans. Similarities between microbiota, immune response and anatomical structure allowed the murine model to become the prominent choice when considering studies of intestinal injury (108). Inflammatory disease in the gut is a highly complex issue, therefore utilization of an appropriate model is necessary. There are currently over 50 animal models of IBD available, and they can be categorized based on the causative mechanism of inflammation (109). Among chemically-induced models, the dextran sulphate sodium (DSS) model is the most favored one. It's relatively simple and cheap, nevertheless revealed important aspects of IBD pathogenesis (110). Furthermore, there are genetically engineered models that employ transgenic animals, e.g. STAT4 transgenic and HLA-B27/β2-microglobulin transgenic mice, or knockout (KO) mice, e.g. *IL-10*-KO and *IL-2*-KO mice. These models are well established and provided new insights into IBD therapy, e.g. anti-Th1-targeted therapies, such as anti-IFN-γ antibodies (111). There are also T cell transfer models available, among which the $CD4^{+}CD45Rb^{high}$ transfer model is the most commonly used. It has been very well described and highlighted Th1 and Th17 cells as key players in gut inflammation (112). Lastly, several spontaneous models, where mutation causes chronic gut inflammation (e.g. the C3H/HeJBir mouse model) were established (113). Taken together, all these approaches reflect certain aspects of colitis very well, but their potential to study pathophysiology of the inflammatory reaction as well as contribution of the gut microbiota is limited.

Recently, some robust and reproducible bacteria-induced models of colitis have been described. In particular, two mouse models, i.e. a *C. rodentium* infection model and a model of *S. enterica* infection in streptomycin pretreated mice, are of great importance. In both mouse models, the acute inflammatory response in the intestinal mucosa is triggered by a well-characterized pathogen. These mucosal infection models allow investigation of the mechanisms associated with colonization resistance. Moreover, they are excellent tools for studying function of the gut mucosal system, as well as the importance of gut homeostasis. Thus, these models provide great input into unravelling the nature of inflammatory reactions in the intestine (114).

Since its first introduction, the *S. enterica* infection model has become a great tool to investigate innate and adaptive mucosal defense mechanisms involved in the development and course of intestinal disease (101, 115). Streptomycin is a broad-spectrum antibiotic that particularly suppresses

the growth of gram-negative bacteria (116). It has been confirmed by several studies that streptomycin treatment prior to *S. enterica* infection eliminates the majority of the intestinal microbiota. This allows the pathogen to overcome the host's resistance, colonize the mucosa and trigger an inflammatory reaction (117–120). Therefore, this model has been shown to be principally useful in studying early phases of intestinal *Salmonella* infection, e.g. interactions between host and microbiota during development of inflammatory reaction (121), description of mechanisms responsible for compromised resistance against pathogenic bacteria (122), or analysis of bacterial virulence mechanisms involved in the development of intestinal inflammation (123).

1.6 Aim of the study

The incidence and prevalence of chronic inflammatory bowel diseases are rising worldwide. Even though these disorders show a relatively low mortality, the patients suffer severely from pain, cramps, and bloody diarrhea (124). The pathogenesis of the inflammatory reaction in the intestine seems to be multifactorial, in particular genetic predisposition, environmental factors as well as an inappropriate immune response are discussed as main causes (125). Recently, the role of the microbiota and pathogenic bacteria in disturbance of intestinal immunity has been highlighted (126). Combination of several mechanisms seems to affect the permeability of the intestinal epithelium, leading to invasion of commensals and pathogens into the gut mucosa. This triggers an unrestricted immune response causing further destruction of the epithelial barrier. The constant influx of antigens sustains the immune reaction and results in chronic inflammation (82). GF mice do not develop clinical symptoms of intestinal disease, which confirms contribution of bacteria in the development of IBD. Moreover, in addition to the microbiota, pathogenic microorganisms are discussed as causative agents (126). For instance, infection of the intestine with *Yersinia enterocolitica* or *Mycobacterium avium ssp. paratuberculosis* triggers acute relapse of the disease in CD patients (127, 128). Furthermore, clinical symptoms of *Shigella spp.* or *Campylobacter spp.* infection greatly resemble the pathology of UC (129). Also, involvement of *Salmonella enterica* in the pathogenesis of IBD has been discussed (130). Even though, there are multiple references indicating contribution of bacteria, until now there are still no fully reliable animal models available that allow investigation of bacteria-driven inflammation, especially in the chronic phase. The most commonly used chemically induced model, i.e. DSS colitis, demonstrates the destruction of mucosal barrier in the gut as a consequence of administration of a chemical agent. This model can probably reflect the initial phase

of IBD, however its ability to illustrate natural mechanisms, which cause onset of inflammatory reaction in the gut, as well as development of the chronic phase of the disease is limited (131).

Therefore, the aim of this study was to establish a bacteria-induced mouse model, which allows studying the role of commensal versus pathogenic bacteria in the development of chronic intestinal inflammation. A previously proposed model of *S. enterica* infection preceded by streptomycin administration has mainly delivered data considering the acute phase of infection (132). In contrast, the mouse model developed in the present work focused on analyzing the chronic inflammatory reaction caused by S. *enterica* infection. Moreover, differences between the acute and chronic phase of infection should be analyzed. Additionally, it should be investigated whether treatment with antibiotics, i.e. streptomycin, changed the course of the systemic or mucosal immune response against *S. enterica.*

Once the mouse model of chronic *S. enterica*-induced colitis is established, a special focus of this work was to investigate the role of IL-22 in this model since increased expression of this cytokine has been observed in colonic tissue of patients with IBD (63). In summary, this study aimed to provide significant input on the role of the microbiota and pathogens, as well as antibiotic therapy in the development and course of chronic inflammatory reactions in the gut.

2 Materials and Methods

2.1 Materials

2.1.1 Tools

Table 1: Used tools

Tool	Source
20G Oral Dosing Needle 38 mm CVD	Veterinary Instrumentation Limited; Sheffield, UK
BD Microtainer SST™ Tubes	Becton, Dickinson and Company; New Jersey, USA
Cell strainers 100 µm	VWR International GmbH; Dresden, Germany
Cover slips 24x60 mm	VWR International GmbH; Darmstadt, Germany
Dako Pen	Dako Deutschland GmbH; Hamburg, Germany
Disposable Drigalski spatulas made from ABS	VWR International GmbH; Dresden, Germany
Disposable needles STERICAN® ø 0.45 x 12mm, 26G x 1/2	B. Braun Melsungen AG; Melsungen, Germany
Disposable syringes NORM-JECT 1 ml	Becton, Dickinson and Company; New Jersey, USA
Embedding cassettes- green for biopsies	neoLab Migge Laborbedarf-Vertriebs GmbH; Heidelberg, Germany
Eppendorf-tubes (0.5 - 2 ml)	Eppendorf AG; Hamburg, Germany
Falcon™ Tubes (15 ml, 50 ml)	Greiner Bio-One GmbH; Frickenhausen, Germany
Microtiter plates: PolySorp™ F96-Plates	Thermo Scientific; Schwerte, Germany
Microtome blades	Leica Biosystems Nussloch GmbH; Nussloch, Germany
Nitrile disposable gloves	Ansell; Richmond, Australia

Microscope slides Superfrost Ultra-Plus	Gerhard Menzel GmbH; Braunschweig, Germany
Pipettes, Pipetman (1-1000 µl)	Greiner Bio-One GmbH; Frickenhausen, Germany
Pipette tips (10-1000 µl)	Greiner Bio-One GmbH; Frickenhausen, Germany
Rotilabo Alufoil	Carl Roth GmbH&Co. KG; Karlsruhe, Germany
Seals for microtiter plates	Laborfachhandel Schubert; Leipzig, Germany
Sterile preparation tools	Various manufacturers
XLD-Agar plates	VWR International GmbH; Dresden, Germany
Hand dispenser Multipette® plus	Eppendorf AG; Wesseling-Berzdorf, Germany

2.1.2 Chemicals and reagents

Table 2: Used chemicals and reagents

Chemical	Source
2-Propanol ≥ 99.5 %	Carl Roth GmbH&Co. KG; Karlsruhe, Germany
3,3′,5,5′-Tetramethylbenzidine (TMB)	Moss, Inc., Pasadena, USA
Acetic acid ≥ 99.5 %	Carl Roth GmbH&Co. KG; Karlsruhe, Germany
Acid sulfur (96%) (H_2SO_4)	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Activated carbon	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Calciumchloride ($CaCl_2$) ≥ 30 %	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Casein	Sigma-Aldrich; Steinheim, Germany

Citric acid monohydrate (C6H8O7 x H2O) ≥ 99.5 %	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
De-ionised water	in-house production
Disodium hydrogen phosphate dodecahydrate (Na_2HPO_4 x 12 H2O)	Sigma-Aldrich; Steinheim, Germany
Entellan®	Merck KGaA; Darmstadt, Germany
Eosin Y	Sigma-Aldrich; Steinheim, Germany
Ethanol ≥ 99.8 % denaturated	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Ethylene diamine tetra-acetic acid (EDTA) ≥ 99 %	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
ExtrAvidin-Cy3 (Streptavidin-Cy3 conjugate)	Sigma-Aldrich; Steinheim, Germany
Fetal calf serum (FCS)	Biochrom AG; Berlin, Germany
Fluoroshield™ with DAPI	Sigma-Aldrich; Steinheim, Germany
Hem alum solution acid acc. to Mayer	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Hydrochloric acid, 1 M	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Hydrochloric acid, concentrated	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
N,N-dimethylformamide (C_3H_7NO)	Sigma-Aldrich; Steinheim, Germany
Paraffin type 3	Thermo Scientific; Schwerte, Germany
Paraffin type 6	Thermo Scientific; Schwerte, Germany
Pararosaniline	Sigma-Aldrich; Steinheim, Germany
Periodic acid (H_5IO_6)	Merck KGaA; Darmstadt, Germany
Potassium chloride (KCl)	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Potassium dihydrogen phosphate (KH_2PO_4)	Carl Roth GmbH + Co. KG, Karlsruhe, Germany

Proteinase K	Qiagen GmbH; Hilden, Germany
Roti®-Histofix 4 %	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Sodium bicarbonate ($NaCO_3$)	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Sodium bisulfate ($Na_2S_2O_5$)	Bernd Kraft GmbH; Duisburg, Germany
Sodium chloride (NaCl)	Th. Geyer GmbH&Co. KG; Renningen, Germany
Sodium citrate dihydrate ($C_6H_5Na_3O_7$ x 2 H_2O)	Sigma-Aldrich; Steinheim, Germany
Sodium hydroxide (NaOH)	AppliChem GmbH; Darmstadt, Germany
Streptavidin-HRP conjugate	GE Healthcare UK Limited; Buckinghamshire, UK
Streptomycin sulfate	Sigma-Aldrich; Steinheim, Germany
Tris(hydroxymethyl)-aminomethane (TRIS) ≥ 99.9 %	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Triton X-100	Sigma-Aldrich; Steinheim, Germany
Tween 20	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Xylol ≥ 98 %	Carl Roth GmbH + Co. KG, Karlsruhe, Germany

2.1.3 Buffers and solutions

Table 3: Used buffers and solutions

Name	Composition
3 % $NaHCO_3$-PBS solution	• 357 mM $NaHCO_3$ • PBS (sterile)
Casein blocking buffer	• 0.5 % Casein • PBS
Citrate buffer pH=6 adjusted with HCl or NaOH	• 5 mM $C_6H_8O_7$ x H_2O • 5 mM $C_6H_5Na_3O_7$ x 2 H_2O • 0.05 % Tween 20 • Aqua bidest.
Eosin solution	• 1 g Eosin Y • 1.7 mM acetic acid
PBS (10x) pH=7.4 (w/o Ca^{2+}/Mg^{2+})	• 27 mM KCl • 65 mM Na_2HPO_4 x 12 H_2O • 15 mM KH_2PO_4 • 1.5 M NaCl • Aqua bidest.
PBS-T	• 0.05% Tween 20 • PBS
Protein-blocking buffer	• 1% FCS • PBS-T
Schiff's reagent	acc. to the European Pharmacopoeia (monography H-Z 2014)
Streptomycin solution	• 137 mM Streptomycin sulfate • Aqua bidest. (autoclaved)
TRIS-EDTA-$CaCl_2$ buffer pH=8 adjusted with HCl or NaOH	• 50 mM TRIS • 1 mM EDTA • 5 mM $CaCl_2$ • 0.5 % Triton X-100 • Aqua bidest.
TRIS-EDTA-Puffer pH=9 adjusted with HCl or NaOH	• 10 mM TRIS • 1 mM EDTA • 0.05 % Tween 20 • Aqua bidest.

2.1.4 Antibodies

Table 4: Used unconjugated primary antibodies for Immunohistochemistry

Antigen	Original species	Used concentration	Source
CD3	Rabbit (polyclonal)	1.2 µg/ml	Dako Denmark A/S; Glostrup, Denmark
Claudin-1	Rabbit (polyclonal)	1 µg/ml	LSBio; Seattle, WA, USA
IFN-γ	Rabbit (polyclonal)	1 µg/ml	Biorbyt Ltd.; Cambridge, UK
IgA	Goat (polyclonal)	0.2 µg/ml	Southern Biotech; Birmingham AL, USA
IL-22	Rabbit (polyclonal)	5 mg/ml	Abcam plc; Cambridge, UK
RORγt	Mouse (monoclonal)	5 µg/ml	BD Bioscience; San Diego, CA, USA

Table 5: Used secondary antibodies for Immunohistochemistry

Target protein	Original species	Labeling	Used concentration	Source
Goat IgG	Rabbit	Biotin	3 µg/ml	Jackson ImmunoResearch Europe Ltd; Suffolk, UK
Rabbit IgG	Goat	Biotin	2 µg/ml	Life Technologies/ Thermo Fisher Scientific; Waltham, MA, USA
Mouse IgG	Goat	Biotin	2.8 µg/ml	Jackson ImmunoResearch Europe Ltd; Suffolk, UK
Rat IgG	Donkey	Biotin	1.6 µg/ml	Jackson ImmunoResearch Europe Ltd; Suffolk, UK

Table 6: Used detection antibodies for ELISA

Antigen	Original species	Labeling	Used concentration	Source
Mouse IgA	Goat (polyclonal)	horseradish peroxidase	0.4 µg/ml	SouthernBiotech; Birmingham, AL, USA
Mouse IgG1	Goat (polyclonal)	horseradish peroxidase	0.4 µg/ml	SouthernBiotech; Birmingham, AL,USA
Mouse IgG2c	Goat (polyclonal)	horseradish peroxidase	0.4 µg/ml	SouthernBiotech; Birmingham, AL, USA
Mouse IgM	Goat (polyclonal)	horseradish peroxidase	0.4 µg/ml	SouthernBiotech; Birmingham, AL, USA

2.1.5 Devices

Table 7: Used devices

Name	Source
Analytical scale CPA 324 S	Sartorius AG; Göttingen, Germany
AxioScan.Z1	Carl Zeiss Jena GmbH; Jena, Germany
Clean bench Herasafe™ KS (NSF) Class II	Thermo Scientific; Schwerte, Germany
Cooling plate COP30	Medite; Burgdorf, Germany
Euthanasia box	Agnthos AB; Lidingö, Sweden
High-purity water facility	Thermo Scientific; Schwerte, Germany
Microplate reader TECAN Safire2TM	Tecan Group Ltd.; Männedorf, Switzerland
Microplate washer TECAN 96 Plate Washer™	Tecan Group Ltd.; Männedorf, Switzerland
Paraffin-embedding station Leica EG1150H	Leica Biosystems Nussloch GmbH; Nussloch, Germany
pH-Meter	SI-Analytics GmbH; Mainz, Germany

Precision scale Extend	Sartorius AG; Göttingen, Germany
Refrigerated centrifuge 5427 R	Eppendorf; Wesseling-Berzdorf, Germany
Rotary microtome Leica RM2255	Leica Biosystems Nussloch GmbH; Nussloch, Germany
Staining module Leica ST5020	Leica Biosystems Nussloch GmbH; Nussloch, Germany
Table centrifuge Sprout Mini-Centrifuge	Heathrow Scientific LLC; Vernon Hills, IL, USA
Tissue processor Leica TP1020	Leica Biosystems Nussloch GmbH; Nussloch, Germany
Vortex Genie 2 Mixer	OMNILAB-LABORZENTRUM GmbH & Co. KG; Bremen, Germany
Water bath	GFL GmbH; Burgwedel, Germany
Water-bath-flattening table combo	MDS GmbH; Buseck, Germany

2.1.6 Animals

Table 8: Used animals

Animals	Strain	Sex	Source
Mice	C57BL/6JRj	Female	Janvier; Saint-Berthevin, France
Mice	C57BL6/JOlaHsd	Female	Ludwig Institute for Cancer Research; Brussels, Belgium
Mice	C57BL6/JOlaHsd-*IL22* knock-out	Female	Ludwig Institute for Cancer Research; Brussels, Belgium

2.1.7 Software

Table 9: Used software

Method	Software (source)
Picture analysis	ImageJ 1.46r (Wayne Rasband, National Institutes of Health; Bethesda, MD, USA)
Picture acquisition	Zen2 (Carl Zeiss Jena GmbH; Jena, Germany)
Graphic work and Statistics	GraphPad Prism 5 (GraphPad Software Inc.; La Jolla, CA,USA)

2.2 Methods

2.2.1 *In vivo* experiments

All experimental procedures using mice were approved by the State Animal Care and Use Committee (Landesdirektion Sachsen, Leipzig, Germany, TVV 56/13) and were carried out in accordance with the European Communities Council Directive (86/609/EEC) for the Care and Use of Laboratory Animals. All efforts were made to minimize suffering of the animals. Animals were kept at the Centre for Experimental Medicine (TEZ) at Fraunhofer Institute for Cell Therapy and Immunology in Leipzig. Mice were housed as six animals per cage in a temperature- and light/dark cycle-controlled environment (23 °C, 12 h/12 h light/dark, 50 % humidity). They had free access to pelleted standard rodent chow and water *ad libitum*. Animals were acclimatized to the environmental conditions for 14 days before starting the experiment.

2.2.1.1 Induction of *S. enterica*-colitis in wild-type animals

Female C57BL/6JRj mice (10 weeks old; 20-24 g; n=48) were divided into 8 groups. Selected groups received a single application of streptomycin at a dose of 20 mg per mouse 24 h prior to infection. To prepare a streptomycin solution, 800 mg of streptomycin powder was dissolved in 8 ml of autoclaved water to obtain concentration of 100 mg/ml. Mice were administered 200 µl of streptomycin solution orally per mouse. Some groups received oral application of autoclaved water only.

Certain groups of mice were inoculated with 10^8 colony forming units (cfu) of low-virulent *S. enterica* serovar Enteritidis (SE; 64/03; ade^+/his^+), using an oral dosing needle. To prepare a bacteria solution, a stock solution stored at -150 °C containing $7x10^9$ cfu was thawed and centrifuged at 4000 x g, 4 °C for 5 min. The supernatant was discarded and bacteria pellet was washed with sterile 3 % $NaHCO_3$-PBS solution. After repeating the centrifugation process, the pellet was resuspended in 14 ml of sterile 3 % $NaHCO_3$-PBS solution and stored on ice. For the control of viability of used *S. enterica* stock, part of the prepared bacteria solution was applied on selective xylose lysine deoxycholate (XLD) agar plates and incubated for 24 h at 37 °C, 5 % CO_2. Afterwards, grown colonies were counted. 200 µl ($1x10^8$ cfu) of the 3 % $NaHCO_3$-PBS bacteria solution was used for the oral inoculation per mouse. Control groups received oral application of the sterile 3 % $NaHCO_3$-PBS solution.

Figure 1 represents a design and course of the experiment.

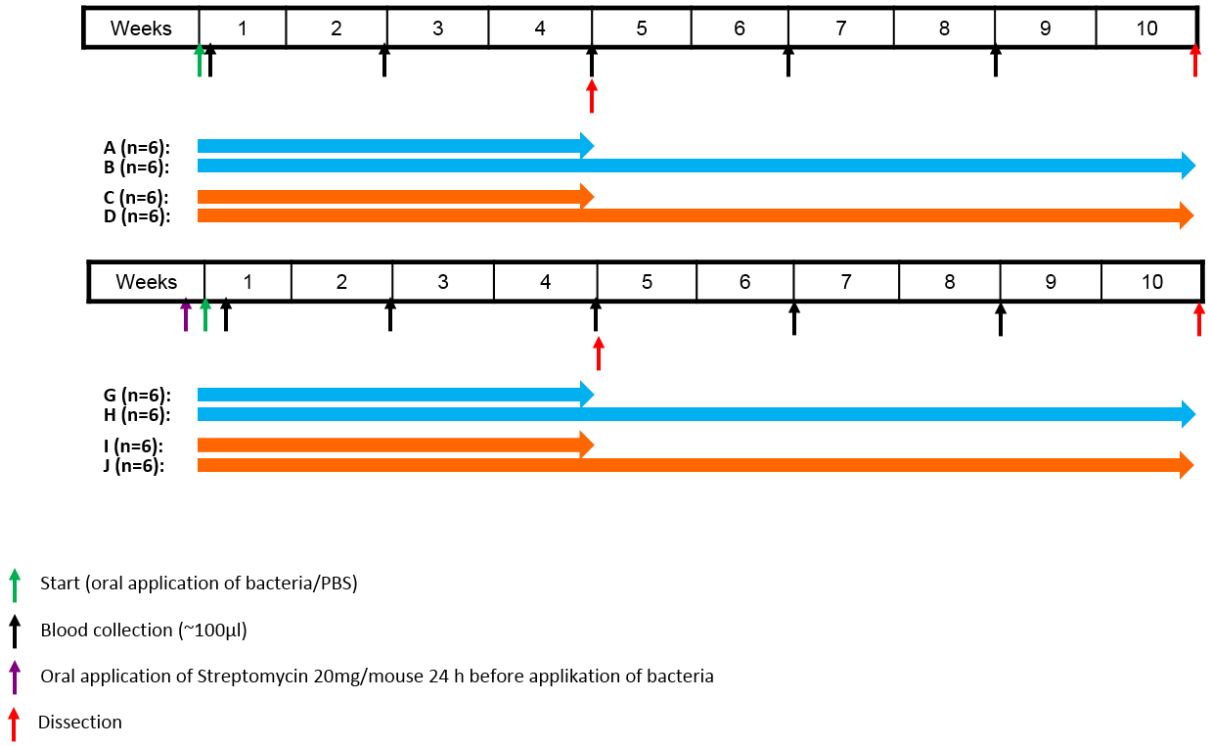

Figure 1: General design of the *in vivo* experiment using WT C57BL/6JRj mice.

2.2.1.2 Induction of *S. enterica*-colitis in IL-22-deficient animals

Female C57BL6/JOlaHsd mice (10 weeks old; n=18) and female C57BL6/JOlaHsd *IL-22* knock out mice (10 weeks old; n=18) were divided into 8 groups. Selected groups received a single application of streptomycin at a dose of 20 mg per mouse 24 h prior to infection. Streptomycin solution was prepared as described above (2.2.1.1). Mice were administered 200 µl of streptomycin solution orally per mouse. Some groups received oral application of autoclaved water only. Certain groups of mice were inoculated with 10^8 cfu of low-virulent *S. enterica* serovar Enteritidis (SE; 64/03; ade+/his+), using an oral dosing needle. Bacteria solution was prepared as described above (2.2.1.1). 200 µl ($1x10^8$ cfu) of the 3 % $NaHCO_3$-PBS bacteria solution was used for the oral inoculation per mouse. Control groups received oral application of the sterile 3 % $NaHCO_3$-PBS solution.

Figure 2 represent a design and course of the experiment.

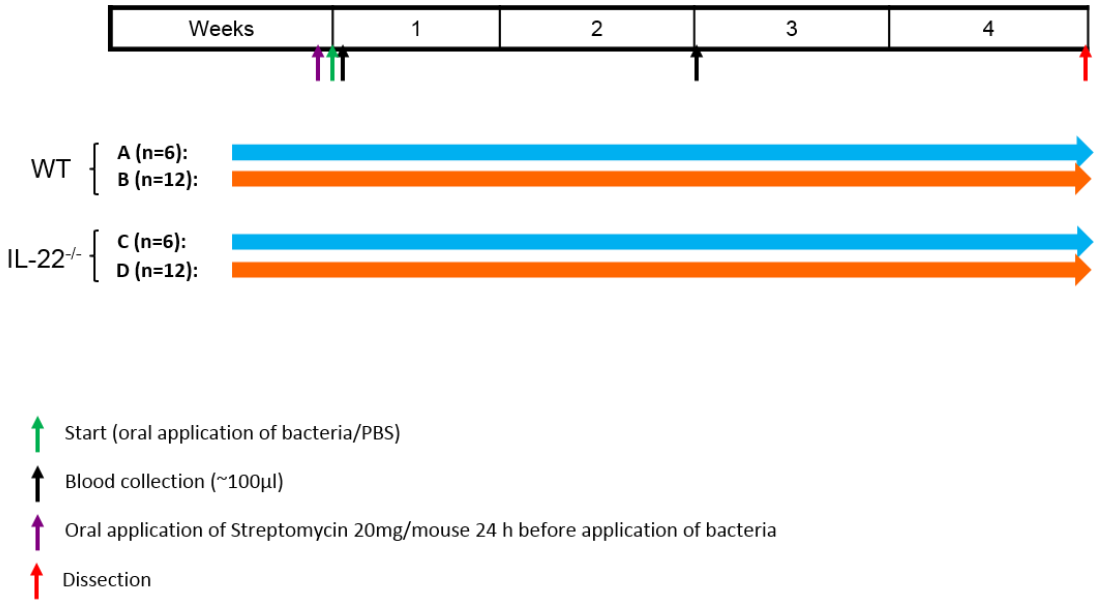

Figure 2: General design of the *in vivo* experiment using IL-22 deficient C57BL6/JOlaHsd mice.

2.2.2 Observation of clinical symptoms of colitis and determination of the clinical score

Animals were inspected daily for overall appearance. Body weight loss score as well as a score for stool consistency were assessed. Scoring systems are shown in tables 10 and 11. The clinical score was calculated as the average of the scores for both factors.

Table 10: Scoring system for weight loss

Weight loss in comparison to starting weight [%]	Points
0	0
< 2	0.4
2 – 4	0.8
4 – 6	1.2
6 – 8	1.6
8 – 10	2.0
10 – 12	2.4
12 – 14	2.8
14 – 16	3.2
16 – 18	3.6
18 – 20	4.0
> 20	5

Table 11: Scoring system for stool consistency

Stool consistency	Points
normal	0
soft, but still formed pellet	0.4
very soft, no pellet formed	0.8
diarrhea	1.2

2.2.3 Dissection and collection of samples

Starting 24 h post infection (p.i.), blood samples were collected from mice every two weeks. Blood was taken from the sublingual vain and transferred into BD Microtainer SST™ tubes. Serum was prepared by 30-60 min incubation at room temperature (RT) followed by centrifugation at 1800 x g for 10 min. Sera were transferred into 0.5 ml tubes and stored at -80 °C until analysis.

On days 29 and 71 p.i., accordingly, animals were sacrificed using carbon dioxide (following Society of Laboratory Animal Science (GV-SOLAS) directions). Afterwards, animals were

aseptically dissected. Blood samples were collected by cardiac puncture, transferred into BD Microtainer SST™ tubes and processed as described above. Two parts of the most distal and proximal colon were taken for histopathological analysis and transferred into 2-ml tubes containing Roti® Histofix 4 %, which is a formaldehyde solution fixing the tissue.

2.2.4 Histopathological analysis

2.2.4.1 Preparation of the colon tissue

Dehydration of the tissue

Tissue samples were fixed in Roti®-Histofix 4 % for 24 h at 4 °C and afterwards transferred into embedding cassettes. To remove the rests of formalin solution, cassettes were washed with distilled water for several times and located in the tissue processor Leica TP 1020. The samples ran through a dehydration process, including upgraded series of alcohols, followed by incubation in xylol and paraffin type 3. The course of dehydration process is shown in the table 12.

Table 12: Dehydration process of the colon tissue

Step	Chemicals / Reagents	Time [min]
Upgraded series of alcohols	Ethanol 70 %	60
	Ethanol 70 %	90
	Ethanol 80 %	90
	Ethanol 96 %	60
	Ethanol 96 %	90
	Ethanol 100 %	90
	Ethanol 100 %	90
	Isopropanol	90
	Xylol	90
	Xylol	120
Paraffin incubation	Paraffin type 3	120
	Paraffin type 3	180

Embedding of tissue samples in paraffin blocks

Embedding station Leica EG1150H was used in order to embed colon tissue samples in paraffin blocks. Cassettes containing tissue samples were transferred from the fluid paraffin into a heated chamber. Paraffin type 6 was melted at 70 °C and filled into metal forms, where tissue samples were aligned afterwards using preheated forceps. Metal forms were moved on a cooling plate (-14 °C) to harden the paraffin blocks immediately. After 10 min, the blocks were removed from the metal forms.

Production of tissue sections

Previously prepared paraffin blocks were cooled at a -14 °C plate. Using a rotary microtome Leica RM2255, 3-µm thick sections from various regions of the tissue were cut and transferred into water bath (50 °C). Sections were mounted on microscope slides and dried on a heating plate (70 °C) in order to remove rests of water.

2.2.4.2 Histochemical stainings

Haematoxylin and eosin (H&E) staining / histopathological score

The H&E staining was used to get a general overview of pathomorphology in the colon tissue. Haematoxylin is a basic dye, which binds to negatively charged regions, e.g. phosphate groups of DNA in the cell nucleus. Blue staining is created after change of pH to slightly basic by incubation in tap water. The synthetic acidic dye Eosin which binds to plasma proteins and stains them red is used afterwards as counterstain. The H&E staining was realized using staining module Leica ST5020 and single steps are presented in the table 13. Downgraded series of alcohol allowed removal of paraffin and rehydration of tissue sections. After H&E staining procedure, slides were dehydrated in upgraded series of ethanol and after incubation in xylol, covered with Entellan®.

Table 13: Process of the H&E staining in Leica ST 5020 staining module

Step	Chemicals / Reagents	Time
Downgraded series of alcohols	Xylol	5 min
	Xylol	5 min
	Ethanol 100 %	5 min
	Ethanol 90 %	5 min
	Ethanol 80 %	5 min
	Ethanol 70 %	5 min
	Ethanol 50 %	5 min
	high-purity water	≥ 5 min
H&E staining	Hem alum solution acid acc. to Mayer, filtered	30 sec
	tap water, flowing	10 min
	Eosin Y solution, filtered	2 min
Upgraded series of alcohols	Ethanol 70 %	shortly rinse
	Ethanol 90 %	shortly rinse
	Ethanol 100 %	30 sec
	Xylol	≥ 1 min

Pictures of stained sections were taken using a slide scanner AxioScan.Z1. Histopathological score was determined after stained sections were evaluated by a trained pathologist (Dr. Klaus Weber; AnaPath GmbH, Switzerland) with regard to the extent of inflammation and fibrosis, as well as the infiltration of immune cells into the tissue. Considered criteria are presented in table 14. The histopathological score was calculated as a mean value of assigned grades.

Table 14: Histopathological score

Category	Grade	Criteria
Granulocytes / HPF (20x)	1	10/HPF
	2	20/HPF
	3	50/HPF
	4	big, multi-focal infiltration
	5	Infiltration with a dense presence of granulocytes
Lymphocytes / HPF (20x)	1	10/HPF
	2	20/HPF
	3	50/HPF
	4	big, multi-focal infiltration, affecting up to 50 % of the analyzed tissue surface
	5	dense infiltration of lymphocytes, the whole tissue affected
Macrophages / HPF (20x)	1	10/HPF
	2	20/HPF
	3	50/HPF
	4	big, multi-focal infiltration, affecting up to 50 % of the analyzed tissue surface
	5	dense infiltration of macrophages, the whole tissue affected
Fibrosis (mucosa)	1	focal lesion
	2	several focal lesions
	3	up to 50 % of tissue replaced
	4	up to 80 % of tissue replaced
	5	100 % of tissue replaced
Fibrosis (submucosa)	1	focal lesion
	2	several focal lesions
	3	up to 50 % of tissue replaced
	4	up to 80 % of tissue replaced
	5	100 % of tissue replaced

Periodic acid-Schiff (PAS) staining and quantification

PAS reaction was used in order to visualize mucus and mucus-producing goblet cells in the colon tissue. Periodic acid is a strong oxidant, which oxidates 1,2-glycoles into aldehydes. These bind afterwards to fuchsin sulphurous acids contained in Schiff reagent and stain therefore mucus glycoproteins in magenta purple colour. Staining module Leica ST5020 was used to process the tissues through downgraded series of alcohols. Afterwards, tissues were transferred into glass cuvettes and stained following the steps shown in table 15. After the counterstaining with haematoxylin, tissue sections were dehydrated in the staining module and covered with Entellan®. Pictures of stained colon sections were taken using the slide scanner AxioScan.Z1

Table 15: Process of the PAS staining in Leica ST 5020 staining module / glass cuvettes

Step	Chemicals / Reagents	Time
Downgraded series of alcohols	Xylol	5 min
	Xylol	5 min
	Ethanol 100 %	5 min
	Ethanol 90 %	5 min
	Ethanol 80 %	5 min
	Ethanol 70 %	5 min
	Ethanol 50 %	5 min
	high-purity water	≥ 5 min
PAS reaction	periodic acid solution 1%	10 min
	tap water	3 min flowing 1.5 min standing
	Schiff's reagent	15 min
	lukewarm tap water	5 min
Counterstaining	Hem alum solution acid acc. to Mayer, filtered	1 min
	flowing tap water	until water is clean
	tap water	10 min
Upgraded series of alcohols	Ethanol 70 %	2 min
	Ethanol 90 %	2 min
	Ethanol 96 %	2 min
	Ethanol 100 %	5 min
	Isopropanol	5 min
	Xylol	≥ 5 min

ImageJ™ software was used to quantify the PAS staining. Software tool “Color deconvolution” allowed color dispersion on the tissue section and the violet canal was used for the PAS-stained regions. The analysis region was defined by the edge of the outer muscle layer of the colon cross section. The intensity of the colored area was related to the total area of the analyzed region.

2.2.4.3 Immunohistochemical (IHC) staining

For immunofluorescent staining of specific cell markers, i.e. surface markers, transcription factors and epithelial markers, an indirect immunohistochemical (IHC) staining was used.
Tissue sections were subjected to dewaxing and rehydration through downgraded series of alcohols. Prior fixation of the colon tissue might cause crosslinking of proteins and therefore antigen masking, i.e. the epitope of interest can be no longer detectable for the primary antibody. However, this issue can be avoided by practicing antigen retrieval techniques, which were applied depending on the used antibody (Table 16):

a) Heat-induced epitope retrieval (HIER): after the downgraded alcohol series, tissue sections were incubated for 25 min in a heated (98 °C) citrate buffer (pH=6) or TRIS-EDTA buffer (pH=9). Subsequently, the sections were cooled down for 20 min at RT and then transferred to glass cuvettes filled with PBS.
b) Protease-induced epitope retrieval (PIER): after passing through the downgraded alcohol series, tissue sections were transferred into glass cuvettes, which were filled with TRIS-EDTA-CaCl2 buffer and incubated in a preheated water bath (37 °C) for 15 min. The slides were then incubated with proteinase K at a dilution of 1:1000 for 5 min at 37 °C. The enzymatic reaction was stopped by rinsing the tissue sections with PBS.

Following the antigen retrieval, individual tissue sections were outlined with wax rings using a Dako Pen, which creates a hydrophobic barrier in order to facilitate the application of antibody solutions in the subsequent steps. To block non-specific binding, the slides were incubated for 30 min at RT in a protein-blocking buffer containing 1% fetal calf serum (FCS). Afterwards, appropriate dilution of the primary antibody was applied to the individual sections and incubated in a humid chamber for 2 h at RT or overnight at 4 ° C (see Table 16). After washing with PBS-T, slides were incubated with an appropriate dilution of the corresponding secondary antibody in a humid chamber. Following threefold washing with PBS-T, slides were further incubated with a streptavidine-Cy3 conjugate (1:1000) for 30 min at RT. After subsequent washing step, stained sections were

mounted with a 4′,6 diamidin-2-phenylindol (DAPI)-containing mounting medium. DAPI is a fluorescent dye, which binds mainly to DNA and stains cell nuclei.

Table 16: Antigen retrieval methods used in immunohistochemical staining

Antigen	Antigen retrieval method	Incubation time with a primary antibody
CD3	HIER, Tris-EDTA buffer (pH=9)	2 h
Claudin-1	HIER, Citrate buffer (pH=6)	2 h
IFN-γ	PIER	overnight
IgA	HIER, Citrate buffer (pH=6)	2 h
IL-22	HIER, Citrate buffer (pH=6)	2 h
RORγt	PIER	2 h

Pictures of stained colon sections were taken and digitized using an image scanner Axio.Scan™ Z1. Signals for Cy3 and DAPI were measured in a previously defined region of interest (ROI) applying previously saved greyscale limits. By excitation of Cy3 within a wavelength range of 526-562 nm and measurement of the emission at 561 nm, the investigated cell markers could be visualized. The excitation of DAPI at 333-387 nm and subsequent measurement of the emission at 465 nm enabled the localization of the cell nuclei. The quantification of the individual cell markers was carried out via image analysis using the program ImageJ™ 1.46r. For each treatment group, two sections per animal were analyzed. The analysis area was defined and included the area within the outer muscle layer of the colon. Unspecific coloured regions in the intestinal lumen were excluded from the analysis region. The border areas for the intensity of the channels were determined for each staining in a preliminary experiment. The software decomposed the channels in red, yellow and blue colours. Therefore, Cy3 could be analyzed by red and DAPI by blue channel. For both channels, the same area of analysis was determined and the intensity was measured. Expression of the markers was presented as Cy3-positive area relative to the DAPI-positive area [%].

2.2.5 Determination of *S. enterica* load in faecal pellets

To analyse colonization of the colon with *S. enterica,* faecal samples were collected every two weeks, starting 24 h p.i., immediately transferred into cold sterile PBS and stored on ice. The faecal pellets were processed under a clean bench: crushed with a sterile pipette tip and
vigorously vortexed to obtain a homogenous solution. Afterwards, appropriate dilutions of samples were prepared and 100 µl of each was applied on selective XLD agar plates and incubated for 24 h at

37 °C, 5 % CO_2. The cfu of *S. enterica* per pellet were determined by counting characteristic black colonies, which appear black due to the metabolism of sodium thiosulfate into hydrogen sulfate by *S. enterica*.

2.2.6 Analysis of *S. enterica*-specific antibodies in serum

To investigate specific antibodies against *S. enterica* present in serum of mice, an enzyme-linked immunosorbent assay (ELISA) was performed. First, 96-well plates were coated with *S. enterica* antigens. Therefore, *S. enterica* antigen solution prepared previously within the working group was diluted to a concentration of 1 µg/ml and 100 µl of the solution was applied to each well. The plates were sealed and stored overnight at 4 °C. Subsequently, the plates were washed twice with 300 µl of PBS-T. To block unspecific antibody binding, 0.5 % casein block buffer was added to each well, incubated for 1 h in a humid chamber at RT and washed three times with 300 µl of PBS-T. Serum samples were diluted 1:200 in PBS and 100 µl of each dilution was applied into an appropriate well. As a buffer control, 100 µl of PBS was used. The specificity of the antibody binding was checked by a conjugate control (100 µl PBS + detection antibody), without serum. Plates were sealed and incubated for 2 h in a humid chamber at RT. To remove non-specifically bound antibodies, plates were washed three times with 300 µl of PBS-T. Next, 100 µl of diluted immunoglobulin-specific antibody (IgA, IgM, IgG1 or IgG2c) conjugated with horseradish peroxidase were applied to each well (Tale 6). PBS-T served as a buffer control. Following application, plates were sealed and incubated for 1 h in a humid chamber at RT, protected from light. Subsequently, the washing step was repeated and 100 µl of 3,3', 5,5'-tetramethylbenzidine (TMB) solution per well was applied for detection. During light-protected incubation for 5 min in a humid chamber at RT, the horseradish peroxidase oxidized the chromogenic TMB in the presence of hydrogen peroxide. Reaction was stopped by addition of 100 µl of 0.5 M sulfuric acid to each well. The amount of oxidized substrate was quantified by measuring the optical density (OD) at 450 nm using a conventional microplate reader Tecan Safire2. The measured OD values of the buffer controls were subtracted from the OD values of the samples. Since the amount of oxidized substrate depends on the amount of horseradish peroxidase-conjugated antibody, the *S.enterica*-specific antibodies present in the blood serum could be qualitatively compared.

2.2.7 Statistical analysis

All data were analyzed using GraphPad™ Prism 6. Clinical data are presented as mean or median ±SD. Data for histological analysis and *S. enterica* load in feces are presented as individual data points. Significant differences between data sets were estimated by Kruskal-Wallis one-way ANOVA (if normally distributed) or by One-way ANOVA on Ranks (if the normality test failed) followed by Dunn's multiple comparison test. Differences between data sets for clinical score and specific antibodies in serum were analyzed by Two-way ANOVA followed by Holm-Sidak's multiple comparison test. Values were considered significantly different if $P< 0.05$, with $P< 0.01$, $P< 0.001$ or $P< 0.0001$ denoting higher significance.

3 Results

3.1 Oral infection with *S. enterica* induces clinical symptoms of colitis, which are exacerbated by streptomycin pretreatment in the acute phase of infection

To establish a mouse model of chronic bacterial infection, mice were inoculated with *S. enterica* with or without a single dose of streptomycin treatment 24 h prior to infection. Clinical symptoms, including body weight change and stool consistency, were examined daily over a period of 10 weeks. A clinical score was determined for each mouse as the mean of the scores for body weight change and stool consistency. Figure 3 shows the course of the clinical score over the time of the experiment.

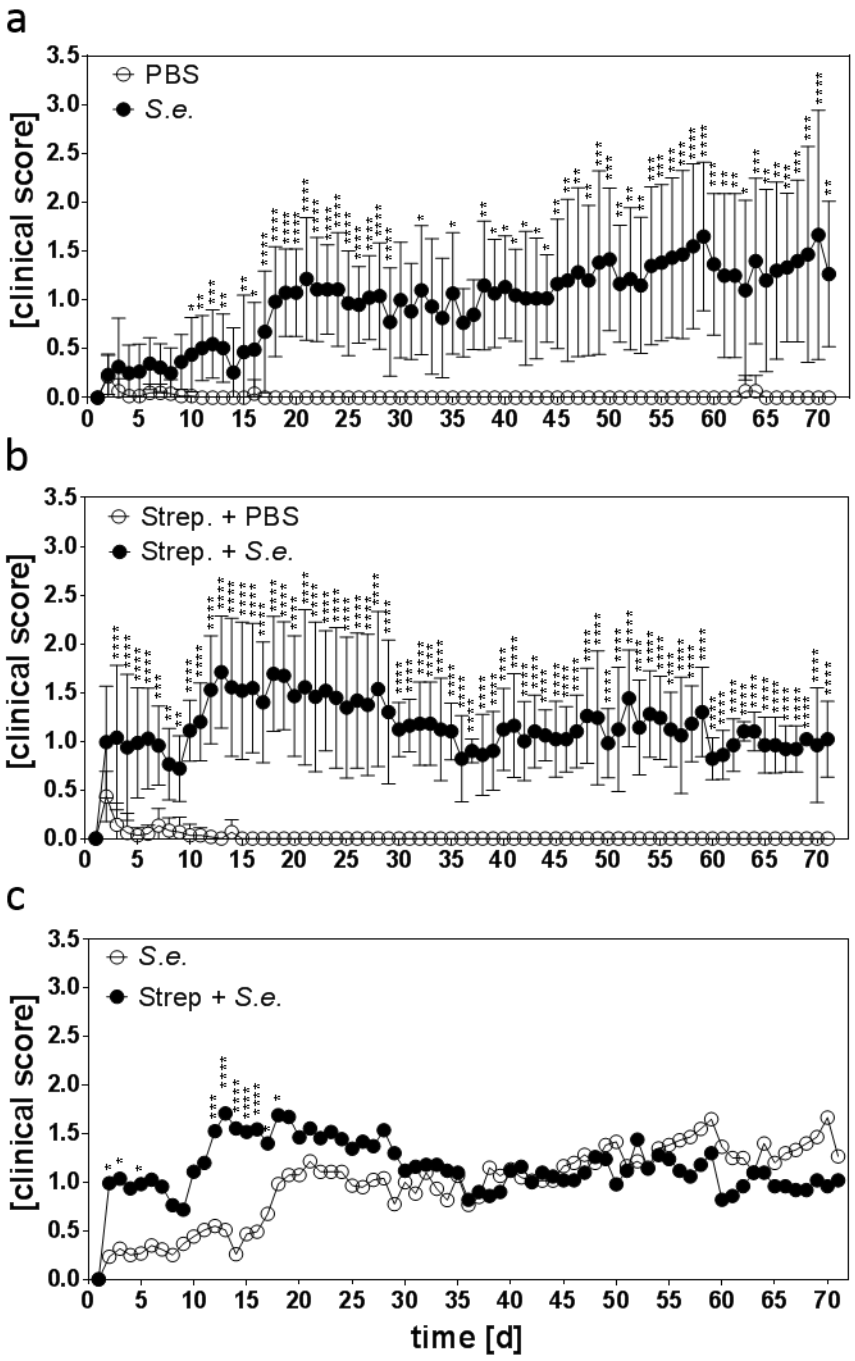

Figure 3: Course of the clinical score in the mouse model of chronic *S. enterica* infection. Chronic infection was induced in C57BL/6 mice by oral inoculation with *S. enterica* (*S.e.*) without (a) or with (b) a single dose of streptomycin (Strep.; 20 mg/mouse) 24 h prior to infection. Animals treated with PBS were used as negative control. The clinical score was determined daily and is shown as mean ± SD (a-b) or mean (c) for n=12 animals (0-29 days) or n=6 (30-70 days) per group. Statistics: Two-way ANOVA followed by Holm-Sidak's multiple comparisons test. *P< 0.05; **P< 0.01; ***P< 0.001; ****P< 0.0001.

Starting from day 9, infected mice showed loss of body weight as well as persisting diarrhea. In comparison to the PBS control group, infected animals without antibiotic pretreatment showed significantly elevated ($P<0.05$) clinical symptoms at day 10 p.i. (Figure 3 a). In mice pretreated with streptomycin, a significantly ($P<0.0001$) elevated clinical score was already observed on day 3 p.i. (Figure 3 b). In both cases, clinical symptoms persisted until the end of the experiment. In comparison to *S. enterica* infection alone, streptomycin pretreatment caused a significant ($P<0.05$) increase of the clinical score as soon as 2 days p.i. This effect was observable from day 3 to 6 p.i. and even more pronounced from day 12 to day 18 p.i. ($P<0.001$). After day 18, clinical symptoms in both groups settled at approximately the same level (Figure 3 c).

3.1.1 IL-22-deficiency causes exacerbation of clinical symptoms in the early phase of infection

To investigate whether IL-22 significantly contributes to the immune response against *S. enterica* infection, the model of chronic infection introduced above was used. Mice were infected with *S. enterica* and a single dose of streptomycin was administered 24 h prior to bacterial inoculation. Clinical symptoms, including body-weight change and stool consistency were assessed daily for 4 weeks. Based on these parameters, the clinical score for each mouse was determined. Figure 4 shows development of the clinical score throughout the course of the experiment.

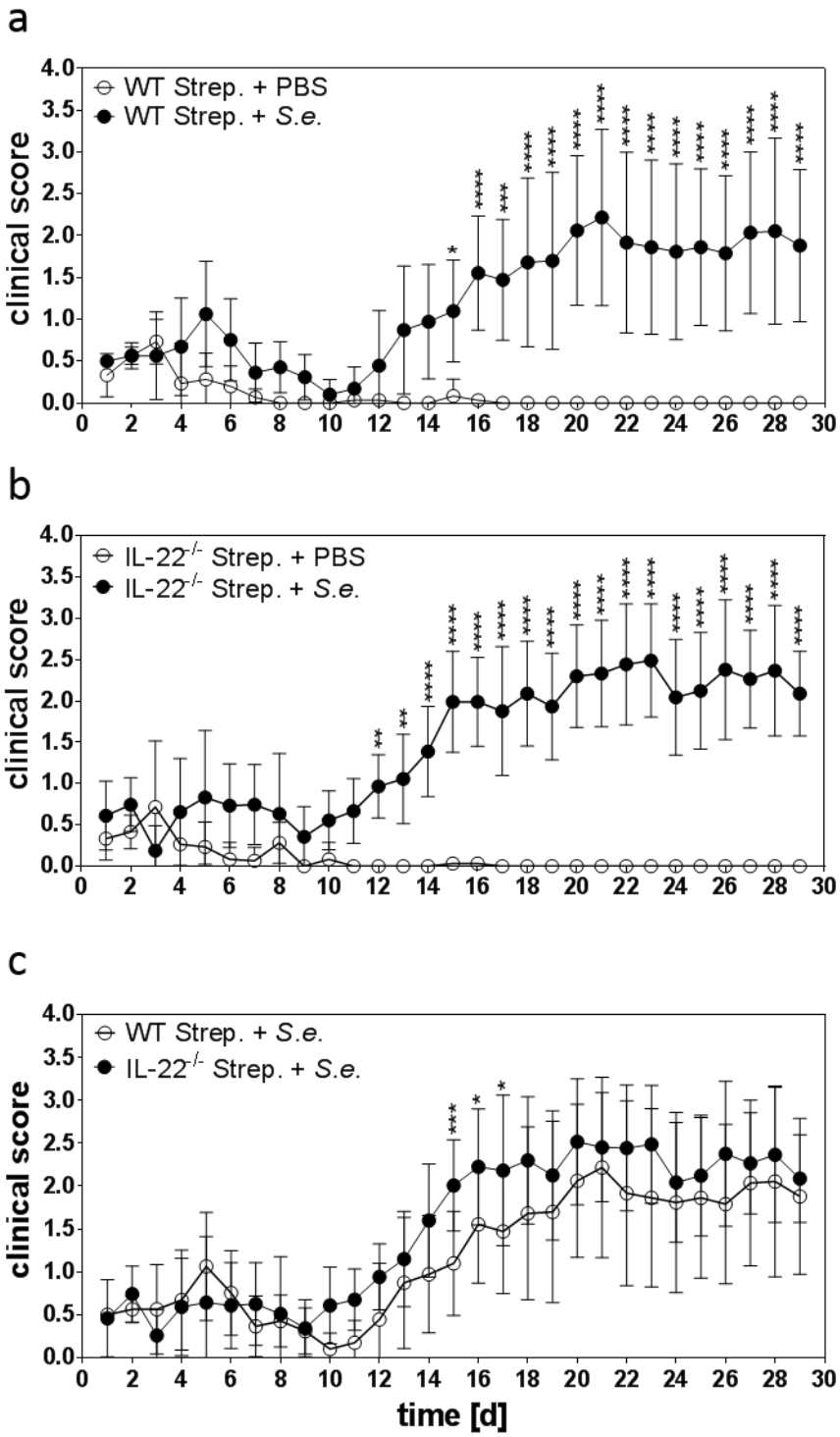

Figure 4: Course of the clinical score over 4 weeks post infection with *S. enterica*. Chronic infection was induced in wild-type (WT) or IL-22-deficient (IL-22$^{-/-}$) C57BL/6 mice by oral inoculation with *S. enterica* (*S.e.*) with a single dose of streptomycin (Strep.; 20 mg/mouse) 24 h prior to infection. Animals treated with PBS were used as negative control. The clinical score was assessed daily and is shown as mean ± SD for WT Strep. + PBS n=6, WT Strep. + *S.e.* n=11, IL-22$^{-/-}$ Strep. + PBS n=6, IL-22$^{-/-}$ Strep. + *S.e.* n=12 animals (a-c). Statistics: Two-way ANOVA followed by Holm-Sidak's multiple comparisons test. *P< 0.05; **P< 0.01; ***P< 0.001, ****P<0.0001.

Starting at day 15 p.i., an increase in the clinical score in WT animals was observed, whereas IL-22-defficient mice showed significant clinical symptoms already at day 12 p.i. (Figure 4 a, b). Both groups maintained a significantly elevated clinical score until the end of experiment. In direct comparison, IL-22-defficient animals developed clinical symptoms significantly faster than WT animals, and the clinical score stayed on a significantly higher level up to day 17 p.i. However, the difference between the groups was less pronounced in the later phase of the experiment (Figure 4 c).

3.2 Histopathological aberrations in the colon tissue caused by oral infection with *S. enterica* are exacerbated by antibiotic pretreatment

To investigate the pathohistlogical changes in the colon tissue caused by infection with *S. enterica*, a histopathological score based on H&E-stained cross sections of the proximal and distal colon was assessed by a trained pathologist at two time points of the experiment (4 weeks p.i. and 10 weeks p.i.). The H&E staining allowed investigation of general structural aberrations as well as functional changes in the tissue. The histopathological score is a crucial parameter for evaluating tissue degeneration and inflammation and considers the extent of fibrosis in mucosa and submucosa, the infiltration of lymphocytes, granulocytes and macrophages, as well as the formation of edema.

The histological analysis revealed moderate inflammatory infiltration affecting the mucosa and submucosa, which was very rich in cells and consisted mainly of lymphocytes and granulocytes (Figure 5 a; *S.e.* 4 weeks p.i., *S.e*. 10 weeks p.i., Strep. + *S.e*. 4 weeks p.i., Strep. + *S.e.* 10 weeks p.i.). Fibrocytes and fibroblasts forming fibrotic lesions as well as some cases of perforation were also noticeable in the colon tissue of streptomycin-pretreated mice (Figure 5 a; Strep.+ *S.e.* 10 weeks p.i. proximal and distal). In summary, routine sections revealed lesions that were mainly characterized by an inflammatory infiltrate affecting the mucosa and submucosa. Furthermore, the infiltrate formed a cuneiform shape that was often related to a perforating lesion. The morphological character was similar to the one described in human UC.

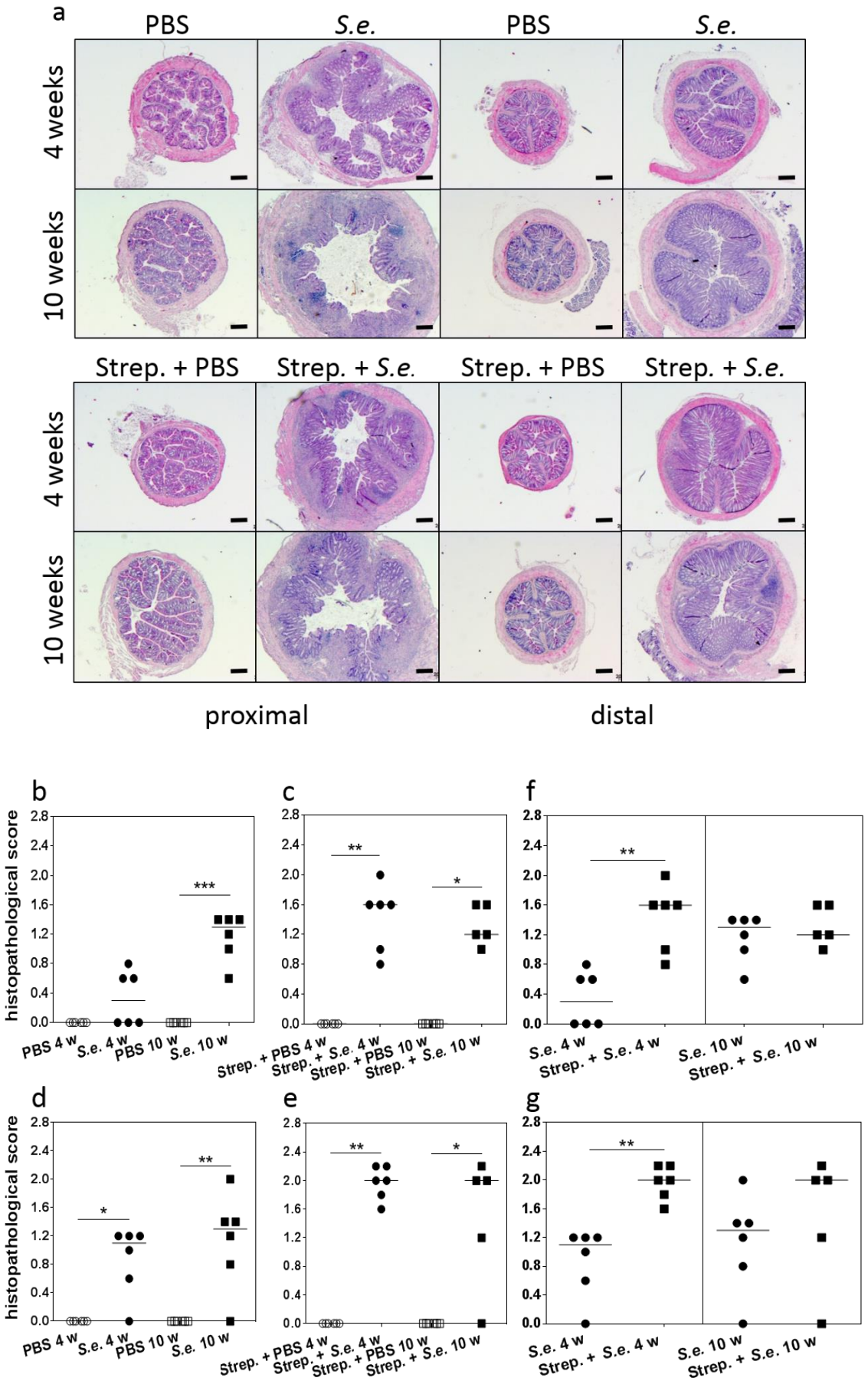
a
PBS
S.e.
PBS
S.e.
4 weeks
10 weeks
Strep. + PBS
Strep. + S.e.
Strep. + PBS
Strep. + S.e.
4 weeks
10 weeks
proximal
distal
b
c
f
histopathological score
PBS 4 w
S.e. 4 w
PBS 10 w
S.e. 10 w
Strep. + PBS 4 w
Strep. + S.e. 4 w
Strep. + PBS 10 w
Strep. + S.e. 10 w
S.e. 4 w
Strep. + S.e. 4 w
S.e. 10 w
Strep. + S.e. 10 w
d
e
g
histopathological score
PBS 4 w
S.e. 4 w
PBS 10 w
S.e. 10 w
Strep. + PBS 4 w
Strep. + S.e. 4 w
Strep. + PBS 10 w
Strep. + S.e. 10 w
S.e. 4 w
Strep. + S.e. 4 w
S.e. 10 w
Strep. + S.e. 10 w

Figure 5: Histopathological changes assessed 4 and 10 weeks after infection with *S. enterica*. Chronic infection was induced in C57BL/6 mice by oral inoculation with *S. enterica* (*S.e*.) without (a) or with (b) a single dose of streptomycin (Strep.; 20 mg/mouse) 24 h prior to infection. Animals treated with PBS were used as negative control. The histopathological score was evaluated based on H&E-stained cross sections (a) of the proximal (b, c, f) and distal (d, e, g) colon for 6 animals per group, Strep. + *S.e.* 10 w n=5 animals; scale bars: 200 µm. The median of individual data points is indicated. Statistics: One-way ANOVA followed by Dunn's multiple comparisons test. *P<0.05; **P<0.01; ***P<0.001.

If compared to the PBS control group, a significantly (P<0.001) elevated histopathological score was observed for the tissue from the proximal colon of mice infected with *S. enterica* without pretreatment only at 10 weeks p.i. (Figure 5 b). However, in the proximal colon of streptomycin-pretreated mice, significant (P<0.01) symptoms of tissue inflammation were already observed 4 weeks p.i. (P<0.01) but also at 10 weeks p.i. (Figure 5 c). Histopathological findings in the distal colon were similar, but in contrast to the proximal colon significant (P<0.05) pathological changes in *S. enterica*-infected mice were already observed at 4 weeks p.i. (Figure 5 d). Pretreatment with streptomycin resulted in a significantly exacerbated histopathological score at 4 weeks (P<0.01) and 10 weeks (P<0.05) p.i. (Figure 5 e). Direct comparison of groups with and without antibiotic pretreatment showed that streptomycin caused a significant (P<0.01) increase in the severity of tissue inflammation in both the proximal and distal colon at 4 weeks p.i. (Figures 5 f and 5 g). However, at 10 weeks p.i. this difference was no longer present since mice without pretreatment revealed symptoms of similar severity as found in streptomycin-treated animals at this period of infection.

3.2.1 Histological aberrations caused by oral infection with *S. enterica* are not dependent on presence of IL-22

To investigate pathohistological changes in the colon tissue caused by infection with *S. enterica* and a possible contribution of IL-22 a histopathological score based on H&E-stained cross-sections of the proximal and distal colon was assessed by a trained pathologist 4 weeks p.i. Figure 10 shows H&E staining (6 a) and histopathological scores determined for WT and IL-22-deficient mice 4 weeks p.i (6 b-d).

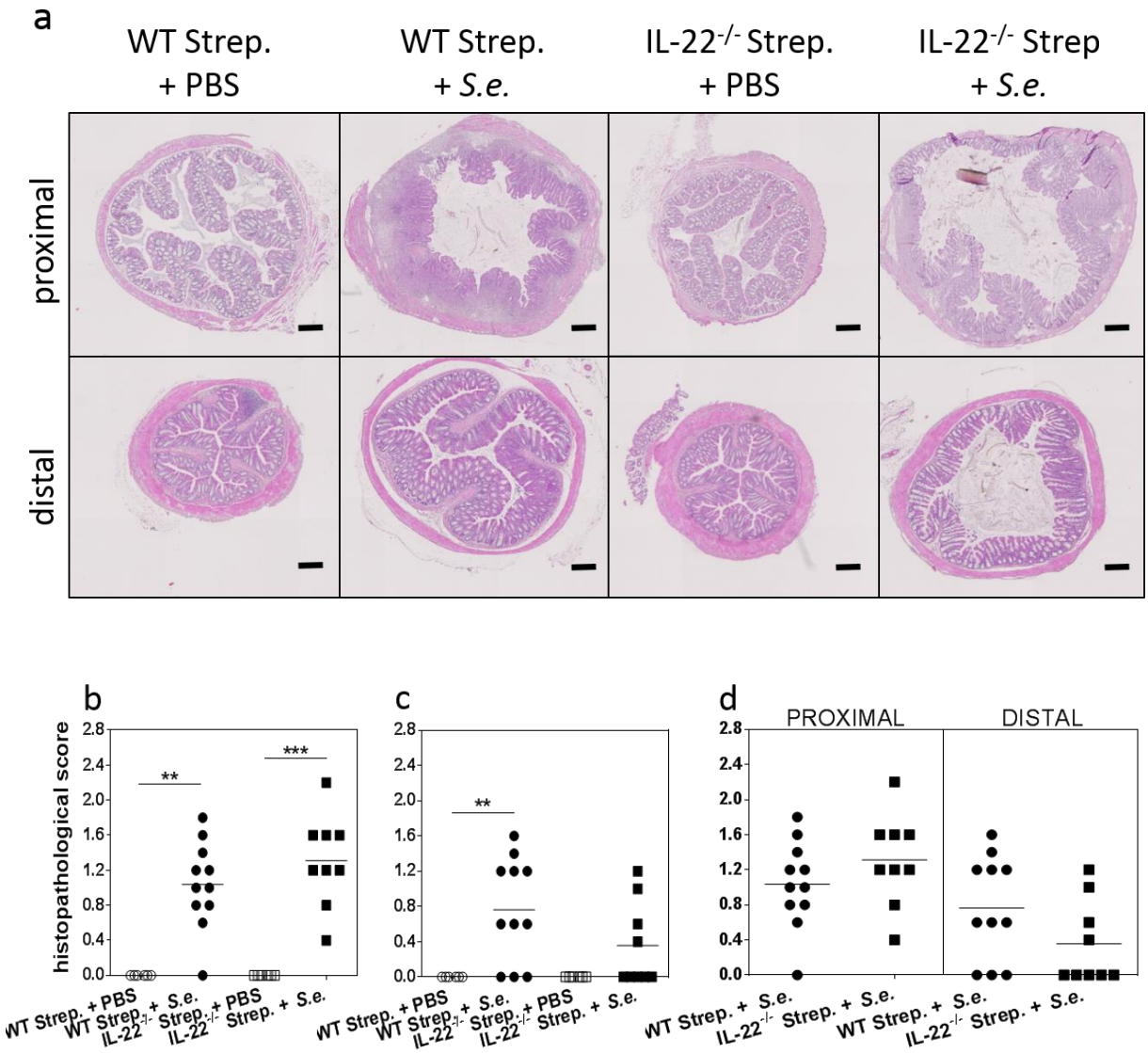

Figure 6: Histopathological changes assessed 4 weeks post infection with *S. enterica*. Chronic infection was induced in wild-type (WT) or IL-22-deficient (IL-22$^{-/-}$) C57BL/6 mice by oral inoculation with *S. enterica* (*S.e.*) and administration of a single dose of streptomycin (Strep.; 20 mg/mouse) 24 h prior to infection. The histopathological score was evaluated based on H&E-stained cross-sections (a) of the proximal (b) and distal (c) colon for groups: WT Strep. + PBS, IL-22$^{-/-}$ Strep. + PBS n=6, WT Strep. + *S.e.* n=12, IL-22$^{-/-}$ Strep. + *S.e.* n=9. Direct comparison of the histopathological scores between WT and IL-22$^{-/-}$ mice is shown (d). The median of individual data points is indicated. Statistics: One-way ANOVA followed by Dunn's multiple comparisons test. **P<0.01; ***P<0.001.

If compared to healthy control animals, a significantly elevated histopathological score could be observed in the proximal colon of both WT and IL-22-deficient mice (Figure 6 b). In addition, WT mice showed significant morphological changes in the tissue of the distal colon, whereas these changes could not be observed in IL-22-deficient mice (Figure 6 c). Comparison of the histopathological scores

between WT and IL-22-deficient mice revealed no significant difference, neither in the proximal nor in the distal colon (Figure 6 d).

3.2.2 IL-22 deficiency causes increased loss of goblet cells in the proximal colon of *S. enterica*-infected mice

Goblet cells play a crucial role in the defense against pathogenic infection of the gut by producing mucus, thus creating a barrier that is hard to penetrate by invading bacteria. Loss of goblet cells is a typical symptom of the inflammatory reaction in the colon tissue, as observed in patients with IBD. Therefore, cross-sections of the colon from WT and IL-22-deficient mice were stained with PAS, which allows visualization of mucus and mucus-producing cells as purple-stained regions. Figure 5 shows microphotographs of PAS-stained cross-sections (Figure 7 a) and quantification of PAS-positive areas in the analyzed colon tissue (Figure 7 b-d).

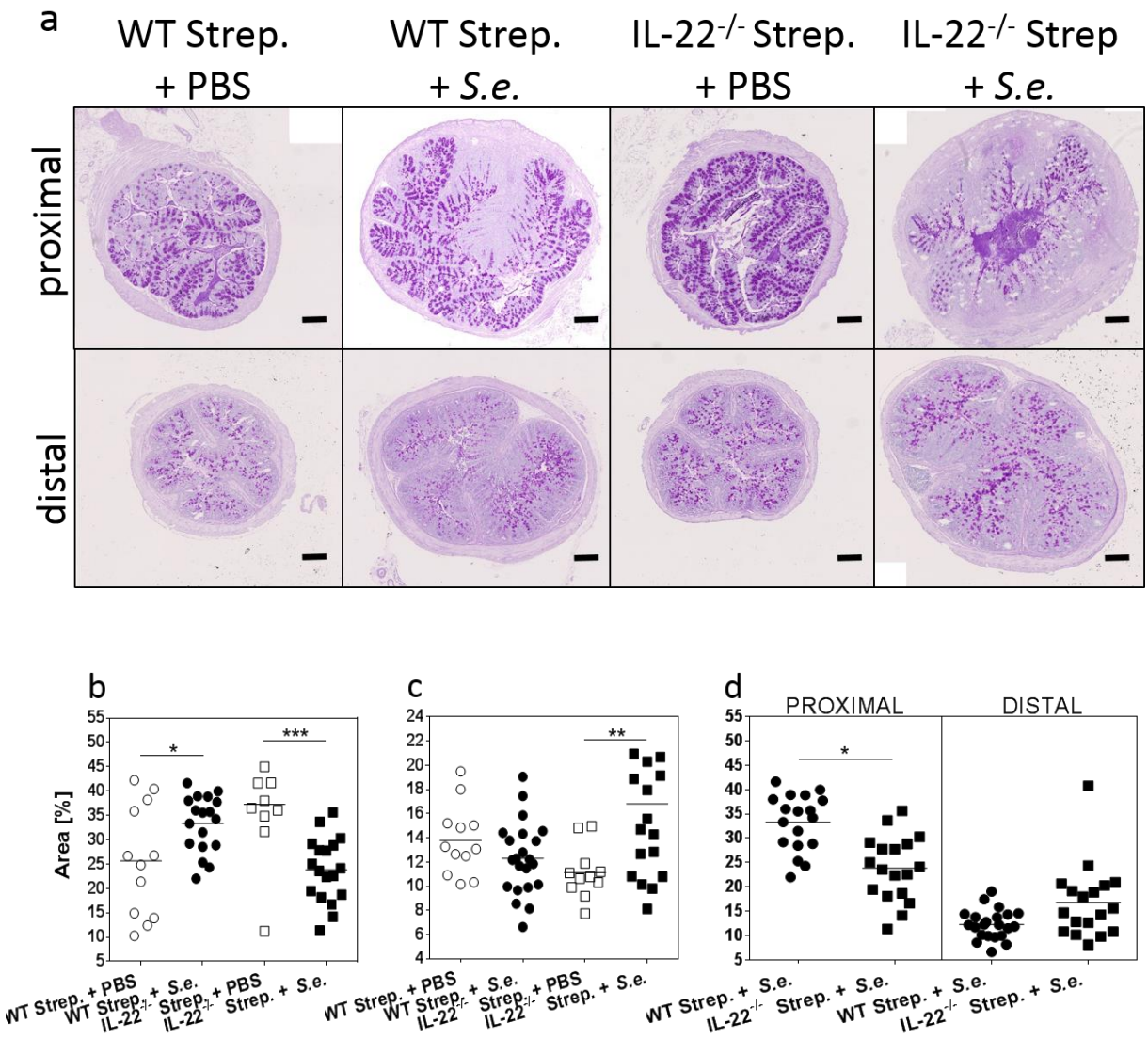

Figure 7: Quantification of mucus and mucus-producing cells in the colon of *S. enterica*-infected mice. Chronic infection was induced in wild-type (WT) or IL-22-deficient (IL-22$^{-/-}$) C57BL/6 mice by oral inoculation with *S. enterica* (*S.e.*) with a single dose of streptomycin (Strep.; 20 mg/mouse) 24 h prior to infection. The areas containing mucus or mucus-producing cells were quantified based on PAS-stained cross sections (a) of the proximal (b) and distal (c) colon for groups: WT Strep. + PBS, IL-22$^{-/-}$ Strep. + PBS n=6, WT Strep. + *S.e.* n=12, IL-22$^{-/-}$ Strep. + *S.e.* n=9. Direct comparison of the quantification of mucus production in the colon between WT and IL-22-deficient mice is shown (d). The median of individual data points is indicated. Statistics: One-way ANOVA followed by Dunn's multiple comparisons test. *P<0.05; **P<0.01; ***P<0.001.

Significantly (P<0.05) expanded areas containing mucus were observed in the proximal colon of WT animals in comparison to the healthy control group. At the same time point, an opposite tendency was found for IL-22-deficient animals, which showed a significantly (P<0.001) reduced amount of mucus or mucus-producing cells (Figure 7 b). In the distal colon, IL-22-deficient mice showed a significantly (P<0.001) increased mucus-positive area if compared to the healthy PBS control.

However, there were no observable differences between healthy and *S.e.*-infected WT animals (Figure 7 c). In direct comparison, IL-22-deficient animals showed a significantly ($P<0.05$) reduced production of mucus in the proximal tissue, whereas no detectable differences were observed for the distal part of the gut (Figure 7 d).

3.3 Streptomycin alters the course of *S. enterica* colonization in the gut

To analyze colonization of the gut after inoculation with *S. enterica,* fecal pellets were collected every 2 weeks starting 24 h p.i. Samples were homogenized and plated onto XLD agar to differentiate *S. enterica* colonies from other bacteria present in the gut. The cfu per fecal pellet were determined by counting characteristic colonies formed on XLD agar, which appear black-colored due to the metabolism of sodium thiosulfate into hydrogen sulfate by *S. enterica*. Figure 8 shows the *S. enterica* load in the colon of infected mice determined on different time points of the experiment.

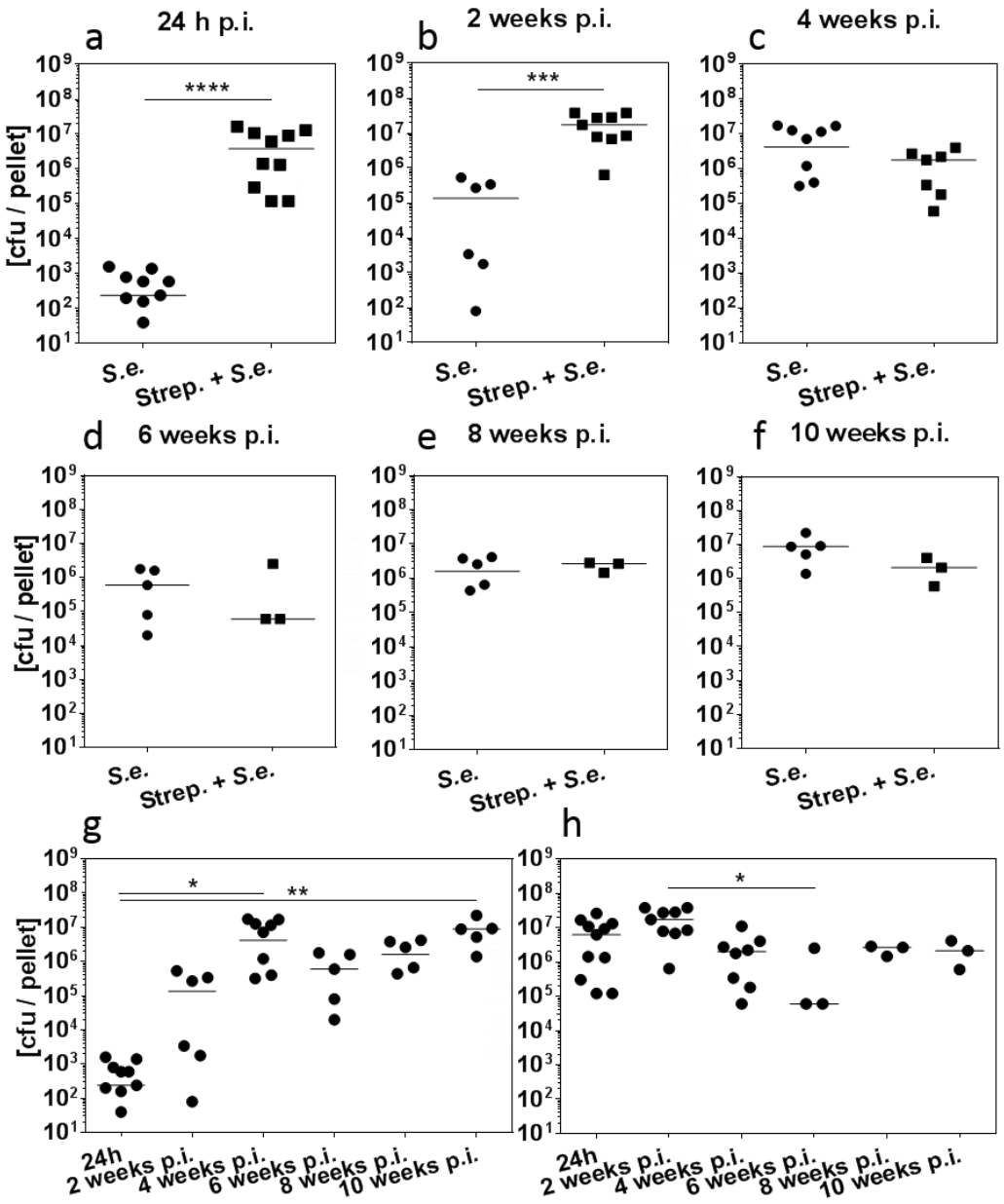

Figure 8: Number of *S. enterica* cfu per fecal pellet assessed over 10 weeks post infection. Chronic infection was induced in C57BL/6 mice by oral inoculation with *S. enterica* (*S.e.*) without (g) or with (h) a single dose of streptomycin (Strep.; 20 mg/mouse) 24 h prior to infection. Fecal samples were collected from each mouse every 2nd week, homogenized and applied on XLD agar. After 24 h, *S. enterica* colonies were counted and are displayed as cfu/pellet (a-f). Bacteria load determined throughout the course of the experiment in infected mice is shown (g, h). The median of individual data points is indicated. Statistics: Mann-Whitney test (a-f) and One-way ANOVA followed by Dunn's multiple comparisons test (g, h). Number of animals per group: n=3-12. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

Already at 24 h p.i., a significantly (P<0.0001) higher load of the pathogen could be detected in mice pretreated with streptomycin (Figure 8 a). At 2 weeks p.i., there was still a significant (P<0.001) difference between the antibiotic-treated vs. untreated group. However, the load of *S. enterica* in the

colon of pretreated animals remained at the same high level (range 10^7 cfu), whereas the bacterial load in the group without streptomycin pretreatment had slightly increased from range 10^2 up to 10^5 cfu (Figure 6 b). This trend continued at later time points, where bacterial loads of both groups were found at similar high levels, i.e. 10^6-10^7 cfu. Observed reduced cfu level in streptomycin-pretreated group 6 weeks p.i. was most likely due to limited number of samples (Figures 8 c-f). This observation was consistent with the development of the clinical scores in both groups, which also converged at later time points (Figure 3 c). Analysis of the bacterial burden throughout the course of the experiment revealed that inoculation with *S. enterica* only caused an increase in colonization of the gut over time (Figure 8 g). Streptomycin pretreatment caused immediate raise of the bacterial load, which remained on a similar high level until week 10 p.i. (Figure 8 h).

3.3.1 Mice lacking IL-22 are more susceptible to the colonization with *S. enterica*

To analyze colonization of the gut with administered *S. enterica*, stool samples were collected at 24 h, 2 weeks and 4 weeks p.i. Fecal pellets were homogenized and plated on XLD agar. After 24 h of incubation at 37 °C, the cfu numbers per each pellet were determined by counting characteristic black colonies (Figure 9).

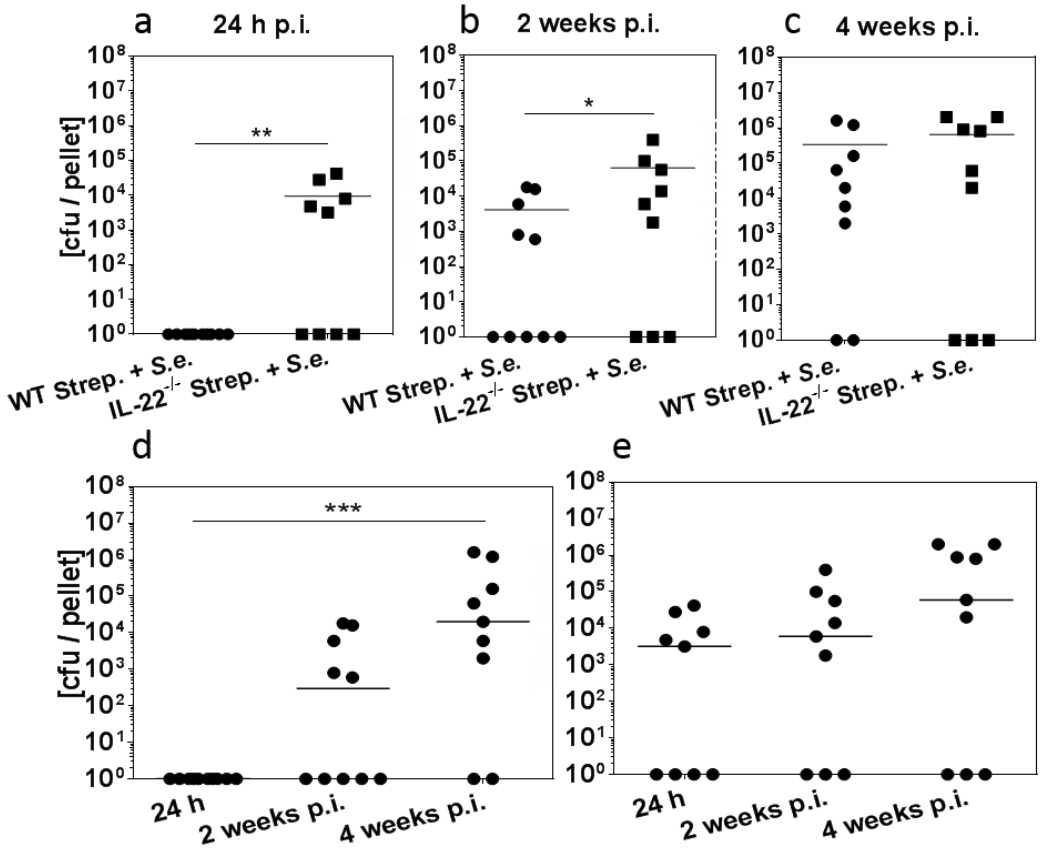

Figure 9: *S. enterica* cfu in fecal pellets from mice infected with *S. enterica*. Chronic infection was induced in wild-type (WT) or IL-22-deficient (IL-22$^{-/-}$) C57BL/6 mice by oral inoculation with *S. enterica* (*S.e.*) and administration of a single dose of streptomycin (Strep.; 20 mg/mouse) 24 h prior to infection. Fecal pellets were collected from mice at 24 h, 2 weeks and 4 weeks p.i., homogenized and plated onto XLD-agar. After 24 h, *S. enterica* colonies were counted and are displayed as cfu/pellet (a-c). Bacteria load determined throughout the course of the experiment in infected WT (d) and IL-22-deficient (e) mice is shown. The median of individual data points is indicated. Statistics: Mann-Whitney test (a-c) and One-way ANOVA followed by Dunn's multiple comparisons test (d-e). Number of animals per group: n=9-11. *P<0.05, **P<0.01, ***P<0.001.

Already 24 h p.i., about half of the IL-22-deficient group manifested a significant (P<0.01) load of *S. enterica*, whereas no bacterial load was observed in WT animals at this time point (Figure 9 a). 2 weeks p.i., the average number of cfu was still significantly higher in IL-22-deficient mice (Figure 9 b), whereas this difference was no more observable 4 weeks p.i. (Figure 9 c). Analysis of the *S. enterica* load throughout the course of the experiment showed that colonization of the gut increased over time in WT mice and was significantly (P<0.001) higher 4 weeks p.i. (Figure 9 d). In contrast, bacterial colonization in IL-22-deficient mice occurred rapidly and remained on a similar high level until the end of the experiment (Figure 9 e).

3.4 Streptomycin affects the local immune response in the colon tissue of *S. enterica*-infected mice

To further investigate the local immune response to *S. enterica* invasion in the colon tissue, cross-sections of the distal colon were analyzed by immunofluorescence staining. The analysis focused on exemplary markers relevant for the mucosal immune response in the gut tissue, i.e. cluster of differentiation (CD) 3, Interferon-gamma (IFN-γ), IL-22 and secretory immunoglobulin A (sIgA).

3.1.1. CD3

CD3 is a basic marker of T cells expressed on their surface. Staining of the CD3-positive ($CD3^+$) cells provided an overview of the infiltration of T cells into the inflamed tissue, indicating the role of these cells in the immune reaction against *S. enterica* infection. Figure 10 shows microphotographs of the CD3-stained cross-sections (Figure 10 a), as well as quantification of CD3 expression in stained cross-sections (Figures 10 b-d).

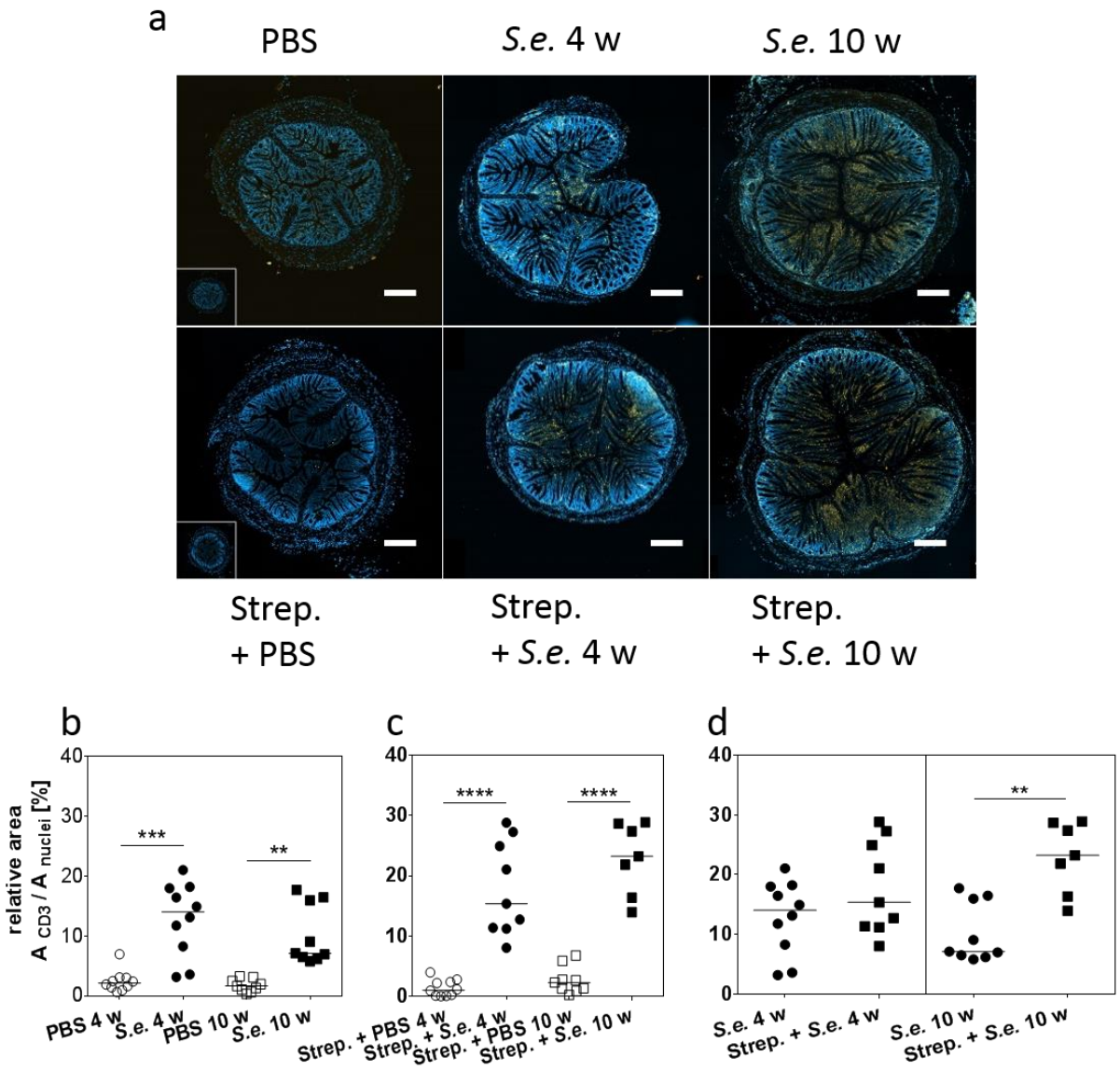

Figure 10: Analysis of CD3 expression in the colon tissue of mice infected with *S. enterica*. Chronic infection was induced in C57BL/6 mice by oral inoculation with *S. enterica* (*S.e.*) without or with administration of a single dose of streptomycin (Strep.; 20 mg/mouse) 24 h prior to infection. Animals treated with PBS were used as negative control. Cross-sections of the distal colon were stained for the T cell marker CD3; scale bars: 200 µm (a). Stained cross-sections (2 sections/mouse) were digitized by means of a slide scanner (AxioScan.Z1, Zeiss). Expression was quantified using the software program ImageJ® and is shown as marker-positive area relative to the DAPI-positive area (b-d). The median of individual data points is indicated. Statistics: One-way ANOVA followed by Dunn's multiple comparisons test. Number of animals per group: n=6, Strep. + *S.e.* 10 w n=5 animals. **P<0.01; ***P<0.001; ****P<0.0001.

In comparison to the PBS control group the numbers of infiltrating T cells were significantly higher in the colon tissue of *S. enterica*-infected mice at 4 weeks p.i. (P<0.001) and 10 weeks p.i. (P<0.01) (Figure 10 b). In mice pretreated with streptomycin, differences to the healthy control group were even more pronounced (P<0.0001; Figure 10 c). If compared directly, pretreated mice showed a

significantly ($P<0.01$) higher expression of CD3 in the colon tissue than mice without antibiotic pretreatment at 10 weeks p.i. (Figure 10 d).

3.4.1 IFN-γ

IFN-γ, a cytokine produced by NK cells and activated T cells, plays an important role in the host response to intracellular pathogens, such as *S. enterica*. Analysis of IFN-γ expression therefore provided further insight into the T-cell-mediated immune response to *S. enterica* infection in the gut. Figure 11 shows microphotographs of the IFN-γ-stained cross-sections (Figure 11 a), and the quantification of IFN-γ expression in stained cross-sections (Figures 11 b-d).

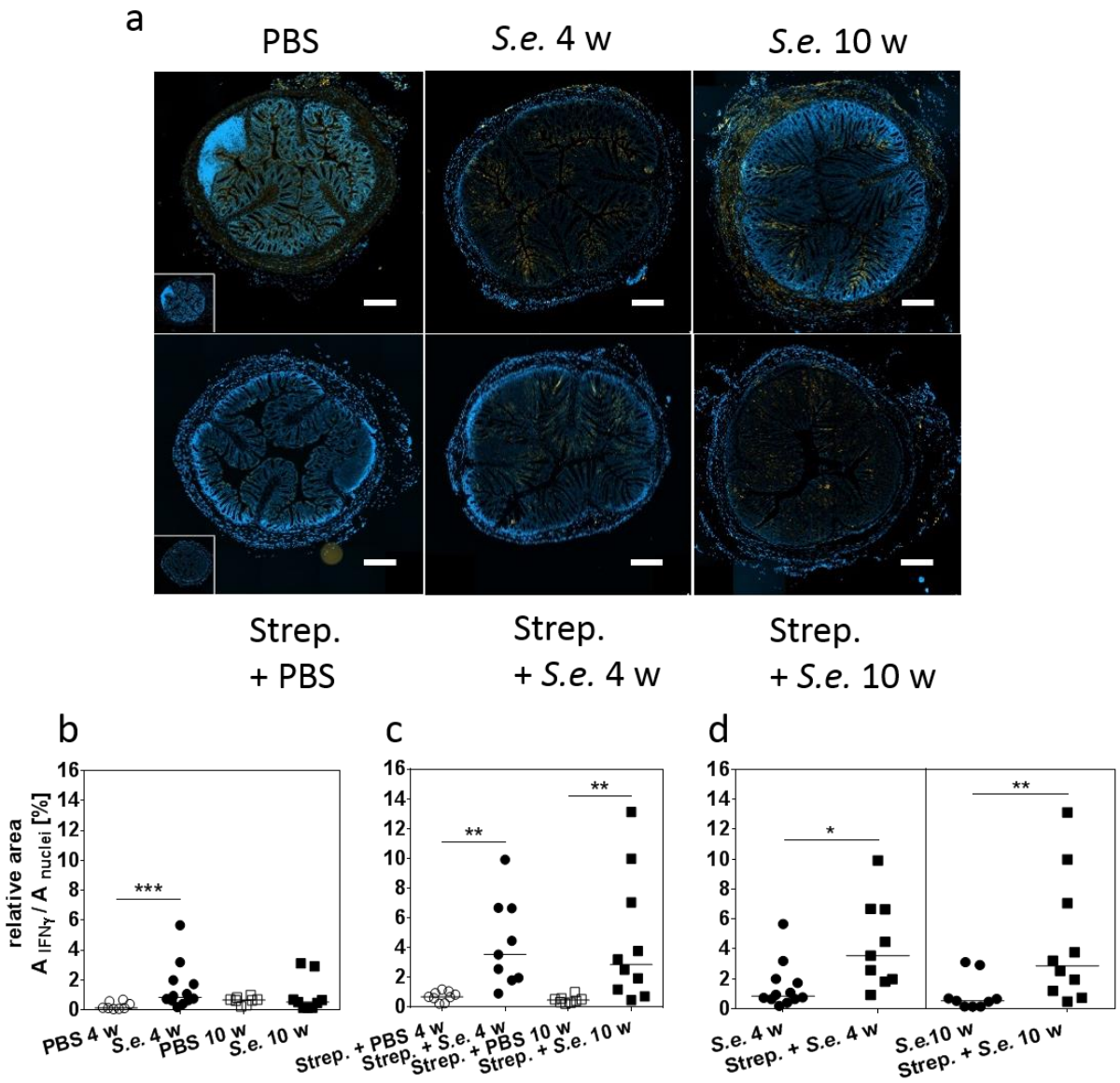

Figure 11: Analysis of IFN-γ expression in the colon tissue of mice infected with *S. enterica*. Chronic infection was induced in C57BL/6 mice by oral inoculation with *S. enterica* (*S.e.*) without or with administration of a single dose of streptomycin (Strep.; 20 mg/mouse) 24 h prior to infection. Animals treated with PBS were used as negative control. Cross-sections of the distal colon were stained for the inflammatory marker IFN-γ; scale bars: 200 µm (a). Stained cross-sections (2 sections/mouse) were digitized by means of a slide scanner (AxioScan.Z1, Zeiss). Expression was quantified using the software program ImageJ® and is shown as marker-positive area relative to the DAPI-positive area (b-d). The median of individual data points is indicated. Statistics: One-way ANOVA followed by Dunn's multiple comparisons test. Number of animals per group =6, Strep. + *S.e.* 10 w n=5 animals. *P< 0.5;**P< 0.01; ***P< 0.001.

Consistent with the analysis of CD3 expression, a significantly (P<0.001) higher level of IFN-γ expression was observed in colon sections of *S. enterica*-infected mice at 4 weeks p.i., but the

level decreased again until 10 weeks p.i. (Figure 11 b). In animals pretreated with streptomycin, expression of IFN-γ was significantly ($P<0.01$) elevated at both 4 weeks p.i. and 10 weeks p.i. (Figure 11 c). In direct comparison, antibiotic pretreatment resulted in an increased expression of IFN-γ in the colon tissue of infected animals at both analytical time points 4 weeks p.i. ($P<0.05$) and 10 weeks p.i. ($P<0.01$) (Figure 11 d).

3.4.2 IL-22

IL-22, produced by different cells of the innate and adaptive immune system (i.e. ILC3, Th17, Th22 cells) contributes to tissue regeneration and plays an important role in the host defense at barrier surfaces. Therefore, the quantification of IL-22 expression in the colon tissue provided important information on its role in the mucosal immune response against *S. enterica*. Figure 12 shows microphotographs of the IL-22-stained cross-sections (Figure 12 a), as well as the quantification of IL-22 expression in stained cross-sections (Figures 12 b-d).

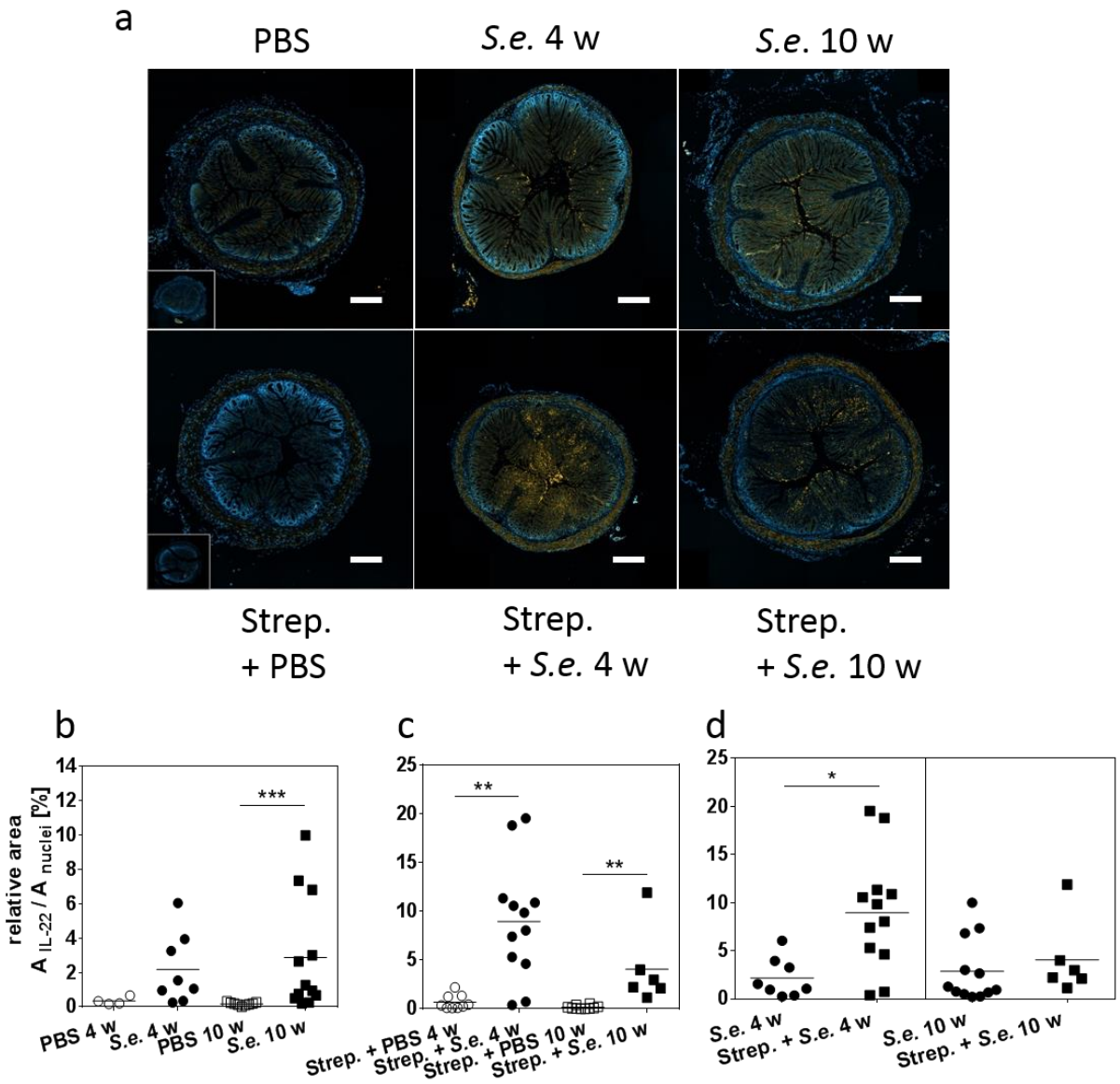

Figure 12: Analysis of IL-22 expression in the colon tissue of mice infected with *S. enterica*. Chronic infection was induced in C57BL/6 mice by oral inoculation with *S. enterica* (*S.e.*) without or with administration of a single dose of streptomycin (Strep.; 20 mg/mouse) 24 h prior to infection. Animals treated with PBS were used as negative control. Cross-sections of the distal colon were stained for IL-22; scale bars: 200 µm (a). Stained cross-sections (2 sections/mouse) were digitized by means of a slide scanner (AxioScan.Z1, Zeiss). Expression was quantified using the software program ImageJ® and is shown as marker-positive area relative to the DAPI-positive area (b-d). The median of individual data points is indicated. Statistics: One-way ANOVA followed by Dunn's multiple comparisons test. Number of animals per group = 6, Strep. + *S.e.* 10 w n=5 animals. *P< 0.5;**P< 0.01.

If compared to the PBS control group, the number of IL-22-expressing cells was not elevated in the colon tissue of *S. enterica*-infected animals at 4 weeks p.i. However, a significant increase of IL-22-positive cells was detectable at 10 weeks p.i. (P<0.001) (Figure 12 b). In streptomycin-

pretreated animals, a significant increase of IL-22-positive cells was observed already 4 weeks p.i. ($P<0.01$) that lasted until the end of the experiment ($P<0.01$) (Figure 12 c). If compared directly, antibiotic-pretreated *S.e.*-infected mice showed significantly higher ($P<0.05$) expression of IL-22 shown as IL-22-positive cells in the colon tissue than animals only infected with *S.e.* without streptomycin pretreatment at 4 weeks p.i., whereas this difference was no more detectable at 10 weeks p.i. (Figure 12 d).

3.4.3 Secretory IgA

sIgA is a vital component of the innate immune system, which plays a critical role in the mucosal immune responses. sIgA is secreted into the mucus and gut lumen and is an important marker for the mucosal immune response. Figure 13 shows microphotographs of the IgA-stained cross-sections (Figure 13 a) and the quantification of IgA expression in stained cross-sections (Figures 13 b-d).

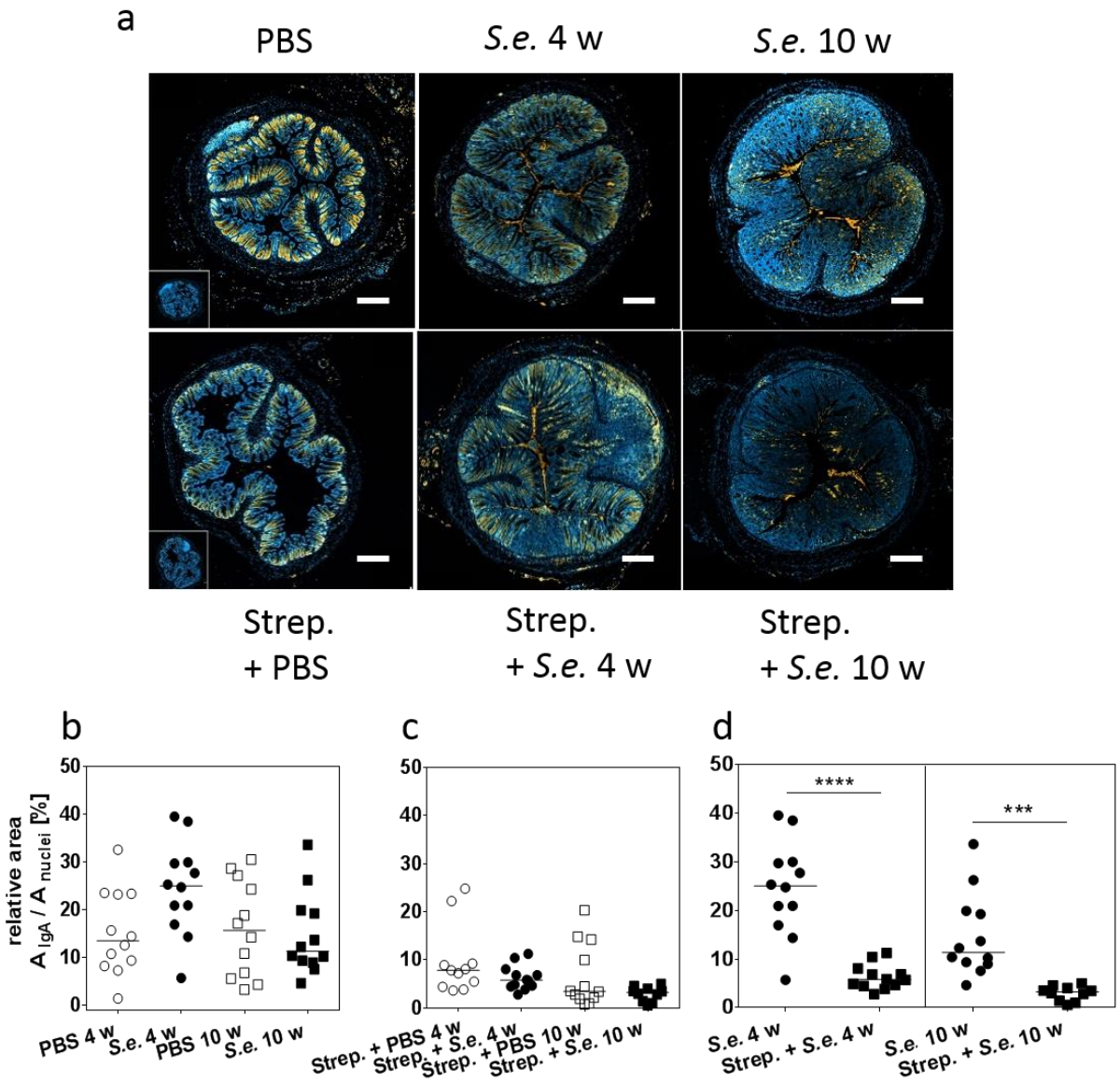

Figure 13: Analysis of IgA expression in the colon tissue of mice infected with *S. enterica*. Chronic infection was induced in C57BL/6 mice by oral inoculation with *S. enterica* (*S.e.*) without or with administration of a single dose of streptomycin (Strep.; 20 mg/mouse) 24 h prior to infection. Animals treated with PBS were used as negative control. Cross-sections of the distal colon were stained for immunoglobulin A; scale bars: 200 µm (a). Stained cross-sections (2 sections/mouse) were digitized by means of a slide scanner (AxioScan.Z1, Zeiss). Expression was quantified using the software program ImageJ® and is shown as marker-positive area relative to the DAPI-positive area (b-d). The median of individual data points is indicated. Statistics: One-way ANOVA followed by Dunn's multiple comparisons test. Number of animals per group = 6, Strep. + *S.e.* 10 w n=5 animals. ***P< 0.001, ****P< 0.0001.

No significant differences in the expression of IgA were observed between healthy and infected mice irrespective of antibiotic pretreatment at 4 or 10 weeks p.i. (Figures 13 b-c). However, if compared to *S.e.*-infected mice without streptomycin pretreatment the IgA expression in *S.e.*-infected animals

with antibiotic pretreatment was significantly reduced at both 4 weeks p.i. ($P<0.0001$) and 10 weeks p.i. ($P<0.001$) (Figure 13 d).

3.5 IL-22 deficiency impairs the intestinal epithelial barrier of the mice infected with *S. enterica*.

Investigation of the *S. enterica*-induced gut inflammation revealed contribution of IL-22 to the intestinal immune reaction against the pathogen. Therefore, additional aim of this study was to analyze the role of IL-22 in the development of chronic inflammation in the gut tissue in the model of bacteria-induced colitis.To investigate whether IL-22 depletion alters the local immune response against *S. enterica* infection in the gut, cross sections of the distal colon were stained using immunofluorescence. The analysis focused on exemplary markers relevant for the immune and function of epithelial barrier in the gut, i.e. CD3, RORγt, and Claudin-1.

3.5.1 CD3

Staining of the $CD3^+$ cells provided an insight into T cell number and location in the inflamed colon tissue. Thus, it allowed preliminary analysis of T cell contribution to the immune reaction against *S. enterica*, as well as the influence of IL-22-deficiency on the T cell response in the gut. Figure 14 shows microphotographs of CD3-stained cross-sections (Figure 14 a) and quantification of CD3 expression in stained cross-sections (Figures 14 b-d).

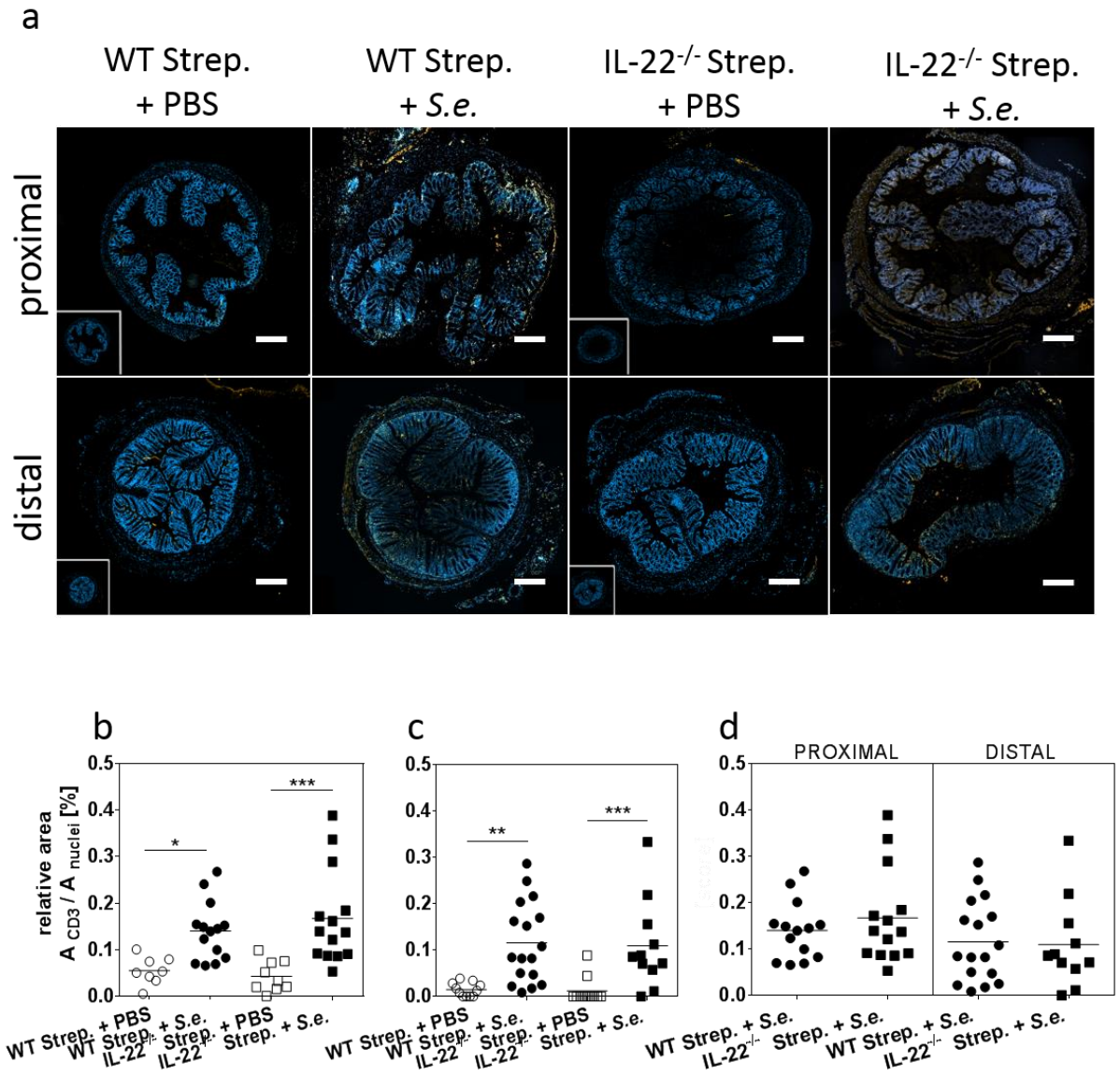

Figure 14: Analysis of CD3 expression in the colon tissue of mice infected with *S. enterica*. Chronic infection was induced in wild-type (WT) or IL-22-deficient (IL-22$^{-/-}$) C57BL/6 mice by oral inoculation with *S. enterica* (*S.e.*) with administration of a single dose of streptomycin (Strep.; 20 mg/mouse) 24 h prior to infection. Animals treated with PBS were used as negative control. Cross-sections of the proximal (b) and distal (c) colon were stained for the T cell marker CD3; scale bars: 200 µm (a). Stained cross-sections (2 sections/mouse) were digitized by means of a slide scanner (AxioScan.Z.1, Zeiss). Expression was quantified using the software program ImageJ® and is shown as marker-positive area relative to the DAPI-positive area (b-d) The median of individual data points is indicated. Statistics: One-way ANOVA followed by Dunn's multiple comparisons test. Number of animals per group: WT Strep. + PBS, IL-22$^{-/-}$ Strep. + PBS n=6, WT Strep. + *S.e.* n=12, IL-22$^{-/-}$ Strep. + *S.e.* n=9. *P<0.05; **P<0.01, ***P<0.001.

Four weeks post infection with *S. enterica*, WT animals showed significantly ($P<0.01$) elevated expression of CD3 in the analyzed cross-sections from both proximal and distal part of the colon if compared to the healthy control animals. This effect was even more pronounced ($P<0.001$) in the IL-22-deficient group (Figure 14 b-c). However, in direct comparison, there were no significant differences in the number of infiltrating T cells caused between WT and IL-22-deficient mice (Figure 14 d).

3.5.2 RORγt

Retinoic-acid related orphan receptor gamma t (RORγt) is one of the isoforms of the RORγ transcription factors and is expressed by various Th cells, e.g. Th17 and Th22 cells, which express IL-22 as well. Analysis of RORγt-positive (RORγt$^+$) cells provided insight whether IL-22-associated T cells were involved in the immune response against *S. enterica* infection in the colon tissue. Figure 15 shows microphotographs of RORγt-stained cross-sections (Figure 15 a) and the quantification of RORγt expression in stained cross-sections (Figures 15 b-d).

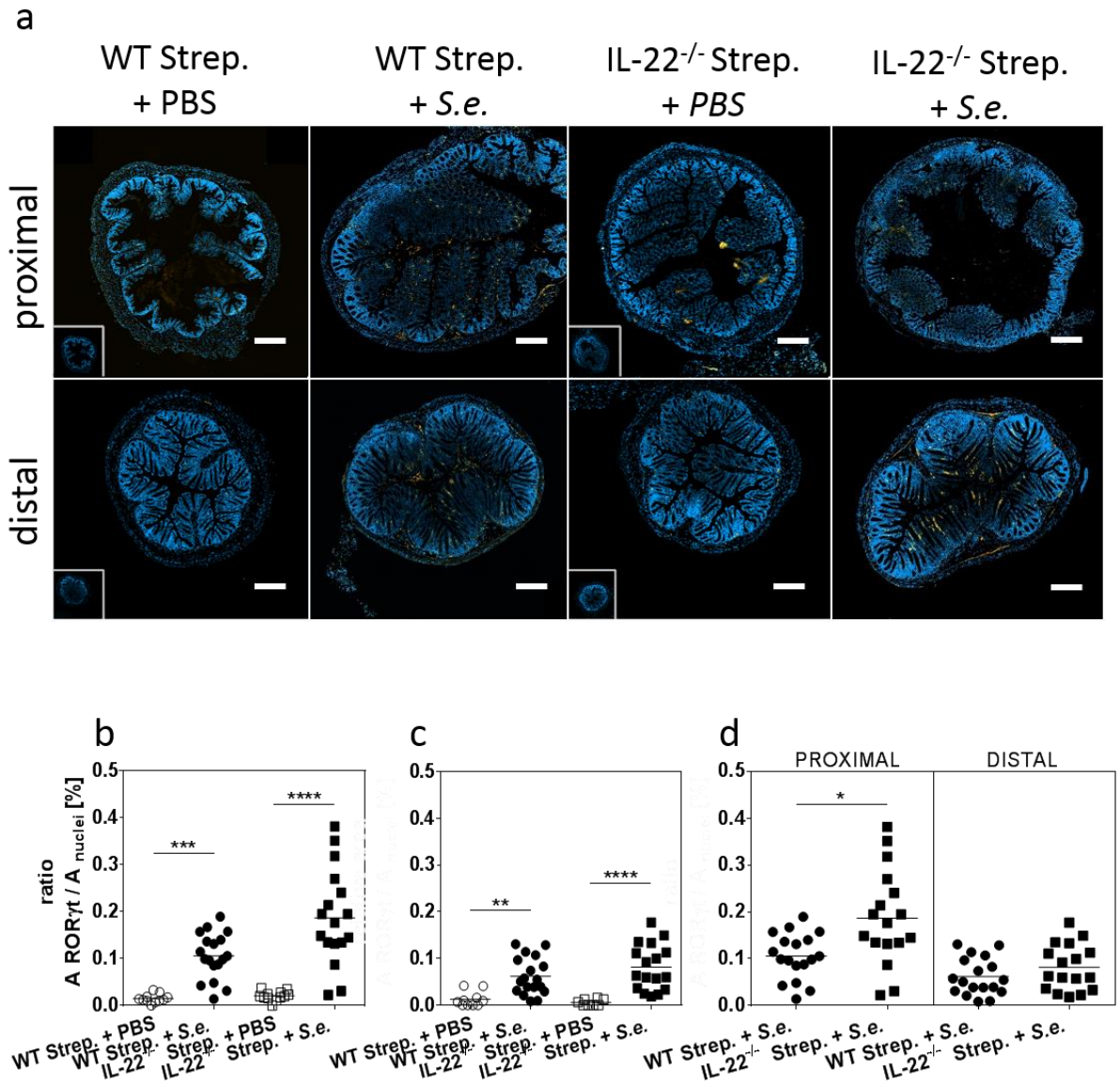

Figure 15: Analysis of RORγt expression in the colon tissue of mice infected with *S. enterica*. Chronic infection was induced in wild-type (WT) or IL-22-deficient (IL-22$^{-/-}$) C57BL/6 mice by oral inoculation with *S. enterica* (*S.e.*) with administration of a single dose of streptomycin (Strep.; 20 mg/mouse) 24 h prior to infection. Animals treated with PBS were used as negative control. Cross-sections of the proximal (b) and distal (c) colon were stained for the transcription factor RORγt; scale bars: 200 µm (a). Stained cross-sections (2 sections/mouse) were digitized by means of a slide scanner (AxioScan.Z.1, Zeiss). Expression was quantified using the software program ImageJ® and is shown as marker-positive area relative to the DAPI-positive area (b-d) The median of individual data points is indicated. Statistics: One-way ANOVA followed by Dunn's multiple comparisons test. Number of animals per group: WT Strep. + PBS, IL-22$^{-/-}$ Strep. + PBS n=6, WT Strep. + *S.e.* n=12, IL-22$^{-/-}$ Strep. + *S.e.* n=9. *P<0.05, **P<0.01, ****P<0.0001.

If compared to the PBS control group, a significantly ($P<0.001$) increased expression of RORγt was determined in the proximal colon tissue of WT animals at the 4 weeks p.i. This observation was even more pronounced ($P<0.0001$) in the IL-22-deficient group (Figure 15 b). The relation was similar in the distal part of the colon, where infiltration of RORγt-producing cells into the intestinal tissue was significantly higher ($P<0.01$) in infected WT mice and even more pronounced in infected IL-22-deficient mice (Figure 15 c). When the WT and IL-22$^{-/-}$ groups were directly compared at 4 weeks p.i., IL-22$^{-/-}$ mice revealed somewhat higher amounts of infiltrating RORγt^{+}, however this difference was only weakly significant ($P<0.05$) and occurred only in the proximal, but not in the distal part of the colon (Figure 15 d).

3.5.3 Claudin-1

Intestinal barrier is a crucial mechanism, protecting from invasion of pathogens into the mucosa during infection. Claudin-1 is a tight-junction protein and an important component of the connections between cells in the gut epithelium. Figure 16 shows microphotographs of Claudin-1-stained cross-sections (Figure 16 a) and the quantification of Claudin-1 expression in stained cross sections (Figures 16 b-d).

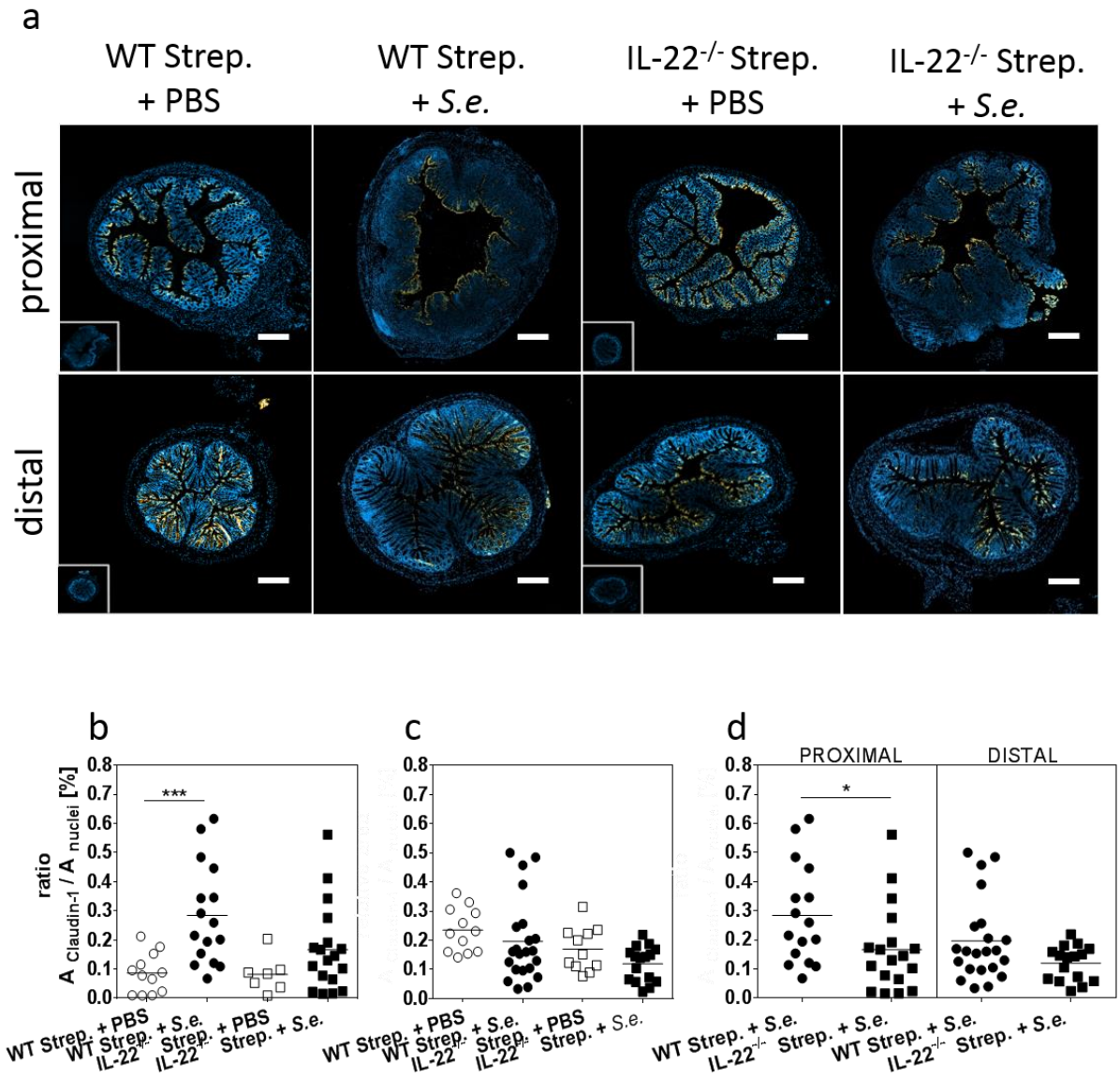

Figure 16: Analysis of Claudin-1 expression in the colon tissue of mice infected with *S. enterica*: Chronic infection was induced in wild-type (WT) or IL-22-deficient (IL-22$^{-/-}$) C57BL/6 mice by oral inoculation with *S. enterica* (*S.e.*) with administration of a single dose of streptomycin (Strep.; 20 mg/mouse) 24 h prior to infection. Animals treated with PBS were used as negative control. Cross-sections of the proximal (b) and distal (c) colon were stained for the TJ protein Claudin-1; scale bars: 200 µm (a). Stained cross-sections (2 sections/mouse) were digitized by means of a slide scanner (AxioScan.Z.1, Zeiss). Expression was quantified using the software program ImageJ® and is shown as marker-positive area relative to the DAPI-positive area (b-d) The median of individual data points is indicated. Statistics: One-way ANOVA followed by Dunn's multiple comparisons test. Number of animals per group: WT Strep. + PBS, IL-22$^{-/-}$ Strep. + PBS n=6, WT Strep. + *S.e.* n=12, IL-22$^{-/-}$ Strep. + *S.e.* n=9. *P<0.05; ***P<0.001.

WT mice infected with *S. enterica* showed significantly (P<0.001) higher expression of Claudin-1 in the proximal part of the colon in comparison to the PBS control group 4 weeks p.i. (Figure 16 b). At the same time point, expression of Claudin-1 in the proximal colon of IL-22-deficient mice stayed at the same level as in untreated animals. Analysis of Claudin-1 expression in the distal part of the colon did not show any significant differences caused by infection with the pathogen (Figure 16 c). In direct comparison, following *S. enterica* infection IL-22-defficient mice had significantly (P<.0.05) lower expression of Claudin-1 in the proximal part of the colon than WT animals. There were no differences observed in the distal colon (Figure 16 d).

3.6 The systemic *S. enterica*-specific antibody response is temporary increased by pretreatment with streptomycin

Starting 24 h p.i., peripheral blood was collected every 2 weeks and serum was analyzed by ELISA for *S. enterica*-specific antibodies (IgA, IgM, IgG1, and IgG2c). The obtained OD values proportionally related to the concentration of the analyzed antibodies and thus allowed the comparison of antibody levels in the serum of *S. enterica*-infected animals. IgA and IgM are markers of acute infection, whereas IgG is detectable in blood for an extended period after infection. Figure 17 shows the course of antibody levels in the sera of mice over the whole experiment.

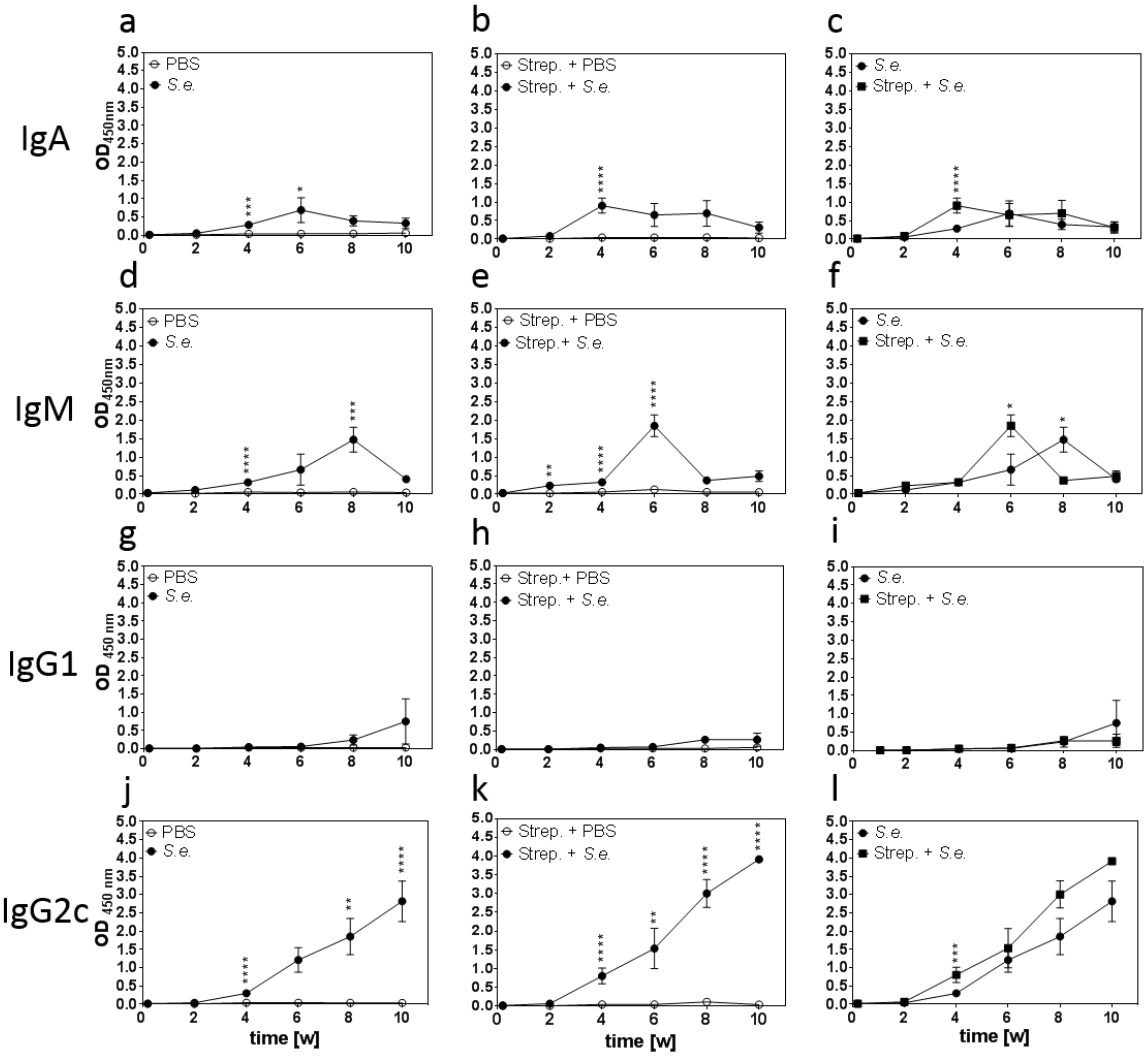

Figure 17: Course of the systemic antibody response analyzed in sera of mice infected with *S. enterica*. Chronic infection was induced in C57BL/6 mice by oral inoculation with *S. enterica* (*S.e.*) without or with administration of a single dose of streptomycin (Strep.; 20 mg/mouse) 24 h prior to infection. Animals treated with PBS were used as negative control. Blood was collected from mice every two weeks and serum was prepared and stored at -80 °C until analysis. Titers of the *S. enterica*-specific antibodies IgA (a-c), IgM (d-f), IgG1 (g-i), and IgG2c (j-l) were determined by ELISA. The OD values were measured at a wavelength of 450 nm using a microplate reader (Safire2, Tecan) and are shown as mean ± SD. Statistics: Two-way ANOVA followed by Holm-Sidak's multiple comparisons test. Number of animals per group: n=12 (0-4 w) or n=6 (6-10 w). *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

A significantly (P<0.001) higher concentration of IgA was detected in infected mice at 4 weeks p.i. (Figure 17 a). At the same time point, there was also a significantly elevated (P<0.0001) level of IgA in the serum of streptomycin-pretreated mice (Figure 17 b). In direct comparison, a significantly (P<0.0001) higher pathogen-specific IgA response could be observed after antibiotic pretreatment at 4 weeks p.i. (Figure 17 c). Similar to IgA, specific IgM antibodies were significantly (P<0.0001) increased in the serum of infected mice at 4 weeks p.i. (Figure 17 d). In streptomycin-

pretreated mice, a significantly (P<0.01) higher specific IgM serum level was already detected at 2 weeks p.i. and lasted until 6 weeks p.i. (P<0.0001) (Figure 17 e). Thus, streptomycin pretreatment caused an earlier specific IgM response (Figure 17 f). The amount of *S. enterica*-specific IgG antibodies was measured to investigate the course of the immune response in a later phase of infection. The titers of IgG2c and IgG1 isotypes served as markers for Th1 and Th2 response, respectively. IgG2c was found to be the dominant isotype, as only a minimal elevation of IgG1 was detected in the serum of infected mice but did not show a significant difference between healthy and infected mice (Figure 17 g-i). Analysis of the IgG2c response showed a significantly (P<0.0001) elevated IgG2c level in the serum of infected animals at 4 weeks p.i. (Figure 17 j). At the same time point, the IgG2c level was also significantly (P<0.0001) elevated in streptomycin-pretreated mice (Figure 17 k). In both groups, the pathogen-specific IgG2c response was accruing throughout the course of the experiment, with antibody levels in antibiotic-pretreated mice being significantly (P<0.001) higher at 4 weeks p.i. (Figure 17 l).

3.6.1 IL-22-deficient mice develop increased systemic IgA response against *S. enterica* in the early phase of infection

To investigate the impact of IL-22 on the systemic *S. enterica*-specific antibody response, starting 24 h p.i., peripheral blood was collected every 2 weeks and serum was analyzed by ELISA for *S. enterica*-specific antibodies (IgA, IgM and IgG2c). Analysis of antibody levels allowed investigation of the infection status in tested animals, considering its acute and chronic phase. Figure 18 shows the course of antibody levels in the sera of mice over the whole experiment.

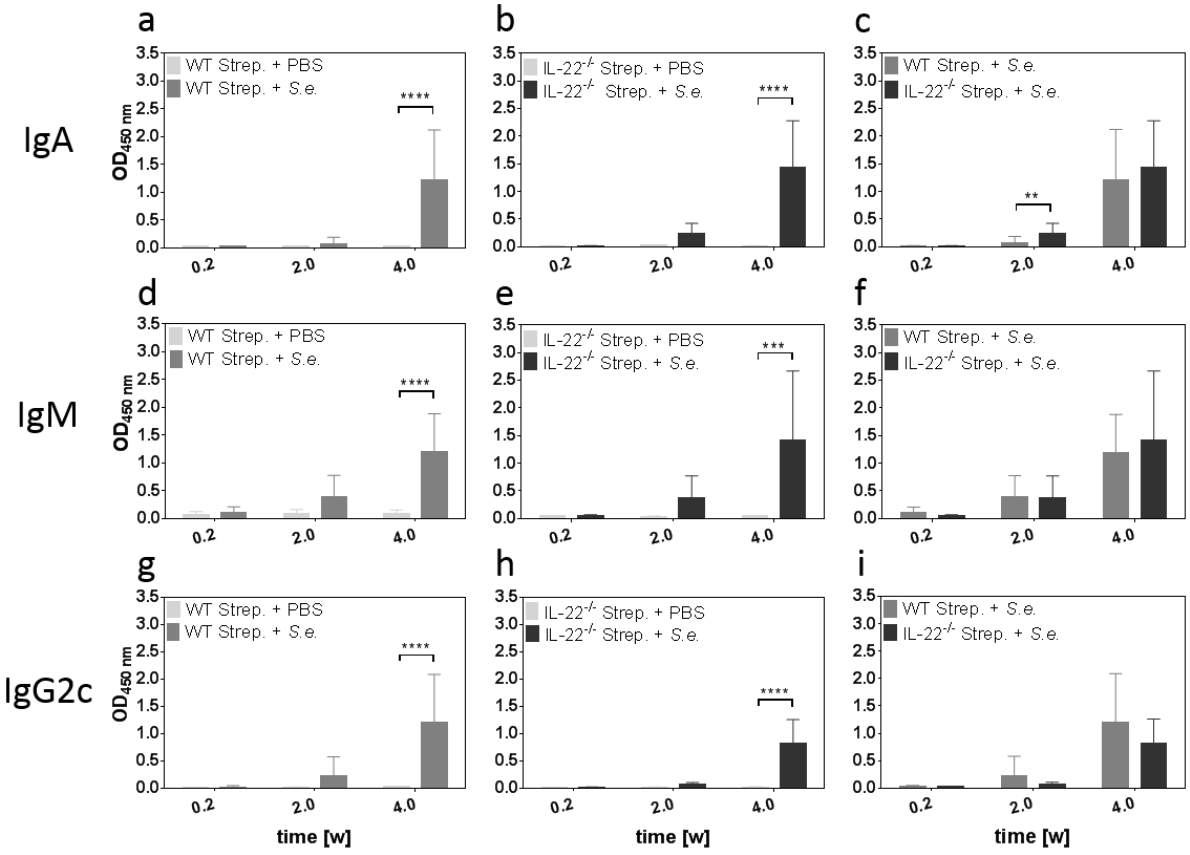

Figure 18: Course of the systemic antibody response analyzed in sera of mice infected with *S. enterica*. Chronic infection was induced in wild-type (WT) or IL-22-deficient (IL-22$^{-/-}$) C57BL/6 mice by oral inoculation with *S. enterica* (*S.e.*) with administration of a single dose of streptomycin (Strep.; 20 mg/mouse) 24 h prior to infection. Animals treated with PBS were used as negative control. Blood was collected from mice every two weeks and serum was prepared and stored at -80 °C until analysis. Titers of the *S. enterica*-specific antibodies IgA (a-c), IgM (d-f) and IgG2c (g-i) were determined by ELISA. The OD values were measured at 450 nm using a microplate reader (Safire 2, Tecan) and are shown as mean ± SD. Statistics: Two-way ANOVA followed by Holm-Sidak's multiple comparisons test. Number of animals per group: WT Strep. + PBS n=6, WT Strep. + *S.e.* n=11, IL-22$^{-/-}$ Strep. + PBS n=6, IL-22-/- Strep. + *S.e.* n=12 animals. **P<0.05; ***P< 0.001; ****P< 0.0001.

A significantly (P<0.0001) higher level of *Salmonella*-specific IgA was detected in infected WT mice at 4 weeks p.i. (Figure 18 a). At the same time point, there was also a significantly elevated (P< 0.0001) level of IgA in the serum of IL-22-deficient mice (Figure 18 b). In direct comparison, a significantly (P<0.05) higher increase of specific IgA antibodies was observed in IL-22$^{-/-}$ animals if compared to WT mice at 2 weeks p.i. (Figure 18 c). Similar to IgA, specific IgM antibodies were significantly increased in the serum of infected mice in both WT (P<0.0001) and IL-22-deficient (P<0.001) mice if compared to the PBS control group at 4 weeks p.i. (Figure 18 d-e). There were no significant differences in the titer of IgM in serum between WT and IL-22$^{-/-}$ mice at any time point of the experiment (Figure 18 f). The amount of *S. enterica*-specific IgG antibodies was measured to investigate the course of the

immune response in a later phase of infection. Analysis of the IgG2c response showed a significantly ($P< 0.0001$) elevated level in the serum of infected WT animals at 4 weeks p.i. (Figure 18 g). At the same time point, the IgG2c level was also significantly ($P< 0.0001$) elevated in IL-22-deficient animals (Figure 18 h). In both groups, the pathogen-specific IgG2c response increased over the course of the experiment in a similar kinetic (Figure 18 i).

4 Discussion

4.1 *S. enterica* infection in combination with streptomycin pretreatment provides a relevant model of intestinal inflammation

By causing numerous health issues, inflammatory diseases of the GI tract tremendously impair the quality of life of many people in both developed and developing countries. Therefore, profound understanding of the underlying mechanisms causing chronic inflammation in the intestine is indispensable in order to develop appropriate therapeutic and prevention strategies. Since the etiology of these disorders is multifactorial and still enigmatic, investigation in appropriate animal models with similar disease parameters is essential (133). Interestingly, IBD is often associated with an acute inflammatory incident triggered by bacteria or virus infection that disturbs homeostasis of the gut and results in dysregulation of the intestinal immune response (134). Considering this, the focus of this study was the establishment and scientific characterization of a mouse model using an appropriate biological agent to elicit an acute inflammatory reaction in the gut that finally results in chronic inflammation.

By combining a mouse model, which has been shown to be an appropriate tool for investigation of the key aspects considering intestinal bacterial infection (132) , with self-developed clinical and histopathological score, a comprehensive analysis of the triggered inflammatory reaction as well as key aspects of developed colitis was performed. The present study showed that infection of WT C57BL/6 mice with an attenuated strain of *S. enterica* serovar Enteritidis resulted in persistent infection with typical symptoms of intestinal inflammation, i.e. an elevated clinical score and histopathological aberrations in the colon tissue. Moreover, a single treatment with streptomycin one day prior to inoculation with bacteria caused aggravation of colitis symptoms and exacerbated histopathological aberrations in the colon tissue in the initial phase of infection. In this study, monitoring of colonization in the gut with the pathogen was performed by plating fecal pellets on selective XLD agar plates, which allows reliable recognition of *Salmonella* colonies. The analysis of *S. enterica* load in fecal pellets confirmed the aggravating impact of streptomycin on the acute phase of infection by causing significant pathogen colonization within 24 h p.i. These results confirm previous findings of Bohnhoff and Miller, who introduced the concept of streptomycin pretreatment for the first time (135). Furthermore, colonization of the gut with *S. enterica* was promoted by antibiotic pretreatment up to 2 weeks p.i. Thus, the observed results confirm that streptomycin pretreatment

increased susceptibility of WT mice to infection with *S. enterica* and accelerated development of clinical colitis symptoms as already

shown by other investigators (132). This effect was most likely caused by eradication of the physiological microbiota in the gut of these animals by antibiotic pretreatment, which allowed *S. enterica* to colonize more efficiently (136). Clinical signs of intestinal inflammation did not seem to be influenced by streptomycin pretreatment in the later phase of infection, since *S. enterica* load in fecal pellets was similar in mice with or without pretreatment starting from 4 weeks p.i. Even though streptomycin pretreatment was shown to eliminate >80% of the microbiota from the intestinal tract (137), animals most likely managed to partially restore a protective gut microbiota. Disruption of the gut microbiota composition has been shown to influence the susceptibility of the host to different diseases. IBD is associated with decreased colonization with *Faecalibacterium prausnitzii*, which is a commensal bacterium showing anti-inflammatory effects (138). Disturbed proportions of Firmicutes and Bacteroidetes combined with decreased diversity of the gut microbiota has been recognized in several mouse models and human patients (139–141) and was associated with the development of obesity. Certain species of the intestinal microbiota are promoting cellular immune responses against pathogens, e.g. segmented filamentous bacteria (SFB)-induced Th17 responses were shown to be competent in fighting intestinal infection with *C. rodentium* (142). Therefore, the differences between pretreated and not pretreated mice in the acute phase of infection observed within our study could be substantial in investigating the association between the intestinal microbiome and the host.

Composition of the commensal microbiota is dynamic and susceptible to changes associated with age, diet or antibiotic treatment. These factors can alter the gut homeostasis and therefore trigger the onset and exacerbation of IBD (143). Several studies have shown that the gut microbiota composition is significantly shifted in IBD patients (144, 145). It has also been demonstrated that the gut microbiota of patients suffering from IBD consisted of fewer bacterial species and was less stable if compared to healthy subjects (146). Moreover, different mouse models of IBD confirmed an expansion of *Enterobacteriaceae* and species belonging to this group, such as *Salmonella*, which altered the host immune defense in order to support their own colonization (147, 148). Additionally, many pathogen infections have been linked to the pathogenesis of IBD, particularly *Mycobacterium paratuberculosis* or *Escherichia coli* (149, 150). Also, an episode of *Salmonella* gastroenteritis has been recognized as a factor increasing the risk to develop IBD (151). According to the results of this study, *S. enterica* infection was able to disturb homeostasis in the gut and elicit an inflammatory reaction, which caused significant

alterations in the local immune response. Moreover, streptomycin pretreatment had a significant impact on the acute and even more explicitly on the chronic phase of inflammatory reaction *in situ*.

It has been shown that *Salmonella* invasion induced T cell activation (102). Furthermore, T cells were shown to be important triggers of intestinal inflammation after *S.* Typhimurium infection and depletion of $CD3^+$ T cells significantly attenuated intestinal immune responses against the pathogen (152). To analyze the immune response in the colon tissue in more detail, we analyzed T cells and inflammatory markers *in situ* by immunohistochemistry which is a prominent method for identifying and locating certain cells and proteins within a tissue, therefore it was possible to confirm that $CD3^+$ T cells were significant participants in the intestinal inflammatory reaction against *S. enterica*. Moreover, streptomycin pretreatment enhanced the infiltration of T cells into the tissue not only 4 weeks, but even more pronounced at 10 weeks p.i. CD4+ T lymphocytes are considered key players in the pathogenesis of human IBD, which was confirmed by their infiltration into the inflamed gut mucosa in IBD patients. Furthermore, a therapy based on a monoclonal anti-CD4 antibody showed efficacy in treatment of patients with chronic steroid-resistant IBD by immediate depletion of CD4 cells from the circulation (153, 154). Additionally, the essential role of T cells has been demonstrated in experimental model of adoptive transfer of naïve T cells into lymphopenic mice (155), which was dependent on the presence of the gut microbiota.

Further investigation of the local intestinal immune response in *S. enterica* infection confirmed a significant role of IFN-γ, which is an important factor in the host defense against microbial pathogens, in particular facultative intracellular bacteria (156). Additionally, mucosal expression of IFN-γ is essential for development and maintenance of the inflammatory reaction. It has been shown that this proinflammatory cytokine participates in modulation of the severity of CD (157). We demonstrated that *S. enterica* invasion caused an increase of IFN-γ-producing cells in the gut mucosa at 4 weeks p.i. This effect was amplified by streptomycin pretreatment, which induced a further increase of IFN-γ during the first 4 weeks p.i., but also up to 10 weeks p.i. Thus, our findings confirmed that IFN-γ significantly contributes to acute mucosal inflammation (158) and, even more important, is a main trigger of chronic gut inflammation in IBD (159).

IL-22 is a cytokine present on mucosal surfaces and plays an important role in the maintenance of a protective barrier. Furthermore, IL-22 is dramatically upregulated in response to various pathogenic infections (160). Our study confirmed that infection with *S. enterica* caused recruitment of IL-22-producing cells into the gut mucosa. Moreover, streptomycin pretreatment led to significantly increased IL-22 expression already 4 weeks p.i. IL-22 induction was also

remarkable in the later phase of the infection, supporting the concept that this cytokine not only enhances early host mucosal defense in response to pathogen invasion, but is also an essential part of tissue regeneration mechanisms in the chronic phase of infection (56, 161). IL-22 is
believed to have a strong association with IBD, considering that most of IL-22-associated molecules are encoded by IBD susceptibility genes (162). Thus, this cytokine may also have a therapeutic potential, especially due to its specific influence on tissue responses with no direct impact on the immune system (49).

The innate immune system plays an essential role in eliminating pathogenic bacteria and maintaining a homeostatic environment in the intestinal mucosa (34). However, also the adaptive immune system contributes to the first line of defense against enteric invaders by the production of sIgA antibodies, which bind pathogens already in the gut lumen (163). Nonetheless, production of sIgA is not crucial for pathogen clearance. It has been shown, that IgA-deficient mice infected with *S.* Typhimurium have an equal ability to clear the pathogen from the gut as the wild-type animals (164, 165). In the present study, sIgA production was not significantly induced at 4 and 10 weeks p.i., indicating that in the later phase of *S. enterica* infection rather the maintenance of homeostasis than proinflammatory action is the main role of this antibody. Macpherson et al. demonstrated that even though sIgA is the main immunoglobulin present in healthy intestinal mucosa, patients with active IBD produce higher concentrations of IgG, but not sIgA. This indicates that relapse of IBD is characterized by an exaggerated mucosal antibody response as a result of lost tolerance to the commensal microbiota in the gut (166).

It has been proven that IBD patients presenting the most complex and problematic clinical course of disease possess high serum reactivity of IgA and IgG antibodies against intestinal microbes (167). Furthermore, production of antibodies against extracellular molecules of gut microorganisms has been associated with IBD. Together with specific antibodies directed against certain host structures (e.g. antibodies to exocrine pancreas or anti-neutrophil cytoplasmic antibodies (ANCA)), these antibodies have been recognized as serum biomarkers for CD and UC (168). In this study, the specific humoral immune response against *S. enterica* (i.e. detection of *S. enterica*-specific serum antibodies levels) was quantified using an ELISA that has been previously developed and validated in our laboratory (98). In contrast to the reduced local IgA response in the gut, the production of specific systemic IgA was induced by streptomycin pretreatment after 4 weeks p.i. in infected mice. This result is in accordance with the fact that secretory and systemic IgA are derived from different cellular sources and can be stimulated independently from each other (169, 170). Furthermore, streptomycin pretreatment induced a late and transient specific IgM response after *S. enterica* infection. Additionally, a constantly rising level of *Salmonella*-specific IgG2c antibodies, which was

enhanced by antibiotic pretreatment, indicated development of a chronic inflammation caused by persisting colonization with the pathogen and driven by Th1 cytokines (171). In contrast, a modest specific IgG1 response that was independent of streptomycin pretreatment indicated a weak *Salmonella*-induced Th2 response, obviously not affected by antibiotic pretreatment.

According to several epidemiologic studies and experimental data, environmental factors play a crucial role in the development of IBD. Antibiotic therapy in combination with enteric infections potentially impairs the intestinal barrier as well as the composition of the gut microbiota, which are key elements in the pathogenesis of IBD (172). Moreover, it has been shown that patients diagnosed with IBD were more likely to have received antibiotic therapy 2-5 years before diagnosis (173). Thus, the influence of these two factors on the intestinal immune system may be a critical component, which should be extensively considered in the management of IBD. Our study introduced a mouse model of chronic *S. enterica* infection with combination of streptomycin pretreatment that facilitated to understand the mechanisms involved in the pathophysiology of intestinal inflammation. We observed a chronic inflammation in the colon tissue with typical clinical symptoms, immunological aberrations and pathohistological features, which are also found in patients suffering from IBD. Therefore, one result of this study was the establishment of a relevant model of intestinal inflammation that reflects a possible variant of natural etiology of IBD, and thus, could be of great benefit for the development of efficient therapeutic strategies in human IBD.

4.2 IL-22 plays a protective role in intestinal inflammation by regulating the structure and function of the gut barrier

IL-22 is thought to have a relevant association to intestinal inflammation, including the ability to promote mucosal repair and restoration by inducing proliferative and anti-apoptotic pathways as well as production of antimicrobial peptides (174). Mucosal healing is one of the current therapeutic targets in treatment of IBD. Therefore, further investigation of IL-22 and its clinical relevance concerning inflammatory diseases of the gut may clarify the mechanisms involved in IBD pathogenesis. IL-22 was shown to be protective to the host in many infectious and inflammatory disorders. However, considering its inherent proinflammatory properties, it can also act destructively, especially when released in combination with other cytokines, such as IL-17 (161).

In the first part of this study, investigation of the mouse model of chronic *S. enterica* infection revealed a significant contribution of IL-22 in the immune response against this pathogen in the colon tissue. On the other hand, Behnsen et al. claimed that colonization of the gut with *S.* Typhimurium is

increased in IL-22-expressing mice (160). IL-22 has been shown to alleviate infection by some pathogens, such as *C. rodentium* or *Plasmodium chabaudi* (56, 175), whereas *Toxoplasma gondii* and *Listeria monocytogenes* infection models revealed its pathogenic character (176, 177). Therefore, the second part of this study dealt with the role of IL-22 in the immune response against *S. enterica* infection in the previously established model. In order to explore this question, oral *Salmonella* infection in combination with streptomycin treatment was induced in IL-22-deficient mice.

The analysis of clinical symptoms after pathogen inoculation with streptomycin pretreatment showed that both WT and IL-22-deficient animals were strongly affected by the infection. However, considering the stage of disease where symptoms such as weight loss and diarrhea were accruing in both groups, the mice with IL-22 deficiency suffered from significantly stronger colitis symptoms. This implies a protective function of IL-22 in *S. enterica*-induced gut inflammation, but only in an early phase of the infection. The more pronounced clinical symptoms in the IL-22-deficient mice might have been caused by a compromised barrier in the intestinal mucosa and therefore enhanced invasion of *S. enterica* into the colon tissue. This confirms previous findings showing that IL-22 deficiency leads to facilitated access of pathogenic bacteria into the gut tissue (56). IL-22 promotes maintenance of epithelial barrier integrity by inducing production of AMPs, mucus proteins as well as strengthening the TJs (60). Thus, the absence of this cytokine might have resulted in impaired defense mechanisms against pathogens and therefore increased colonization with *S. enterica*. This hypothesis has been already confirmed in a T cell transfer model, where IL-22 was shown to have a protective effect against experimental colitis (178).

As shown in our model of chronic *S. enterica* infection, this pathogen colonizes the GI tract causing a notable inflammatory reaction in the gut. Therefore, determination of the pathohistological changes in the colon tissue was an essential part of the analysis of the role of IL-22 in the management of bacteria-induced colitis. WT as well as IL-22-deficient animals showed aberrations in the morphology of the colon tissue, which was confirmed by an independent pathologist. Infiltration of immune cells, such as lymphocytes, granulocytes and macrophages, along with lesions in the structure of the tissue were significant. Similar manifestations characteristic for colitis were previously found in other mouse models of IBD as well as in humans (179). Direct comparison of WT and IL-22-deficient mice revealed no

significant differences in the assessed histopathological score, neither in the proximal nor in the distal part of the colon. Considering a similar clinical and histopathological score at the end of the experiment, IL-22 seems to have an impact in the initial rather than the chronic stage of *S. enterica* infection. This suspicion is also reflected by the analysis of *S. enterica* colonization in the

gut, where a significantly higher load of the pathogen was observed in IL-22-deficient mice up to 2 weeks p.i. An important role of IL-22 on the acute-phase response was highlighted by Liang et al., who showed influence of this cytokine on systemic biochemical, cellular, and physiological parameters and local tissue inflammation (180). IL-22 was shown to play a minor role in the immune response during systemic acute infection with *Trypanosoma cruzi* (181), however its protective actions are likely more important at barrier surfaces. Therefore, it would be necessary to test the effect of IL-22 deficiency on histological aberrations based on colon tissue collected at early time points after *S. enterica* infection, which could not be accomplished within this study due to the limited number of IL-22-deficient mice.

Production of mucus is a crucial mechanism involved in protection of the intestinal barrier by limiting adherence of pathogenic bacteria to the intestinal epithelium. As a consequence, infection and inflammation cause an increased activity of mucus-producing goblet cells (182). In order to evaluate the production of mucus in the colon tissue of *Salmonella*-infected mice, a differential staining of goblet cells was performed using the broadly accepted histochemical PAS reaction. Interestingly, this analysis revealed a decreased amount of mucus in the proximal colon of IL-22-deficient mice, whereas in the distal colon it was significantly increased. This is in accordance with the histopathological score that was significantly higher in the proximal part of the colon. Normally, infection with a pathogen and the consequent inflammatory reaction trigger accelerated secretion of mucins. However, prolonged and exhaustive stimulation leads to depletion of goblet cells as well as alterations in mucus layers (183). The results of this study indicate stronger inflammation in the proximal part of the colon in IL-22-deficient mice, causing loss of goblet cells. In direct comparison of WT and IL-22-deficient mice, animals lacking IL-22 showed a significantly decreased amount of mucus in the proximal colon. This was also apparent on the PAS-stained cross-sections, where a massive loss of goblet cells and alterations of the epithelial cell barrier were observed. A similar impact of IL-22 on the regulation of goblet cell differentiation was already shown in a model of intestinal helminth infection, where IL-22-deficient mice had reduced expression of MUC2 if compared to WT animals (184). These findings suggest a significant function of IL-22 in the maintenance of the mucosal barrier. Moreover, it was shown that increased expression of IL-22 improves the condition of goblet cells in a spontaneous mouse model of colitis (104). Considering these data, IL-22 seems to play a protective role during colitis by preventing loss of mucus-producing cells as well as improving structure and function of the mucosal barrier in the intestine which is reflected in our *in vivo* model.

In addition to the analysis of pathohistological aberrations and production of mucus by goblet cells, immunohistochemical staining of certain immune cells and markers was performed to characterize the local immune response against *S. enterica* in the colon in more detail. Considering the massive

infiltration of $CD3^+$ cells in the model of *S. enterica*-induced colitis, it was interesting to determine the impact of IL-22 on T cell recruitment into the inflamed colonic tissue. It has already been shown that IL-22 diminishes the T cell response in an experimental model of malaria (185) and that IL-22 plays a protective role in T-cell mediated hepatitis (186). However, IL-22 deficiency did not cause any changes in CD3 expression. The IL-22 receptor is only present on non-hematopoietic cells, therefore this cytokine can particularly act on epithelial cells, keratinocytes, and hepatocytes (161). Thus, a direct influence of IL-22 on the infiltration of T lymphocytes can be excluded and the function of IL-22 in the GI tract should be related to the maintenance of the epithelial barrier, which supports our results of decreased production of mucus in IL-22-deficient mice.

RORγt is expressed by various cell types, but primarily by potentially IL-22-producing cells, such as Th22, Th17, and ILC3 (49). Treg cells expressing RORγt were shown to act as stable suppressive agents on T effector cells during intestinal inflammation (187). Accordingly, we observed a significantly increased expression of this transcription factor in colon tissue of mice suffering from bacteria-induced colitis. Additionally, infiltration of $ROR\gamma t^+$ cells was notably stronger in the proximal colon of IL-22-deficient mice if compared to the WT group. Enhanced production of IL-22 by ILCs and γδT cells due to induced binding of RORγt to the IL-22 promoter has been reported to attenuate colitis in a mouse model of *C. rodentium* infection (188). In *S. enterica*-induced intestinal inflammation, an increased expression of RORγt could be explained by the stronger inflammatory reaction in the proximal part of the colon due to IL-22 deficiency. However, in order to reveal the identity of RORγt-expressing cells, they would have to be further characterized by specific markers for Th22, Th17, and ILC3. Additionally, double immunohistochemical staining could be helpful to analyze cells responsible for the massive IL-22 expression in the colon tissue of *S. enterica*-infected mice, which was observed in the first part of the study.

The intestinal epithelial monolayer plays a crucial role in preventing invasion of pathogenic bacteria into the tissue by forming a protective mucus barrier. Claudins are membrane proteins that are major structural components of TJs forming the seal between epithelial cells, thereby maintaining the structure of the epithelial barrier (189). Inflammation of the intestinal tissues is associated with altered TJs structures and increased permeability as well as increased production of IL-22 (64). In this study, it was shown that IL-22-deficient mice suffering from *S. enterica*-induced colitis have a reduced expression of Claudin-1 in the colon tissue if compared to WT animals, which was significantly pronounced in the proximal part of the colon. This result suggests a meaningful function of IL-22 in the regulation of intestinal epithelial barrier permeability by regulation of Claudin-1 expression under inflammatory conditions. Other investigators previously showed that IL-22 improved the barrier function of the intestine by upregulating expression of

Claudin-1 (190). Using semiquantitative immunohistochemical staining of Claudin-1 in the mouse model of the bacteria-induced colitis, this study confirmed a stimulating action of IL-22 on the Claudin-1 levels *in vivo*. This result is in agreement with the clinical score, which was higher for the IL-22-deficient animals, implying that deficiency of IL-22 led to aggravated clinical symptoms due to an altered intestinal epithelial barrier function and consequently facilitated bacterial invasion. Loss of TJ proteins and consequently a weakened gut barrier was also observed in IBD patients (191). The impact of IL-22 on the expression of Claudin-1 further confirms a protective role of this cytokine during inflammation of the GI tract.

IL-22 is known to significantly contribute to the host defense against microbes at mucosal sites. Its role in promoting protective systemic immune responses against *Clostridium difficile* was reported to be critical (192). However, this study did not reveal any significant impact of IL-22 on the specific systemic adaptive immunity against *S. enterica*. Merely production of *Salmonella*-specific IgA serum antibodies was significantly faster and higher in IL-22-deficient mice 14 days p.i. However, it was approximately at the same level as in WT animals at day 29 p.i. It is noteworthy that structure and function of IgA present in the serum is notably different to IgA produced in the gut mucosa, which has a direct impact on bacterial colonization, e.g. through obstruction of anchorage to the mucosal cells. In contrast, systemic IgA is additionally produced in bone marrow and upon binding triggers mechanisms that lead to impairment of pathogenic microbes (193). Therefore, immunohistochemical staining of *S. enterica*-specific IgA in the intestinal tissue should be performed in the future.

In recent years, the role of IL-22 in the defense against bacterial and viral infections has been studied in a number of models. Our study provides results that foster the protective function of this cytokine in intestinal infection. It has been shown that IL-22 ameliorates intestinal inflammation in the mouse T-cell transfer and DSS models (178). Here, we demonstrate a similar protective action of IL-22 in the bacteria-induced model of colitis. To further investigate the possible therapeutic potential of IL-22, local treatment of the inflamed gut of *S. enterica*-infected mice with exogenous IL-22 would be a suitable approach. A novel microinjection-based local gene-delivery system that is capable of targeting the inflamed intestine might be a useful for this purpose (59). Thus, detailed analysis of IL-22 function in the intestinal mucosa as well as the microbiota could be aimed in further studies using the mouse model established in this work.

4.3 Streptomycin-mediated eradication of the commensal microbiota caused a more pronounced and long-lasting Th1 response

Infection with *S. enterica* triggers a rapid innate and adaptive immune response. Even though *Salmonella* inhabits intra-macrophage phagosomes, the T cell response is believed to be essential for resolving the infection (100). Th1 cytokines, e.g. IFN-γ and TNF-α, play a crucial role in the early stages of infection by inducing the production of reactive oxygen by macrophages as well as other mechanisms promoting confinement and clearance of the pathogen (98, 194). It has been shown that mice lacking IFN-γ have a significantly compromised ability to resolve *S. enterica* infection (195). One of the major sources of IFN-γ is represented by activated *S. enterica*-specific effector T cells, which are indispensable for control of primary and secondary *Salmonella* infections (98). Oral infection of mice triggers the activation of antigen-specific T cells in PPs and MLNs. Activated T cells acquire Th1 effector functions to finally stimulate infected macrophages through the release of IFN-γ (100). Furthermore, an effective immune response against *S. enterica* infection requires the contribution of B lymphocytes that are stimulated by inflammatory cytokines to produce *S. enterica*-specific antibodies (98, 196, 197). Our study of the model of *S. enterica*-induced colitis revealed a significant contribution of IFN-γ-producing cells in the local immune response in the intestinal tissue. Unexpectedly, streptomycin pretreatment induced a further increase of these cells during the first 4 weeks p.i. that was sustained up to 10 weeks p.i. This result confirmed that IFN-γ efficiently contributes to acute mucosal inflammation (158) and supports the assumption that IFN-γ is a main trigger of chronic gut inflammation in IBD (159). Th1 cells represent the major source of IFN-γ during chronic inflammation, suggesting a strong Th1 bias following streptomycin pretreatment. It was shown that survival of *S.* Typhimurium infection required activation of macrophages by a Th1 response (198). In addition, IFN-γ signaling was shown to be essential for the confinement of pathogen load in the mucosa (199). Our data promotes the concept that IFN-γ is one of the key factors in chronic *S. enterica* infection controlling the immune status of persistently infected mice. The chronically high pathogen load in bacterial faeces indicated that the infection could not be cleared and consequently, IFN-γ was continuously produced to activate infected macrophages. However, expression of this cytokine was not elevated in the tissue of mice not pretreated with the antibiotic. Thus, streptomycin pretreatment most likely led to the dominance of *S. enterica* over commensals in the early phase of infection. As a consequence, *S. enterica*-specific IFN-γ-producing (Th1) cells were activated and maintained their activity throughout the chronic infection phase. In the course of infection without antibiotic pretreatment, the competing exclusion principle prevented invasion of *S. enterica* and consequently critically increased and prolonged *S. enterica*-specific Th1 activation. Moreover, we observed that streptomycin treatment prior to *S. enterica* inoculation

caused significantly decreased sIgA production in the gut mucosa. In combination with the finding of an increased number of IFN-γ-producing cells this suggests that streptomycin promotes a Th1 immune response in the colon tissue. This is supported by the fact that IFN-γ inhibits the development of IgA-producing B cells in the gut and thus reduces the delivery of sIgA (200). Furthermore, the tendency towards a Th1 response in this model might have inhibited sufficient production of sIgA, since B cell differentiation in Peyer's patches requires production of Th2 cytokines (201). In mice, the Th2 cytokine IL-5 represents an important co-factor for the production of IgA. (202) This study showed that streptomycin not only exacerbated the inflammatory response to *S. enterica* infection, but also promoted a local Th1 response against pathogens in the colon as illustrated by a contemporaneous increase of IFN-γ-producing cells and decreased production of sIgA in the gut. This was also confirmed by the profile of systemic *S. enterica*-specific IgG1 and IgG2c antibody responses. *S. enterica*-infected mice developed increased levels of *Salmonella*-specific IgG2c but no IgG1 serum antibodies, indicating a dominant activity of the Th1 lymphocyte subpopulation (98). The same effect was observed after administration of streptomycin prior to infection, thus pretreatment with antibiotic did not influence the systemic Th1/Th2 balance in this model. However, streptomycin pretreatment prolonged the occurrence of a robust local Th1 response in the gut, which might be critical for the development of chronic colitis in this model. Further investigation of the involved T cell subsets and cytokines could reveal the influence of streptomycin on the intestinal immune response in more detail.

5 Summary

Dissertation to obtain the academic grade Dr.rer.med.

Establishment and characterization of a mouse model of chronic *Salmonella enterica* infection as a proposed animal model for human inflammatory bowel disease.

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An appropriate immune response in the intestine against commensal and pathogenic bacteria primarily depends on the intestinal immune system. Presence of the microbiota is essential for an appropriate function of the intestinal immune system and can be severely disrupted by antibiotic treatment, leading to disturbance of the balance between tolerance and immunity and possibly to serious chronic conditions, e.g. IBD. Therefore, the aim of this study was to establish a bacteria-induced mouse model of human IBD applicable for investigation of the role of commensal versus pathogenic bacteria in the onset and development of chronic intestinal inflammation.
The mouse model introduced in the present work focused on chronic inflammatory reaction triggered by *S. enterica* infection. Furthermore, differences between the acute and chronic phase of

infection were analyzed. Additionally, it was investigated, whether antibiotic treatment prior to infection has a significant impact on the course of the systemic or local mucosal immune response against *S. enterica*. Due to the significant increase of the IL-22 level reported from IBD patients, and the assumed crucial role of this immunoregulatory cytokine for human IBD a special focus of this study was to analyze the role of IL-22 in the established mouse model of bacteria-induced chronic colitis.

Inoculation of C57BL/6 mice with *S. enterica* subsequently to streptomycin treatment resulted in a persistent infection and chronic inflammation in the intestine. Antibiotic pretreatment significantly aggravated clinical symptoms and histological features, particularly in the acute phase of infection. IL-22 deficiency caused further exacerbation of the symptoms. An impact of streptomycin pretreatment on the early phase of infection was also confirmed by a significantly increased presence of bacteria in faecal pellets up to 2 weeks p.i. Mice lacking IL-22 were more susceptible to colonization with *S. enterica*, particularly in the early phase of infection. Streptomycin pretreatment caused aggravation of pathohistological changes in the colon tissue caused by oral infection with *S. enterica*. IL-22 deficiency had no significant impact on histological aberrations. However, it increased loss of mucus-producing goblet cells, which is a typical symptom of an inflammatory reaction in the colon tissue. Analysis of the local immune response against *S. enterica* showed that antibiotic pretreatment significantly promoted infiltration of T cells into the colon tissue in both the acute and the chronic phase of infection. Streptomycin pretreatment resulted in a significant alteration of IL-22 production in the colon. Furthermore, the number of IFN-γ-producing cells and the production of secretory IgA were significantly altered following streptomycin pretreatment. Although, IL-22 deficiency had no impact on T cell recruitment it compromised the intestinal epithelial barrier of *S. enterica*- infected mice by reducing the level of claudin-1 in the gut epithelium. Additionally, the number of RORγt-producing cells was significantly increased in the colon tissue of mice lacking IL-22. An impact of streptomycin pretreatment on the *S. enterica*-specific systemic antibody response resulted in increased levels of IgA and IgM serum antibodies in the early phase of infection. A continuously rising IgG2c response up to 10 weeks p.i was also observed, however was not dependent on administration of the antibiotic. IL-22-deficient mice developed a significantly increased systemic IgA response against *S. enterica* in comparison to WT animals.

In summary, this study convincingly demonstrates that antibiotic pretreatment exacerbates the inflammatory reaction against *S. enterica.* Combination of *S. enterica* infection with streptomycin pretreatment results in a chronic inflammation of the colon tissue with typical clinical symptoms, immunological aberrations, and pathohistological features that are characteristic for IBD. Therefore, this approach provides a relevant model of chronic intestinal inflammation, which might

reflect a possible scenario of the pathogenesis of human IBD. The present study emphasizes a protective role of IL-22 in intestinal inflammation and its crucial function in maintenance of the gut barrier, therefore further extended analysis of its role in intestinal mucosa should be performed in future studies using this *in vivo* model.

Interestingly, this work showed that eradication of gut microbiota resulting from treatment with streptomycin triggers a more pronounced and robust Th1 response against *S. enterica* infection. This effect might be crucial for the development of chronic colitis in this model and should be further investigated since it might also be relevant for human IBD.

6 Literature

1. Holmgren J. Mucosal immunity and vaccines. Nat Med 2005; 11(4 Suppl):45-53.

2. Nagler-Anderson C. Man the barrier! Strategic defences in the intestinal mucosa. Nat Rev Immunol 2001; 1(1):59–67.

3. Vighi G, Marcucci F, Sensi L, Di Cara G, Frati F. Allergy and the gastrointestinal system. Clin Exp Immunol 2008; 153 Suppl 1:3–6.

4. Mattos BRR de, Garcia MPG, Nogueira JB, Paiatto LN, Albuquerque CG, Souza CL, Fernandes LG, Tamashiro WM, Simioni PU. Inflammatory Bowel Disease: An Overview of Immune Mechanisms and Biological Treatments. Mediators Inflamm 2015; 2015:493012.

5. Prince BT, Mandel MJ, Nadeau K, Singh AM. Gut Microbiome and the Development of Food Allergy and Allergic Disease. Pediatr Clin North Am 2015; 62(6):1479–92.

6. Pastorelli L, Salvo C de, Mercado JR, Vecchi M, Pizarro TT. Central role of the gut epithelial barrier in the pathogenesis of chronic intestinal inflammation: lessons learned from animal models and human genetics. Front Immunol 2013; 4:280.

7. Turner JR. Intestinal mucosal barrier function in health and disease. Nat Rev Immunol 2009; 9(11):799–809.

8. Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat Rev Immunol 2014; 14(3):141–53.

9. Kraehenbuhl JP, Neutra MR. Epithelial M cells: differentiation and function. Annu Rev Cell Dev Biol 2000; 16:301–32.

10. Miron N, Cristea V. Enterocytes: active cells in tolerance to food and microbial antigens in the gut. Clin Exp Immunol 2012; 167(3):405–12.

11. Zihni C, Mills C, Matter K, Balda MS. Tight junctions: from simple barriers to multifunctional molecular gates. Nat Rev Mol Cell Biol 2016; 17(9):564–80.

12. Hansson GC, Johansson ME. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. Gut Microbes 2010; 1(1):51–4.

13. Velcich A, Yang W, Heyer J, Fragale A, Nicholas C, Viani S, Kucherlapati R, Lipkin M, Yang K, Augenlicht L. Colorectal cancer in mice genetically deficient in the mucin Muc2. Science 2002; 295(5560):1726–9.

14. van der Sluis M, Koning BAE de, Bruijn ACJM de, Velcich A, Meijerink JPP, van Goudoever JB, Büller HA, Dekker J, Van Seuningen I, Renes IB, Einerhand AW. Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. Gastroenterology 2006; 131(1):117–29.

15. Bevins CL, Salzman NH. Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. Nat Rev Microbiol 2011; 9(5):356–68.

16. Uematsu S, Fujimoto K, Jang MH, Yang B-G, Jung Y-J, Nishiyama M, Sato S, Tsujimura T, Yamamoto M, Kiyono H, Miyasaka M, Ishii KJ, Akira S. Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. Nat Immunol 2008; 9(7):769–76.

17. Bain CC, Mowat AM. Macrophages in intestinal homeostasis and inflammation. Immunol Rev 2014; 260(1):102–17.

18. Kayama H, Ueda Y, Sawa Y, Jeon SG, Ma JS, Okumura R, Kubo A, Ishii M, Okazaki T, Murakami M, Yamamoto m, Yagita H, Takeda K. Intestinal CX3C chemokine receptor 1(high) (CX3CR1(high)) myeloid cells prevent T-cell-dependent colitis. Proc Natl Acad Sci U S A 2012; 109(13):5010–5.

19. Kobayashi M, Kweon M-N, Kuwata H, Schreiber RD, Kiyono H, Takeda K, Akira S. Toll-like receptor-dependent production of IL-12p40 causes chronic enterocolitis in myeloid cell-specific Stat3-deficient mice. J Clin Invest 2003; 111(9):1297–308.

20. Tait Wojno ED, Artis D. Innate lymphoid cells: balancing immunity, inflammation, and tissue repair in the intestine. Cell Host Microbe 2012; 12(4):445–57.

21. Qiu J, Heller JJ, Guo X, Chen Z-mE, Fish K, Fu Y-X, Zhou L. The aryl hydrocarbon receptor regulates gut immunity through modulation of innate lymphoid cells. Immunity 2012; 36(1):92–104.

22. Behnsen J, Raffatellu M. Keeping the peace: aryl hydrocarbon receptor signaling modulates the mucosal microbiota. Immunity 2013; 39(2):206–7.

23. Cella M, Colonna M. Aryl hydrocarbon receptor: Linking environment to immunity. Semin Immunol 2015; 27(5):310–4.

24. Wang J, Wang P, Tian H, Tian F, Zhang Y, Zhang L, Gao X, Wang X. Aryl hydrocarbon receptor/IL-22/Stat3 signaling pathway is involved in the modulation of intestinal mucosa antimicrobial molecules by commensal microbiota in mice. Innate Immun 2018; 24(5):297–306.

25. Mowat AM, Agace WW. Regional specialization within the intestinal immune system. Nat Rev Immunol 2014; 14(10):667–85.

26. Maloy KJ, Kullberg MC. IL-23 and Th17 cytokines in intestinal homeostasis. Mucosal Immunol 2008; 1(5):339–49.

27. Kayama H, Takeda K. Functions of innate immune cells and commensal bacteria in gut homeostasis. J Biochem 2016; 159(2):141–9.

28. Keubler LM, Buettner M, Häger C, Bleich A. A Multihit Model: Colitis Lessons from the Interleukin-10-deficient Mouse. Inflamm Bowel Dis 2015; 21(8):1967–75.

29. Shevach EM. Mechanisms of foxp3+ T regulatory cell-mediated suppression. Immunity 2009; 30(5):636–45.

30. Brandtzaeg P, Johansen F-E. Mucosal B cells: phenotypic characteristics, transcriptional regulation, and homing properties. Immunol Rev 2005; 206:32–63.

31. Brandtzaeg P. Function of mucosa-associated lymphoid tissue in antibody formation. Immunol Invest 2010; 39(4-5):303–55.

32. Forchielli ML, Walker WA. The role of gut-associated lymphoid tissues and mucosal defence. BJN 2005; 93(S1):S41.

33. Corthésy B. Multi-faceted functions of secretory IgA at mucosal surfaces. Front Immunol 2013; 4:185.

34. Alexander KL, Targan SR, Elson CO. Microbiota activation and regulation of innate and adaptive immunity. Immunol Rev 2014; 260(1):206–20.

35. Houghteling PD, Walker WA. Why is initial bacterial colonization of the intestine important to infants' and children's health? J Pediatr Gastroenterol Nutr 2015; 60(3):294–307.

36. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Nageshwar Reddy D. Role of the normal gut microbiota. World J Gastroenterol 2015; 21(29):8787–803.

37. Kamada N, Seo S-U, Chen GY, Núñez G. Role of the gut microbiota in immunity and inflammatory disease. Nat Rev Immunol 2013; 13(5):321–35.

38. Al-Asmakh M, Zadjali F. Use of Germ-Free Animal Models in Microbiota-Related Research. J Microbiol Biotechnol 2015; 25(10):1583–8.

39. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell 2004; 118(2):229–41.

40. Williams AM, Probert CSJ, Stepankova R, Tlaskalova-Hogenova H, Phillips A, Bland PW. Effects of microflora on the neonatal development of gut mucosal T cells and myeloid cells in the mouse. Immunology 2006; 119(4):470–8.

41. Ochi T, Feng Y, Kitamoto S, Nagao-Kitamoto H, Kuffa P, Atarashi K, Honda K, Teitelbaum DH, Kamada N. Diet-dependent, microbiota-independent regulation of IL-10-producing lamina propria macrophages in the small intestine. Sci Rep 2016; 6:27634.

42. Kimura A, Naka T, Nakahama T, Chinen I, Masuda K, Nohara K, Fujii-Kuriyama Y, Kishimoto T. Aryl hydrocarbon receptor in combination with Stat1 regulates LPS-induced inflammatory responses. J Exp Med 2009; 206(9):2027–35.

43. Mørland B, Midtvedt T. Phagocytosis, peritoneal influx, and enzyme activities in peritoneal macrophages from germfree, conventional, and ex-germfree mice. Infect Immun 1984; 44(3):750–2.

44. Mikkelsen HB, Garbarsch C, Tranum-Jensen J, Thuneberg L. Macrophages in the small intestinal muscularis externa of embryos, newborn and adult germ-free mice. J Mol Histol 2004; 35(4):377–87.

45. Reikvam DH, Erofeev A, Sandvik A, Grcic V, Jahnsen FL, Gaustad P, McCoy KD, Macpherson AJ, Meza-Zepeda LA, Johansen FE. Depletion of murine intestinal microbiota: effects on gut mucosa and epithelial gene expression. PLoS ONE 2011; 6(3):e17996.

46. Chung H, Pamp SJ, Hill JA, Surana NK, Edelman SM, Troy EB, Reading NC, Villablanca EJ, Wang S, Mora JR, Umesaki Y, Mathis D, Benoist C, Relman DA, Kasper DL. Gut immune maturation depends on colonization with a host-specific microbiota. Cell 2012; 149(7):1578–93.

47. Ostman S, Rask C, Wold AE, Hultkrantz S, Telemo E. Impaired regulatory T cell function in germ-free mice. Eur J Immunol 2006; 36(9):2336–46.

48. Macpherson AJ, McCoy KD, Johansen F-E, Brandtzaeg P. The immune geography of IgA induction and function. Mucosal Immunol 2008; 1(1):11–22.

49. Li L-J, Gong C, Zhao M-H, Feng B-S. Role of interleukin-22 in inflammatory bowel disease. World J Gastroenterol 2014; 20(48):18177–88.

50. Wang Y, Mumm JB, Herbst R, Kolbeck R, Wang Y. IL-22 Increases Permeability of Intestinal Epithelial Tight Junctions by Enhancing Claudin-2 Expression. J Immunol 2017; 199(9):3316–25.

51. Wolk K, Kunz S, Witte E, Friedrich M, Asadullah K, Sabat R. IL-22 Increases the Innate Immunity of Tissues. Immunity 2004; 21(2):241–54.

52. Parks OB, Pociask DA, Hodzic Z, Kolls JK, Good M. Interleukin-22 Signaling in the Regulation of Intestinal Health and Disease. Front Cell Dev Biol 2015; 3:85.

53. Pickert G, Neufert C, Leppkes M, Zheng Y, Wittkopf N, Warntjen M, Lehr HA, Hirth S, Weigmann B, Wirtz S, Ouyang W, Neurath MF, Becker C. STAT3 links IL-22 signaling in intestinal epithelial cells to mucosal wound healing. J Exp Med 2009; 206(7):1465–72.

54. Brand S, Beigel F, Olszak T, Zitzmann K, Eichhorst ST, Otte J-M, Diepolder H, Marquardt A, Jagla W, Popp A, Leclair S, Herrmann K, Seiderer J, Ochsenkühn T, Göke B, Auernhammer CJ, Dambacher J. IL-22 is increased in active Crohn's disease and promotes proinflammatory gene expression and intestinal epithelial cell migration. Am J Physiol Gastrointest Liver Physiol 2006; 290(4):G827-38.

55. Lejeune D, Dumoutier L, Constantinescu S, Kruijer W, Schuringa JJ, Renauld J-C. Interleukin-22 (IL-22) activates the JAK/STAT, ERK, JNK, and p38 MAP kinase pathways in a rat hepatoma cell line. Pathways that are shared with and distinct from IL-10. J Biol Chem 2002; 277(37):33676–82.

56. Zheng Y, Valdez PA, Danilenko DM, Hu Y, Sa SM, Gong Q, Abbas AR, Modrusan Z, Ghilardi N, de Sauvage FJ, Ouyang W. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. Nat Med 2008; 14(3):282–9.

57. Sanos SL, Vonarbourg C, Mortha A, Diefenbach A. Control of epithelial cell function by interleukin-22-producing RORγt+ innate lymphoid cells. Immunology 2011; 132(4):453–65.

58. Li S, Bostick JW, Zhou L. Regulation of Innate Lymphoid Cells by Aryl Hydrocarbon Receptor. Front Immunol 2017; 8:1909.

59. Sugimoto K, Ogawa A, Mizoguchi E, Shimomura Y, Andoh A, Bhan AK, Blumberg RS, Xavier RJ, Mizoguchi A. IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. J Clin Invest 2008; 118(2):534–44.

60. Schreiber F, Arasteh JM, Lawley TD. Pathogen Resistance Mediated by IL-22 Signaling at the Epithelial-Microbiota Interface. J Mol Biol 2015; 427(23):3676–82.

61. Boniface K, Guignouard E, Pedretti N, Garcia M, Delwail A, Bernard F-X, Nau F, Guillet G, Dagregorio G, Yssel H, Lecron JC, Morel F. A role for T cell-derived interleukin 22 in psoriatic skin inflammation. Clin Exp Immunol 2007; 150(3):407–15.

62. Andoh A, Zhang Z, Inatomi O, Fujino S, Deguchi Y, Araki Y, Tsujikawa T, Kitoh K, Kim-Mitsuyama S, Takayanagi A, Shimizu N, Fujiyama Y. Interleukin-22, a member of the IL-10 subfamily, induces inflammatory responses in colonic subepithelial myofibroblasts. Gastroenterology 2005; 129(3):969–84.

63. Hanash AM, Dudakov JA, Hua G, O'Connor MH, Young LF, Singer NV, West ML, Jenq RR, Holland AM, Kappel LW, Ghosh A, Tsai JJ, Rao UK, Yim NL, Smith OM, Velardi E, Hawryluk EB, Murphy GF, Liu C, Fouser LA, Kolesnick R, Blazar BR, van den Brink MR. Interleukin-22 protects intestinal stem cells from immune-mediated tissue damage and regulates sensitivity to graft versus host disease. Immunity 2012; 37(2):339–50.

64. Sabat R, Ouyang W, Wolk K. Therapeutic opportunities of the IL-22-IL-22R1 system. Nat Rev Drug Discov 2014; 13(1):21–38.

65. Ubeda C, Pamer EG. Antibiotics, microbiota, and immune defense. Trends Immunol 2012; 33(9):459–66.

66. Buffie CG, Pamer EG. Microbiota-mediated colonization resistance against intestinal pathogens. Nat Rev Immunol 2013; 13(11):790–801.

67. Lange K, Buerger M, Stallmach A, Bruns T. Effects of Antibiotics on Gut Microbiota. Dig Dis 2016; 34(3):260–8.

68. Taggart H, Bergstrom L. An Overview of the Microbiome and the Effects of Antibiotics. The Journal for Nurse Practitioners 2014; 10(7):445–50.

69. Vangay P, Ward T, Gerber JS, Knights D. Antibiotics, pediatric dysbiosis, and disease. Cell Host Microbe 2015; 17(5):553–64.

70. Francino MP. Early development of the gut microbiota and immune health. Pathogens 2014; 3(3):769–90.

71. Hviid A, Svanström H, Frisch M. Antibiotic use and inflammatory bowel diseases in childhood. Gut 2011; 60(1):49–54.

72. Wlodarska M, Willing B, Keeney KM, Menendez A, Bergstrom KS, Gill N, Russell SL, Vallance BA, Finlay BB. Antibiotic treatment alters the colonic mucus layer and predisposes the host to exacerbated Citrobacter rodentium-induced colitis. Infect Immun 2011; 79(4):1536–45.

73. Willing BP, Russell SL, Finlay BB. Shifting the balance: antibiotic effects on host-microbiota mutualism. Nat Rev Microbiol 2011; 9(4):233–43.

74. Ivanov II, Frutos RdL, Manel N, Yoshinaga K, Rifkin DB, Sartor RB, Finlay BB, Littman DR. Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. Cell Host Microbe 2008; 4(4):337–49.

75. Hill DA, Hoffmann C, Abt MC, Du Y, Kobuley D, Kirn TJ, Bushman FD, Artis D. Metagenomic analyses reveal antibiotic-induced temporal and spatial changes in intestinal microbiota with associated alterations in immune cell homeostasis. Mucosal Immunol 2010; 3(2):148–58.

76. Matsuoka K, Kanai T. The gut microbiota and inflammatory bowel disease. Semin Immunopathol 2015; 37(1):47–55.

77. Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, Ward DV, Reyes JA, Shah SA, LeLeiko N, Snapper SB, Bousvaros A, Korzenik J, Sands BE, Xavier RJ, Huttenhower C. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. Genome Biol 2012; 13(9):R79.

78. Pitcher MCL. The contribution of sulphate reducing bacteria and 5-aminosalicylic acid to faecal sulphide in patients with ulcerative colitis. Gut 2000; 46(1):64–72.

79. Swidsinski A, Ladhoff A, Pernthaler A, Swidsinski S, Loening-Baucke V, Ortner M, Weber J, Hoffmann U, Schreiber S, Dietel M, Lochs H. Mucosal flora in inflammatory bowel disease. Gastroenterology 2002; 122(1):44–54.

80. Rubin DC, Shaker A, Levin MS. Chronic intestinal inflammation: inflammatory bowel disease and colitis-associated colon cancer. Front Immunol 2012; 3:107.

81. Kim ER, Chang DK. Colorectal cancer in inflammatory bowel disease: the risk, pathogenesis, prevention and diagnosis. World J Gastroenterol 2014; 20(29):9872–81.

82. Hendrickson BA, Gokhale R, Cho JH. Clinical Aspects and Pathophysiology of Inflammatory Bowel Disease. Clin Microbiol Rev 2002; 15(1):79–94.

83. Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. Nat Rev Immunol 2003; 3(7):521–33.

84. Neurath MF. New targets for mucosal healing and therapy in inflammatory bowel diseases. Mucosal Immunol 2014; 7(1):6–19.

85. Becker C, Neurath MF, Wirtz S. The Intestinal Microbiota in Inflammatory Bowel Disease. ILAR J 2015; 56(2):192–204.

86. Loftus EV. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. Gastroenterology 2004; 126(6):1504–17.

87. Bernstein CN, Shanahan F. Disorders of a modern lifestyle: reconciling the epidemiology of inflammatory bowel diseases. Gut 2008; 57(9):1185–91.

88. Bianco AM, Girardelli M, Tommasini A. Genetics of inflammatory bowel disease from multifactorial to monogenic forms. World J Gastroenterol 2015; 21(43):12296–310.

89. Loddo I, Romano C. Inflammatory Bowel Disease: Genetics, Epigenetics, and Pathogenesis. Front Immunol 2015; 6:551.

90. McGovern D, Powrie F. The IL23 axis plays a key role in the pathogenesis of IBD. Gut 2007; 56(10):1333–6.

91. Malik-Kale P, Jolly CE, Lathrop S, Winfree S, Luterbach C, Steele-Mortimer O. Salmonella - at home in the host cell. Front Microbiol 2011; 2:125.

92. Golubeva YA, Sadik AY, Ellermeier JR, Slauch JM. Integrating global regulatory input into the Salmonella pathogenicity island 1 type III secretion system. Genetics 2012; 190(1):79–90.

93. Grassl GA, Finlay BB. Pathogenesis of enteric Salmonella infections. Curr Opin Gastroenterol 2008; 24(1):22–6.

94. Salazar-Gonzalez R-M, Srinivasan A, Griffin A, Muralimohan G, Ertelt JM, Ravindran R, Vella AT, McSorley SJ . Salmonella flagellin induces bystander activation of splenic dendritic cells and hinders bacterial replication in vivo. J Immunol 2007; 179(9):6169–75.

95. Tumbarello M, Tacconelli E, Caponera S, Cauda R, Ortona L. The impact of bacteraemia on HIV infection. Nine years experience in a large Italian university hospital. J Infect 1995; 31(2):123–31.

96. Rydström A, Wick MJ. Monocyte and neutrophil recruitment during oral Salmonella infection is driven by MyD88-derived chemokines. Eur J Immunol 2009; 39(11):3019–30.

97. Mastroeni P, Chabalgoity JA, Dunstan SJ, Maskell DJ, Dougan G. Salmonella: immune responses and vaccines. Vet J 2001; 161(2):132–64.

98. Lehmann J, Springer S, Werner CE, Lindner T, Bellmann S, Straubinger RK, Selbitz HJ, Alber G. Immunity induced with a Salmonella enterica serovar Enteritidis live vaccine is regulated by Th1-cell-dependent cellular and humoral effector mechanisms in susceptible BALB/c mice. Vaccine 2006; 24(22):4779–93.

99. Nauciel C. Role of CD4+ T cells and T-independent mechanisms in acquired resistance to Salmonella typhimurium infection. J Immunol 1990; 145(4):1265–9.

100. Griffin AJ, McSorley SJ. Development of protective immunity to Salmonella, a mucosal pathogen with a systemic agenda. Mucosal Immunol 2011; 4(4):371–82.

101. Lehmann J, Bellmann S, Werner C, Schroder R, Schutze N, Alber G. IL-12p40-Dependent Agonistic Effects on the Development of Protective Innate and Adaptive Immunity Against Salmonella Enteritidis. J Immunol 2001; 167(9):5304–15.

102. Ravindran R, McSorley SJ. Tracking the dynamics of T-cell activation in response to Salmonella infection. Immunology 2005; 114(4):450–8.

103. Kupz A, Scott TA, Belz GT, Andrews DM, Greyer M, Lew AM, Brooks AG, Smyth MJ, Curtiss R 3rd, Bedoui S, Strugnell RA Contribution of Thy1+ NK cells to protective IFN-γ production during Salmonella typhimurium infections. Proc Natl Acad Sci USA 2013; 110(6):2252–7.

104. Pham OH, McSorley SJ. Protective host immune responses to Salmonella infection. Future Microbiol 2015; 10(1):101–10.

105. Ugrinovic S, Menager N, Goh N, Mastroeni P. Characterization and Development of T-Cell Immune Responses in B-Cell-Deficient (Igh-6-/-) Mice with Salmonella enterica Serovar TyphimuriumInfection. Infect Immun 2003; 71(12):6808–19.

106. Michetti P, Porta N, Mahan MJ, Slauch JM, Mekalanos JJ, Blum AL, Kraehenbuhl JP, Neutra MR. Monoclonal immunoglobulin A prevents adherence and invasion of polarized

epithelial cell monolayers by Salmonella typhimurium. Gastroenterology 1994; 107(4):915–23.

107. Iankov ID, Petrov DP, Mladenov IV, Haralambieva IH, Kalev OK, Balabanova MS, Mitov IG. Protective efficacy of IgA monoclonal antibodies to O and H antigens in a mouse model of intranasal challenge with Salmonella enterica serotype Enteritidis. Microbes Infect 2004; 6(10):901–10.

108. Jiminez JA, Uwiera TC, Douglas Inglis G, Uwiera RRE. Animal models to study acute and chronic intestinal inflammation in mammals. Gut Pathog 2015; 7:29.

109. Neurath MF. Animal models of inflammatory bowel diseases: illuminating the pathogenesis of colitis, ileitis and cancer. Dig Dis 2012; 30 Suppl 1:91–4.

110. Chassaing B, Aitken JD, Malleshappa M, Vijay-Kumar M. Dextran sulfate sodium (DSS)-induced colitis in mice. Curr Protoc Immunol 2014; 104:Unit 15.25.

111. Mizoguchi A, Takeuchi T, Himuro H, Okada T, Mizoguchi E. Genetically engineered mouse models for studying inflammatory bowel disease. J Pathol 2016; 238(2):205–19.

112. Eri R, McGuckin MA, Wadley R. T cell transfer model of colitis: a great tool to assess the contribution of T cells in chronic intestinal inflammation. Methods Mol Biol 2012; 844:261–75.

113. Elson CO, Cong Y, Sundberg J. The C3H/HeJBir mouse model: a high susceptibility phenotype for colitis. Int Rev Immunol 2000; 19(1):63–75.

114. Kaiser P, Diard M, Stecher B, Hardt W-D. The streptomycin mouse model for Salmonella diarrhea: functional analysis of the microbiota, the pathogen's virulence factors, and the host's mucosal immune response. Immunol Rev 2012; 245(1):56–83.

115. Broz P, Ruby T, Belhocine K, Bouley DM, Kayagaki N, Dixit VM, Monack DM. Caspase-11 increases susceptibility to Salmonella infection in the absence of caspase-1. Nature 2012; 490(7419):288–91.

116. Kohanski MA, Dwyer DJ, Collins JJ. How antibiotics kill bacteria: from targets to networks. Nat Rev Microbiol 2010; 8(6):423–35.

117. Miller CP, Bohnhoff M. Changes in the mouse's enteric microflora associated with enhanced susceptibility to Salmonella infection following streptomycin treatment. J Infect Dis 1963; 113:59–66.

118. Bohnhoff M, Miller CP, Martin WR. Resistance of the mouse´s intestinal tract to experimental Salmonella infection. I. Factors which interfere with the initiation of infection by oral inoculation. J Exp Med 1964; 120:805–16.

119. Que JU, Hentges DJ. Effect of streptomycin administration on colonization resistance to Salmonella typhimurium in mice. Infect Immun 1985; 48(1):169–74.

120. Stecher B, Macpherson AJ, Hapfelmeier S, Kremer M, Stallmach T, Hardt W-D. Comparison of Salmonella enterica serovar Typhimurium colitis in germfree mice and mice pretreated with streptomycin. Infect Immun 2005; 73(6):3228–41.

121. Stecher B, Robbiani R, Walker AW, Westendorf AM, Barthel M, Kremer M, Chaffron S, Macpherson AJ, Buer J, Parkhill J, Dougan G, von Mering C, Hardt WD. Salmonella enterica serovar typhimurium exploits inflammation to compete with the intestinal microbiota. PLoS Biol 2007; 5(10):2177–89.

122. Spees AM, Wangdi T, Lopez CA, Kingsbury DD, Xavier MN, Winter SE, Tsolis RM, Bäumler AJ . Streptomycin-induced inflammation enhances Escherichia coli gut colonization through nitrate respiration. MBio 2013; 4(4).

123. Hapfelmeier S, Stecher B, Barthel M, Kremer M, Muller AJ, Heikenwalder M, Stallmach T, Hensel M, Pfeffer K, Akira S, Hardt WD. The Salmonella Pathogenicity Island (SPI)-2 and SPI-1 Type III Secretion Systems Allow Salmonella Serovar typhimurium to Trigger Colitis via MyD88-Dependent and MyD88-Independent Mechanisms.J Immunol 2005; 174(3):1675–85.

124. M'Koma AE. Inflammatory bowel disease: an expanding global health problem. Clin Med Insights Gastroenterol 2013; 6:33–47.

125. Matricon J, Barnich N, Ardid D. Immunopathogenesis of inflammatory bowel disease. Self Nonself 2010; 1(4):299–309.

126. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 2009; 9(5):313–23.

127. Lamps LW, Madhusudhan KT, Havens JM, Greenson JK, Bronner MP, Chiles MC, Dean PJ, Scott MA. Pathogenic Yersinia DNA is detected in bowel and mesenteric lymph nodes from patients with Crohn's disease. Am J Surg Pathol 2003; 27(2):220–7.

128. Naser SA, Sagramsingh SR, Naser AS, Thanigachalam S. Mycobacterium avium subspecies paratuberculosis causes Crohn's disease in some inflammatory bowel disease patients. World J Gastroenterol 2014; 20(23):7403–15.

129. Merger M, Croitoru K. Infections in the immunopathogenesis of chronic inflammatory bowel disease. Semin Immunol 1998; 10(1):69–78.

130. Cunliffe RN, Kamal M, Rose FRAJ, James PD, Mahida YR. Expression of antimicrobial neutrophil defensins in epithelial cells of active inflammatory bowel disease mucosa. J Clin Pathol 2002; 55(4):298–304.

131. Hoffmann M, Schwertassek U, Seydel A, Weber K, Falk W, Hauschildt S, Lehmann J. A refined and translationally relevant model of chronic DSS colitis in BALB/c mice. Lab Anim 2018; 52(3):240–52.

132. Barthel M, Hapfelmeier S, Quintanilla-Martinez L, Kremer M, Rohde M, Hogardt M, Pfeffer K, Rüssmann H, Hardt WD. Pretreatment of Mice with Streptomycin Provides a Salmonella enterica Serovar Typhimurium Colitis Model That Allows Analysis of Both Pathogen and Host. Infect Immun 2003; 71(5):2839–58.

133. Ponder A, Long MD. A clinical review of recent findings in the epidemiology of inflammatory bowel disease. Clin Epidemiol 2013; 5:237–47.

134. Lidar M, Langevitz P, Shoenfeld Y. The role of infection in inflammatory bowel disease: initiation, exacerbation and protection. Isr Med Assoc J 2009; 11(9):558–63.

135. BOHNHOFF M, MILLER CP. Enhanced susceptibility to Salmonella infection in streptomycin-treated mice. J Infect Dis 1962; 111:117–27.

136. Croswell A, Amir E, Teggatz P, Barman M, Salzman NH. Prolonged impact of antibiotics on intestinal microbial ecology and susceptibility to enteric Salmonella infection. Infect Immun 2009; 77(7):2741–53.

137. Bohnhoff M, Drake BL, Miller CP. Effect of streptomycin on susceptibility of intestinal tract to experimental Salmonella infection. Proc Soc Exp Biol Med 1954; 86(1):132–7.

138. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, Gratadoux J-J, Blugeon S, Bridonneau C, Furet JP, Corthier G, Grangette C, Vasquez N, Pochart P, Trugnan G, Thomas G, Blottière HM, Doré J, Marteau P, Seksik P, Langella P. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci U S A 2008; 105(43):16731–6.

139. Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. Proc Natl Acad Sci U S A 2005; 102(31):11070–5.

140. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature 2006; 444(7122):1022–3.

141. Turnbaugh PJ, Bäckhed F, Fulton L, Gordon JI. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell Host Microbe 2008; 3(4):213–23.

142. Goto Y, Panea C, Nakato G, Cebula A, Lee C, Diez MG, Laufer TM3, Ignatowicz L2, Ivanov II. Segmented filamentous bacteria antigens presented by intestinal dendritic cells drive mucosal Th17 cell differentiation. Immunity 2014; 40(4):594–607.

143. Lane ER, Zisman TL, Suskind DL. The microbiota in inflammatory bowel disease: current and therapeutic insights. J Inflamm Res 2017; 10:63–73.

144. Tamboli CP, Neut C, Desreumaux P, Colombel JF. Dysbiosis as a prerequisite for IBD. Gut 2004; 53(7):1057.

145. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci U S A 2007; 104(34):13780–5.

146. Andoh A, Kuzuoka H, Tsujikawa T, Nakamura S, Hirai F, Suzuki Y, Matsui T, Fujiyama Y, Matsumoto T. Multicenter analysis of fecal microbiota profiles in Japanese patients with Crohn's disease. J Gastroenterol 2012; 47(12):1298–307.

147. Garrett WS, Gallini CA, Yatsunenko T, Michaud M, DuBois A, Delaney ML, Punit S, Karlsson M, Bry L, Glickman JN, Gordon JI, Onderdonk AB, Glimcher LH. Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. Cell Host Microbe 2010; 8(3):292–300.

148. Hannemann S, Gao B, Galán JE. Salmonella modulation of host cell gene expression promotes its intracellular growth. PLoS Pathog 2013; 9(10):e1003668.

149. Martinez-Medina M, Garcia-Gil LJ. Escherichia coli in chronic inflammatory bowel diseases: An update on adherent invasive Escherichia coli pathogenicity. World J Gastrointest Pathophysiol 2014; 5(3):213–27.

150. Hong SN, Kim HJ, Kim KH, Han S-J, Ahn IM, Ahn HS. Risk of incident Mycobacterium tuberculosis infection in patients with inflammatory bowel disease: a nationwide population-based study in South Korea. Aliment Pharmacol Ther 2017; 45(2):253–63.

151. Gradel KO, Nielsen HL, Schønheyder HC, Ejlertsen T, Kristensen B, Nielsen H. Increased short- and long-term risk of inflammatory bowel disease after salmonella or campylobacter gastroenteritis. Gastroenterology 2009; 137(2):495–501.

152. Godinez I, Haneda T, Raffatellu M, George MD, Paixão TA, Rolán HG, Santos RL, Dandekar S, Tsolis RM, Bäumler AJ. T cells help to amplify inflammatory responses induced by Salmonella enterica serotype Typhimurium in the intestinal mucosa. Infect Immun 2008; 76(5):2008–17.

153. Emmrich J, Seyfarth M, Fleig WE, Emmrich F. Treatment of inflammatory bowel disease with anti-CD4 monoclonal antibody. Lancet 1991; 338(8766):570–1.

154. Larmonier CB, Shehab KW, Ghishan FK, Kiela PR. T Lymphocyte Dynamics in Inflammatory Bowel Diseases: Role of the Microbiome. Biomed Res Int 2015; 2015:504638.

155. Ostanin DV, Bao J, Koboziev I, Gray L, Robinson-Jackson SA, Kosloski-Davidson M, Price VH, Grisham MB. T cell transfer model of chronic colitis: concepts, considerations, and tricks of the trade. Am J Physiol Gastrointest Liver Physiol 2009; 296(2):G135-46.

156. Shtrichman R, Samuel CE. The role of gamma interferon in antimicrobial immunity. Curr Opin Microbiol 2001; 4(3):251–9.

157. Camoglio L, Te Velde AA, Tigges AJ, Das PK, van Deventer SJ. Altered expression of interferon-gamma and interleukin-4 in inflammatory bowel disease. Inflamm Bowel Dis 1998; 4(4):285–90.

158. Ito R, Shin-Ya M, Kishida T, Urano A, Takada R, Sakagami J, Imanishi J, Kita M, Ueda Y, Iwakura Y, Kataoka K, Okanoue T, Mazda O. Interferon-gamma is causatively involved in experimental inflammatory bowel disease in mice. Clin Exp Immunol 2006; 146(2):330–8.

159. Strober W, Fuss IJ. Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. Gastroenterology 2011; 140(6):1756–67.

160. Behnsen J, Jellbauer S, Wong CP, Edwards RA, George MD, Ouyang W, Raffatellu M. The cytokine IL-22 promotes pathogen colonization by suppressing related commensal bacteria. Immunity 2014; 40(2):262–73.

161. Rutz S, Eidenschenk C, Ouyang W. IL-22, not simply a Th17 cytokine. Immunol Rev 2013; 252(1):116–32.

162. Mizoguchi A. Healing of intestinal inflammation by IL-22. Inflamm Bowel Dis 2012; 18(9):1777–84.

163. Mantis NJ, Rol N, Corthésy B. Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. Mucosal Immunol 2011; 4(6):603–11.

164. Uren TK, Wijburg OLC, Simmons C, Johansen F-E, Brandtzaeg P, Strugnell RA. Vaccine-induced protection against gastrointestinal bacterial infections in the absence of secretory antibodies. Eur J Immunol 2005; 35(1):180–8.

165. Endt K, Stecher B, Chaffron S, Slack E, Tchitchek N, Benecke A, Van Maele L, Sirard JC, Mueller AJ, Heikenwalder M, Macpherson AJ, Strugnell R, von Mering C, Hardt WD. The microbiota mediates pathogen clearance from the gut lumen after non-typhoidal Salmonella diarrhea. PLoS Pathog 2010; 6(9):e1001097.

166. Macpherson A, Khoo UY, Forgacs I, Philpott-Howard J, Bjarnason I. Mucosal antibodies in inflammatory bowel disease are directed against intestinal bacteria. Gut 1996; 38(3):365–75.

167. Schoepfer AM, Schaffer T, Mueller S, Flogerzi B, Vassella E, Seibold-Schmid B, Seibold F. Phenotypic associations of Crohn's disease with antibodies to flagellins A4-Fla2 and Fla-X, ASCA, p-ANCA, PAB, and NOD2 mutations in a Swiss Cohort. Inflamm Bowel Dis 2009; 15(9):1358–67.

168. Hevia A, López P, Suárez A, Jacquot C, Urdaci MC, Margolles A, Sánchez B. Association of levels of antibodies from patients with inflammatory bowel disease with extracellular proteins of food and probiotic bacteria. Biomed Res Int 2014; 2014:351204.

169. Mestecky J, McGhee JR. Immunoglobulin A (IgA): molecular and cellular interactions involved in IgA biosynthesis and immune response. Adv Immunol 1987; 40:153–245.

170. McGhee JR, Mestecky J, Elson CO, Kiyono H. Regulation of IgA synthesis and immune response by T cells and interleukins. J Clin Immunol 1989; 9(3):175–99.

171. Almeida LC, Muraro LS, Albuquerque DA. Enhancement of anti-OVA IgG2c production in vivo by enalapril. Braz J Med Biol Res 2016; 49(8).

172. Ananthakrishnan AN. Environmental triggers for inflammatory bowel disease. Curr Gastroenterol Rep 2013; 15(1):302.

173. Shaw SY, Blanchard JF, Bernstein CN. Association between the use of antibiotics and new diagnoses of Crohn's disease and ulcerative colitis. Am J Gastroenterol 2011; 106(12):2133–42.

174. Mizoguchi A, Yano A, Himuro H, Ezaki Y, Sadanaga T, Mizoguchi E. Clinical importance of IL-22 cascade in IBD. J Gastroenterol 2017.

175. Mastelic B, do Rosario APF, Veldhoen M, Renauld JC, Jarra W, Sponaas A-M, Roetynck S, Stockinger B, Langhorne J. IL-22 Protects Against Liver Pathology and Lethality of an Experimental Blood-Stage Malaria Infection. Front Immunol 2012; 3:85.

176. Wilson MS, Feng CG, Barber DL, Yarovinsky F, Cheever AW, Sher A, Grigg M, Collins M, Fouser L, Wynn TA. Redundant and pathogenic roles for IL-22 in mycobacterial, protozoan, and helminth infections. J Immunol 2010; 184(8):4378–90.

177. Graham AC, Carr KD, Sieve AN, Indramohan M, Break TJ, Berg RE. IL-22 production is regulated by IL-23 during Listeria monocytogenes infection but is not required for bacterial clearance or tissue protection. PLoS ONE 2011; 6(2):e17171.

178. Zenewicz LA, Yancopoulos GD, Valenzuela DM, Murphy AJ, Stevens S, Flavell RA. Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. Immunity 2008; 29(6):947–57.

179. Jones-Hall YL, Grisham MB. Immunopathological characterization of selected mouse models of inflammatory bowel disease: Comparison to human disease. Pathophysiology 2014; 21(4):267–88.

180. Liang SC, Nickerson-Nutter C, Pittman DD, Carrier Y, Goodwin DG, Shields KM, Lambert AJ, Schelling SH, Medley QG, Ma HL, Collins M, Dunussi-Joannopoulos K, Fouser LA. IL-22 induces an acute-phase response. J Immunol 2010; 185(9):5531–8.

181. Erdmann H, Behrends J, Hölscher C. During acute experimental infection with the reticulotropic Trypanosoma cruzi strain Tulahuen IL-22 is induced IL-23-dependently but is dispensable for protection. Sci Rep 2016; 6:32927.

182. Johansson MEV, Sjövall H, Hansson GC. The gastrointestinal mucus system in health and disease. Nat Rev Gastroenterol Hepatol 2013; 10(6):352–61.

183. Kim YS, Ho SB. Intestinal goblet cells and mucins in health and disease: recent insights and progress. Curr Gastroenterol Rep 2010; 12(5):319–30.

184. Turner J-E, Stockinger B, Helmby H. IL-22 mediates goblet cell hyperplasia and worm expulsion in intestinal helminth infection. PLoS Pathog 2013; 9(10):e1003698.

185. Sellau J, Alvarado CF, Hoenow S, Mackroth MS, Kleinschmidt D, Huber S, Jacobs T. IL-22 dampens the T cell response in experimental malaria. Sci Rep 2016; 6:28058.

186. Radaeva S, Sun R, Pan H-N, Hong F, Gao B. Interleukin 22 (IL-22) plays a protective role in T cell-mediated murine hepatitis: IL-22 is a survival factor for hepatocytes via STAT3 activation. Hepatology 2004; 39(5):1332–42.

187. Yang B-H, Hagemann S, Mamareli P, Lauer U, Hoffmann U, Beckstette M, Föhse L, Prinz I, Pezoldt J, Suerbaum S, Sparwasser T, Hamann A, Floess S, Huehn J, Lochner M. Foxp3(+) T cells expressing RORγt represent a stable regulatory T-cell effector lineage with enhanced suppressive capacity during intestinal inflammation. Mucosal Immunol 2016; 9(2):444–57.

188. Mielke LA, Jones SA, Raverdeau M, Higgs R, Stefanska A, Groom JR, Misiak A, Dungan LS, Sutton CE, Streubel G, Bracken AP, Mills KH . Retinoic acid expression associates with enhanced IL-22 production by γδ T cells and innate lymphoid cells and attenuation of intestinal inflammation. J Exp Med 2013; 210(6):1117–24.

189. Günzel D, Yu ASL. Claudins and the modulation of tight junction permeability. Physiol Rev 2013; 93(2):525–69.

190. Mizuno S, Mikami Y, Kamada N, Handa T, Hayashi A, Sato T, Matsuoka K, Matano M, Ohta Y, Sugita A, Koganei K, Sahara R, Takazoe M, Hisamatsu T, Kanai T. Cross-talk between RORγt+ innate lymphoid cells and intestinal macrophages induces mucosal IL-22 production in Crohn's disease. Inflamm Bowel Dis 2014; 20(8):1426–34.

191. Ivanov AI, Nusrat A, Parkos CA. The epithelium in inflammatory bowel disease: potential role of endocytosis of junctional proteins in barrier disruption. Novartis Found Symp 2004; 263:115-24; discussion 124-32, 211-8.

192. Hasegawa M, Yada S, Liu MZ, Kamada N, Muñoz-Planillo R, Do N, Núñez G, Inohara N. Interleukin-22 regulates the complement system to promote resistance against pathobionts after pathogen-induced intestinal damage. Immunity 2014; 41(4):620–32.

193. Woof JM, Kerr MA. The function of immunoglobulin A in immunity. J Pathol 2006; 208(2):270–82.

194. Dunlap NE, Benjamin WH, Berry A, Eldridge JH, Briles DE. A ‘safe-site’ for Salmonella typhimurium is within splenic polymorphonuclear cells. Microbial Pathogenesis 1992; 13(3):181–90.

195. Mastroeni P, Villarreal-Ramos B, Hormaeche CE. Role of T cells, TNF alpha and IFN gamma in recall of immunity to oral challenge with virulent salmonellae in mice vaccinated with live attenuated aro- Salmonella vaccines. Microbial Pathogenesis 1992; 13(6):477–91.

196. Mastroeni P, Simmons C, Fowler R, Hormaeche CE, Dougan G. Igh-6(-/-) (B-cell-deficient) mice fail to mount solid acquired resistance to oral challenge with virulent Salmonella enterica serovar typhimurium and show impaired Th1 T-cell responses to Salmonella antigens. Infect Immun 2000; 68(1):46–53.

197. Mittrücker HW, Raupach B, Köhler A, Kaufmann SH. Cutting edge: role of B lymphocytes in protective immunity against Salmonella typhimurium infection. J Immunol 2000; 164(4):1648–52.

198. Nauciel C, Espinasse-Maes F. Role of gamma interferon and tumor necrosis factor alpha in resistance to Salmonella typhimurium infection. Infect Immun 1992; 60(2):450–4.

199. Songhet P, Barthel M, Stecher B, Müller AJ, Kremer M, Hansson GC, Hardt WD. Stromal IFN-γR-signaling modulates goblet cell function during Salmonella Typhimurium infection. PLoS ONE 2011; 6(7):e22459.

200. Bao S, Beagley KW, France MP, Shen J, Husband AJ. Interferon-gamma plays a critical role in intestinal immunity against Salmonella typhimurium infection. Immunology 2000; 99(3):464–72.

201. Gärdby E, Wrammert J, Schön K, Ekman L, Leanderson T, Lycke N. Strong differential regulation of serum and mucosal IgA responses as revealed in CD28-deficient mice using cholera toxin adjuvant. J Immunol 2003; 170(1):55–63.

202. Matthaei KI, Foster P, Young IG. The role of interleukin-5 (IL-5) in vivo: studies with IL-5 deficient mice. Mem Inst Oswaldo Cruz 1997; 92 Suppl 2:63–8.

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Erklärung über die eigenständige Abfassung der Arbeit

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne unzulässige Hilfe oder Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Ich versichere, dass Dritte von mir weder unmittelbar noch mittelbar eine Vergütung oder geldwerte Leistungen für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen, und dass die vorgelegte Arbeit weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde zum Zweck einer Promotion oder eines anderen Prüfungsverfahrens vorgelegt wurde. Alles aus anderen Quellen und von anderen Personen übernommene Material, das in der Arbeit verwendet wurde oder auf das direkt Bezug genommen wird, wurde als solches kenntlich gemacht. Insbesondere wurden alle Personen genannt, die direkt an der Entstehung der vorliegenden Arbeit beteiligt waren. Die aktuellen gesetzlichen Vorgaben in Bezug auf die Zulassung der klinischen Studien, die Bestimmungen des Tierschutzgesetzes, die Bestimmungen des Gentechnikgesetzes und die allgemeinen Datenschutzbestimmungen wurden eingehalten. Ich versichere, dass ich die Regelungen der Satzung der Universität Leipzig zur Sicherung guter wissenschaftlicher Praxis kenne und eingehalten habe.

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- Hoffmann, Maximilian; Schwertassek, Ulla; **Seydel, Aleksandra**; Weber, Klaus; Falk, Werner; Hauschildt, Sunna; Lehmann, Jörg (2017): A refined and translationally relevant model of chronic DSS colitis in BALB/c mice. In *Laboratory animals*, 23677217742681. DOI: 10.1177/0023677217742681.

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