universität wien

MASTERARBEIT

Titel der Masterarbeit

Type I Interferons as Mediators of the Innate Immune Response to Gastrointestinal Infection with *Listeria monocytogenes*

Verfasserin

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angestrebter akademischer Grad

Master of Science (MSc)

Wien, 2012

Studienkennzahl lt. Studienblatt:	A 066 830
Studienrichtung lt. Studienblatt:	Masterstudium Molekulare Mikrobiologie und Immunbiologie
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Abstract

Listeria monocytogenes (*Lm*), a Gram-positive facultatively intracellular bacterium, is a human pathogen and a major challenge to the food industry. It occurs ubiquitously due to its ability to upregulate unique transcriptional profiles under diverse environmental conditions, such as temperature, acid or salt. Its natural route of infection is through the gastrointestinal tract where it can adapt to and invade the host. In biological sciences *Lm* is a widely used model organism that has provided insight into many fundamental principles of immunology or cell biology.

Synthesis of type I interferons (IFN-I) is one of the immediate innate responses to infection with *Lm*. Here, the mouse-adapted LO28InlA* strain, that is capable to interact with the murine epithelial junction protein E-cadherin, was used to study the impact of IFN-I responses on intragastric (i.g.) infection with *Lm*. IFN-I were shown to protect murine hosts in this situation, as mice lacking the IFN-I-receptor-chain 1 (Ifnar1-/-) were more susceptible than Wild-type C57BL/6N mice (Wt). This is in striking contrast with previous reports on systemic *Lm* infection, where IFN-I were described to be detrimental.

Histological analysis of the gut revealed infected cell patches in the mucosal tissue underlying the epithelium and in the gut-associated lymphoid tissue (GALT), but the pattern or extent of infection was highly similar between Wt and Ifnar1-/- mice. This finding was corroborated by histological analysis, studies of gene expression and by the determination of bacterial burden with colony-forming-unit (CFU) assays of intestinal tissue including Peyer's patches (PP) and mesenteric lymph nodes (MLN). The difference arising from i.g. infection between Wt and Ifnar1-/- mice was most pronounced in the liver, an early target organ of *Lm* on its way to systemic spread. Initially, Listeria resided in hepatocytes, following immune cell infiltration of Gr1+ myeloid cells and other leukocytes. In addition, smaller infiltrates containing F4/80+ macrophages were observed. Interestingly F4/80+ cells became increasingly rare as the size of inflammatory infiltrates increased. 48hrs after i.g. infection, massive cell death was found within the infiltrate as well as the surrounding hepatic tissue. About thirty percent of Ifnar1-/- mice displayed this dramatic liver phenotype and this correlated well with the lethality of infection. The majority of mice survived this stage, most likely due to a strong increase of IFNγ production.

Measurement of cytokine profiles during infection showed that the pattern of early IL-6, IFNγ and MCP-1 production appears to be prognostic for the severity of infection. Wt mice infected i.g. displayed the highest levels of these cytokines early after infection. Speculatively, mammalian hosts are better adapted to gut-derived as opposed to systemic bacteria, resulting in a more regulated immune response with IFN-I as one of the first mediators. Ifnar1-/- mice seem to miss an adequate response within the first 24h, a timeframe for *Lm* to colonize its host and replicate. While our studies cannot definitively identify the relevant target organs or cells for IFN-I

action after i.g. infection, they rule out a pronounced role for IFN-I at the site of intestinal entry. Interestingly, an impact of type III interferons (IFN-III) on the intestinal epithelium is suggested by the fact that IRF9-/- mice, that are unresponsive to both IFN-I and IFN-III, show an even higher bacterial burden than Ifnar1-/- mice upon i.g. infection.

Taken together, the results presented in my thesis open a new perspective on the role of IFN-I in bacterial infections. They emphasize the importance of the infection route by demonstrating opposing roles of the cytokines upon infection via gastrointestinal or systemic administration.

Zusammenfassung

Listeria monocytogenes (Lm) ist ein Gram-positives, fakultativ intrazelluläres Bakterium, ein Humanpathogen und damit eine große Herausforderung für die Nahrungsmittelindustrie. Aufgrund seiner Fähigkeit einzigartige transkriptionelle Programme hochzuregulieren und sich dadurch an verschiedene Umweltbedingungen wie Temperatur, Säure oder Salz anzupassen kann *Lm* viele ökologische Nischen besetzen. Der natürliche Infektionsweg beim Menschen ist über den Magen-Darm-Trakt, wo sich das Bakterium adaptieren und den Wirt befallen kann. In der Naturwissenschaft ist *Lm* ein häufig verwendeter Modellorganismus, dessen molekulare Eigenschaften Einblicke in viele fundamentale Prinzipien der Immunologie und Zellbiologie ermöglichen.

Die Synthese von Typ I Interferonen (IFN-I) ist eine der ersten Reaktionen des angeborenen Immunsystems auf Listerieninfektion. Um die Rolle von IFN-I nach oraler Verabreichung (i.g.) zu untersuchen, wurde in der vorliegenden Arbeit der murinisierte LO28InlA* Stamm verwendet, welcher mit murinem E-cadherin auf Epithelzellen interagieren kann. Dabei zeigte sich, dass IFN-I eine protektive Wirkung auf den Wirt haben, da Mäuse ohne IFN-I Rezeptorkette 1 (Ifnar1-/-) anfälliger sind als Wild-typ C57BL/6N Mäuse (Wt). Dies steht im Gegensatz zu früheren Aufzeichnungen, die IFN-I nach systemischer (i.p.) Infektion als nachteilig für den Wirt beschreiben.

Histologische Untersuchungen des Darms ergaben, dass *Lm* nur stellenweise Zellen der Mucosa, unterhalb der Epithelschicht in Darm-assoziierten lymphoiden Geweben (GALT), infiziert. Jedoch waren Infektionsausmaß bzw. -verteilung zwischen Wt und Ifnar1-/- Mäusen sehr ähnlich. Diese Beobachtungen wurden durch histologische Untersuchungen, Genexpressions-analysen und Bestimmung von Bakterienmengen im Darm assoziierten Immungewebe, wie den Peyer's patches (PP) und den mesenterialen Lymphknoten (MLN), bestätigt. Der unterschiedliche Phänotyp zwischen i.g. infizierten Wt und Ifnar1-/- Mäusen war am stärksten ausgeprägt in der Leber, einer wichtigen Replikationsnische von *Lm* am Weg zur systemischen Infektion. Zu Beginn der Infektion befindet sich *Lm* noch in Hepatozyten, gefolgt von Infiltrierung von Gr1+ myeloider Zellen und anderen Leukozyten. Kleinere Infiltrate enthielten auch F4/80+ Makrophagen, die Anzahl dieser Zellen nahm allerdings mit der Größe der Infiltrate ab. 48h nach i.g. Infektion waren die Infiltrate mit massiven Zelltod assoziiert, sowohl innerhalb als auch um das Infiltrat herum. 30% der Ifnar1-/- Mäuse zeigten diesen dramatischen Phänotyp, korrelierend mit der Letalität der Infektion. Dennoch überlebte der Großteil der Mäuse dieses Stadium, höchstwahrscheinlich wegen steigender IFNγ Werte im Blut.

Zytokinmessungen während der Infektion ergaben, dass das Expressionsprofile von frühem IL-6, IFNγ und MCP-1 prognostisch für den Grad der Infektion sind da i.g infizierte Wt

Mäuse hohe Werte dieser Zytokine zeigten. Vermutlich ist der Wirt durch eine besser regulierte Immunantwort, mit IFNα und IFNβ als Mediatoren, besser auf Bakterien aus dem Darm vorbereitet als auf solche die systemisch verabreicht werden, denn Ifnar1-/- Mäusen fehlt eine adäquate Immunantwort innerhalb der ersten 24h. In diesem Zeitfenster können Listerien Fuß fassen und replizieren. In welchem Organ/ Zellen die IFN-I Antwort die wichtigste Rolle spielt steht noch nicht fest. Wir können allerdings eine verstärkte Rolle für IFN-I an der intestinalen Eintrittsstelle ausschließen. Vielmehr vermuten wir eine wichtige Rolle der Typ III Interferone (IFN-III), da IRF9-/- Mäuse, die weder auf IFN-I noch IFN-III Interferone reagieren können, höhere Bakterienanzahlen aufzeigen als Ifnar1-/- Mäuse.

Zusammengefasst öffnen die Ergebnisse meiner Arbeit neue Perspektiven im Hinblick auf die Rolle von IFN-I bei bakteriellen Infektionen. Sie unterstreichen eine herausragende Rolle der Infektionsroute durch den inversen Effekt eines Zytokins nach gastrointestinaler oder systemischer Verabreichung.

Content

Introduction

1. Gut immunity

The gastrointestinal (GI) tract with its unique architecture is responsible for digestion of food and absorbance of nutrients. Further, it represents a large mucosal immune organ with the ability to protect against ingested and potentially harmful microbes and to maintain tolerance against self antigen and a large pool of commensal bacteria. Under physiological conditions, host and environment are separated by a single follicle-associated epithelial barrier, held together by tight junctions. It creates a first physical defence line by secreting e.g. mucin, defensins and secretory antibodies against invading pathogens (1). To establish immune surveillance, transport through the epithelium, antigen processing and presentation to cells of the underlying gut associated lymphoid tissue (GALT) is a prerequisite. M-cells, specialized in transporting pathogens to antigen-presenting cells within the follicle associated epithelium or underlying Peyer's patches (PP), represent an alternative gateway, beside intraepithelial dendritic cells (DC) that exhibit the ability to directly capture bacteria from the luminal content (2) (Fig1).

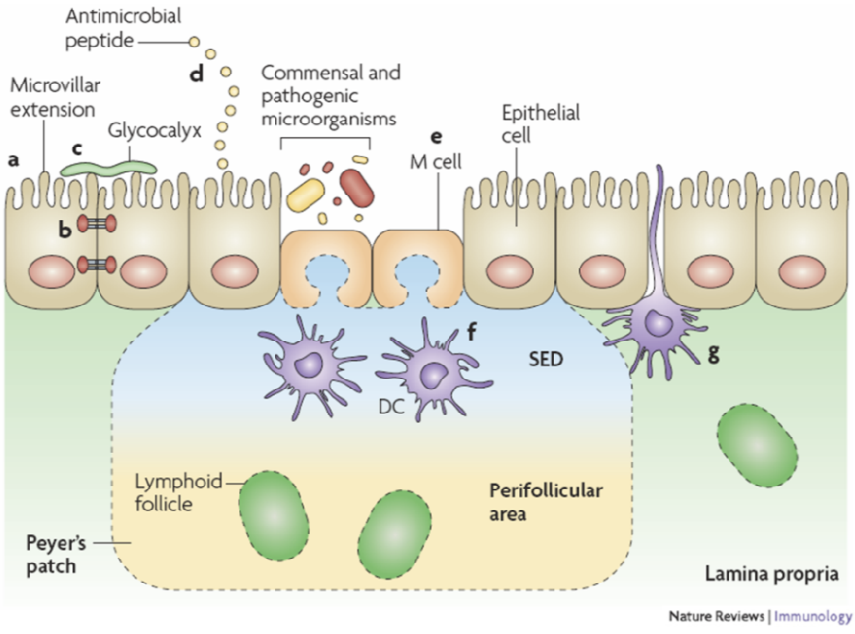

Figure 1 | The intestinal epithelial barrier. Epithelial integrity is established by polarized epithelial cells (a) connected through intercellular tight junction proteins (b) thus, exhibiting apical actin-rich microvilli that facilitate surface enlargement. Important defence mechanisms against bacteria are the secretion of mucin (c) and antimicrobial peptides (d) such as immunoglobulin A (IgA) or defensins. Furthermore, M-cells (e) lack secretion of mucin and promote bacterial trafficking to antigen-presenting cells (f) within the underlying Peyer's patches (PP). In addition, extended dendrites from dendritic cells (g), in the literature described as $CX3CR1^{+}$ $CD103^{-}$ (3), are found in between the epithelial layer which can trap bacteria form the lumen. From: (4)

Barrier dysfunction, as a consequence of e.g. inflammation, infection or injury, is monitored by intraepithelial lymphocytes, mainly CD8+T-cells and numerous immune cells of the underlying lamina propria, comprising CD4+T-cells, CD8+T-cells, macrophages, DCs, mast cells and B-cells. However, new cell populations are constantly indentified which do not fit in the scheme of the classical markers for phenotyping, underlining the complexity of immune surveillance and importance of immune homeostasis in individuals.

Inappropriate responses of the gut mucosa lead to high susceptibility to enteropathogens and disease. In addition, various pathogens have evolved strategies to invade the epithelium and evade the host immune response. Among them is the intracellular pathogen *Listeria monocytogenes (Lm).*

2. *Listeria monocytogenes*

Lm is a Gram-positive, facultatively intracellular bacterium and the causative agent of listeriosis, manifested by gastroenteritis with diarrhea and abdominal pain. It can be a lethal pathogen in immunocompromized patients because of its ability to cross the blood-brain or the feto-placental barrier leading to encephalitis, abortion or sepsis (5). It occurs ubiquitously nevertheless, its natural route of infection is through the GI-tract by ingestion of contaminated food.

After incorporation, *Lm* has the ability to adapt to the environmental conditions like low pH, bile, temperature and osmotic stress by upregulating virulence genes under the stress response transcription factor sigma B (σ^B). Another transcription factor PrfA allows the bacterium to invade the epithelium and to initiate its intracellular life cycle by activating virulence genes clustered in its genome (Fig.2). One of them is the surface protein Internalin A (InlA). Cossart *et al* have demonstrated in 2003 that InlA of *Lm* plays a critical role in the invasion of non-phagocytic cells (6). The pathogen uses InlA to adhere to the intestinal epithelium via its host receptor E-cadherin (E-cad). Signalling events via cytosolic E-cad interaction partners, such as the catenins, lead to the entry and dissemination of *Lm* by a dynamic process including actin polymerization and membrane remodelling.

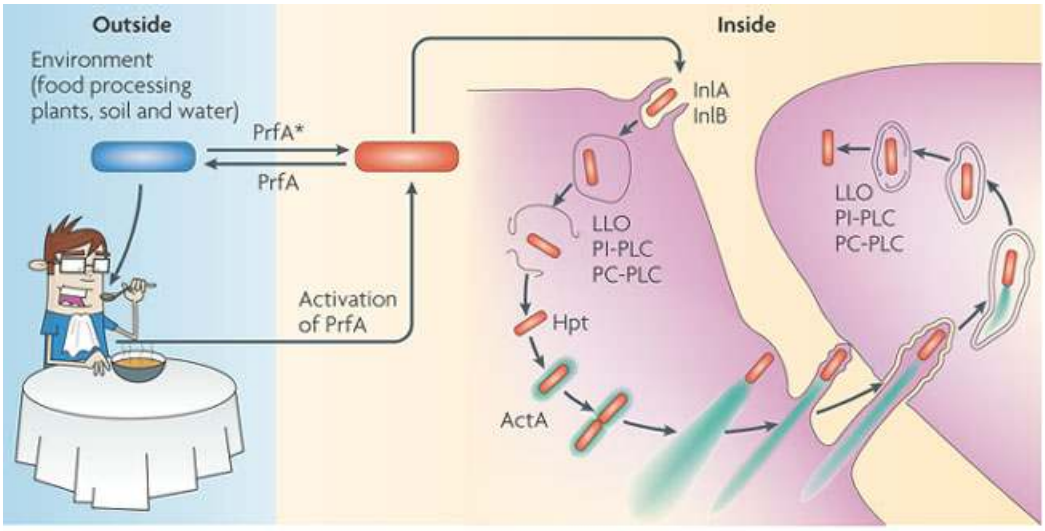

Figure 2 | Schematic representation of Listeria monocytogenes crossing three host barriers. The food-borne bacterium adapts to the conditions of the GI-tract and promotes its uptake by the expression of several virulence genes under the common control of the PrfA transcriptional regulator. Accordingly, it invades the host cell via the interaction of its surface proteins internalinA (InlA) or internalinB (InlB) with corresponding host receptors. After invasion the bacterium is engulfed in a phagocytic vacuole, where it escapes due to the expression of the pore-forming exotoxin ListeriolysinO (LLO) and phospholipaseC (PlcA). Within the cytoplasm the bacterium starts to replicate. Movement within the cytoplasm is driven by actin polymerization, initiated by another virulence factor, ActA. So called "actin comets" are formed that allow the bacterium to move not only within the cell, but also to neighbouring cells and thus to overcome epithelial barriers of the placenta or the central nervous system. Once Listeria has spread to the adjacent cells, it is caught in a double membrane vacuole which is then lysed by LLO, PlcA and a second phospholipaseC (PlcB) leading to the restart of the lifecycle and dissemination. From: (7)

E-cad is a homophilic calcium-depended adherence junction protein located below tight junctions at the basolateral site of the intestinal epithelium (8). Therefore the question arose how *Lm* can reach its extracellular domain (EC1). Pentecost *et al* showed that *Lm* does not actively destroy the epithelial junctions; instead invasion takes place at sites of epithelial cell extrusion at the villous tips (9). Nikitas *et al* suggested that E-cad is luminally accessible at mucus-expelling goblet cells as well as epithelial folds and tips of villi (10). Others stressed the role of passive invasion of Peyer's patches (PP) and overlaying M-cells (11-15) which is also observed by other pathogens such as *S. typhimurium* (16) or *Y. Enterocolitica* (17). The degree to which transcytosis via M-cells or invasion of epithelial cells contribute to intestinal invasion remains controversial.

Apart from InlA, a major virulence factor is the product of the *hly* gene, listeriolysin O (LLO). It is a pore forming exotoxin enabling *Lm* to escape from endosomes and phagosomes. Without LLO the bacterium is completely avirulent (18). LLO activity is supported by *plcA*- and *plcB*-encoded phospholipases, which are suggested to be multifunctional virulence factors. For

instance, there is evidence that these listerial proteins act together with InlA to facilitate bacterial entry by inserting holes into epithelial cells (19). Furthermore, it has been shown that they can mediate entry into hepatocytes (20).

3. Dissemination of *Listeria monocytogenes* to internal organs

Despite the knowledge about molecular invasion mechanisms, several key questions how *Lm* spreads to internal organs remain unanswered. The main reason has been the lack of an animal model, as the mouse is not the natural host for *Lm.* Limitations are given by low affinity between the bacterial InlA and the murine E-cadherin (8) (Fig.3), although listerial passage through the intestinal mucosa was successfully reported in mice gavaged with high inoculum sizes.

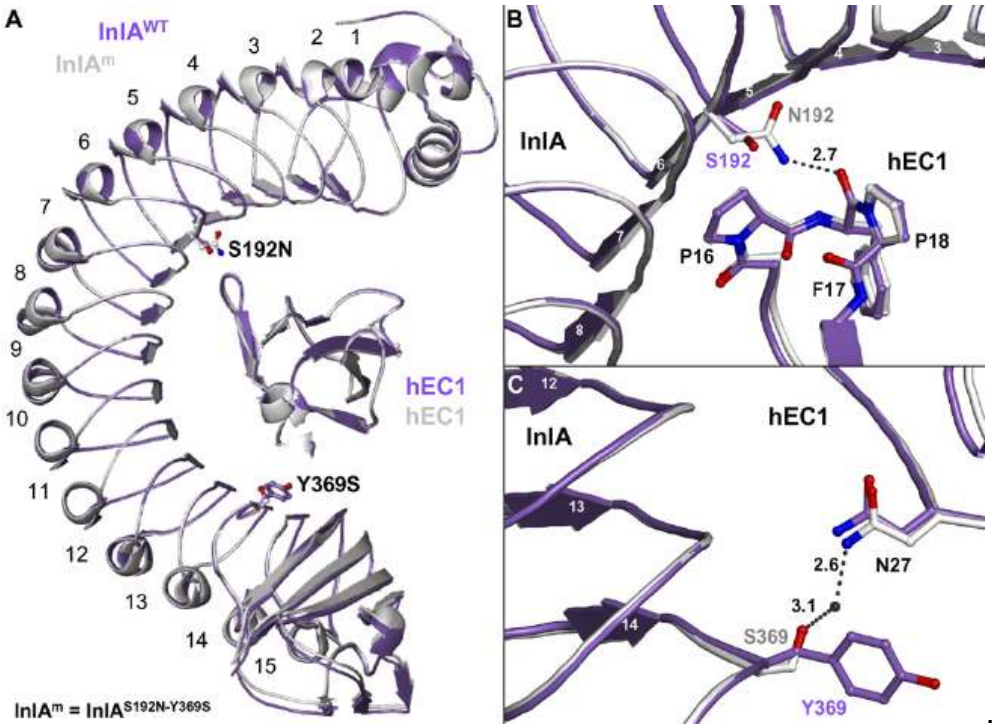

Figure 3 | Structure of the wild-type (violet) and murinized (grey) InlA interacting with the extracellular domain (EC1) of human E-cadherin (A). The replacement of serine192 against asparagine leads to a direct H-bond to phenyalanine17 (B). The replacement of tyrosine369 against serine introduces a water-bridge interaction to asparagine27 (C). Consequently, the binding affinity is identical between InlA/hEcad and $InlA^m$/mEcad. From: (21)

Speculatively, there are at least four possible mechanisms for listerial dissemination. First, intestinal dendritic cells (DCs) might take up *Lm* in the same way as they receive luminal content from M-cells. Accordingly, they can be transported via afferent lymphatic vessels to present antigen to T-cell areas within the paracortical regions of draining lymph nodes (22). The $CD103^+CD11b^+CD8\alpha^-$ DC population was described to be the most promising candidate for CCR7-depended migration to mesenteric lymph nodes (MLN) (23). Second, $CX3CR1^+CD103^-$ DCs can capture bacteria with their dendrites from the intestinal lumen and transport the bacteria to MLN (3), but if this is true for *Lm* needs further investigation. Third, systemic spread can be also achieved by blood monocytes. It has been demonstrated that $CD11b^+Ly6C^+CCR2^+$ monocytes carry *Lm* and are directed to the brain by the chemokine MCP-1 (24). This observation was confirmed by showing that dissemination of *Lm* within monocytes also occurred in mice treated with gentamicin (25). To reach the blood stream *Lm* has to pass at least two cell layers, one composed of enterocytes and one of endothelial cells (14). Where and how uptake into this carrier cell type takes place and whether or not infection occurs through yet another "intermediate" cell type is not understood. Possible candidates are cells of the early innate immune response to *Lm* including neutrophils, NK cells, DCs, macrophages or γδ T-cells (14, 26, 27). The initial recruitment of these cells depends on the synthesis of several cytokines and chemokines. Among them IFN-I play a key role (see 6.1).

As to the last possibility, lessons from other microbes suggest an alternative transport route for enterobacteria to the liver via the portal vein, as suggested by Melton-Witt *et al*. (28). The entry of mucosal *Lm* to blood vessels collected by the portal vein may explain the rapid dissemination to the liver from the gut within a few hours after infection (21, 29).

4. The liver as target organ of *Lm*

The liver is a vital organ fulfilling metabolic, clearance and storage functions including the uptake of nutrients, detoxification and trapping blood-borne bacteria as well as bacterial products. To prevent organ damage following an inadequate immune response against antigens, the liver exhibits unique immunoregulatory functions, making it an ideal target site for pathogens (30). Incoming bacteria are generally considered to be cleared by phagocytes. In the liver, 25% of all mononuclear cells are Kupffer cells being the liver resident macrophages that adhere to endothelial cells lining the liver sinusoids (31). However, it has been demonstrated that the large majority of *Lm* is associated with hepatocytes within the first three days of infection, whereas a relatively small number resides in Kupffer cells (32). The increase in Listeria CFU during the first 3-4 days of infection is stopped by the adaptive immune system which is mainly T-cell dependent and decreases listerial burden to result in sterile immunity (33). Despite the importance of the adaptive response in clearing *Lm*, the innate immune responses efficiently hampers bacterial

growth. In the liver, Kupffer cells, natural killer (NK) cells, incoming macrophages and neutrophils play an important role as mediators of the innate response (34).

5. Recognition of *Listeria monocytogenes* by the innate immune system

The innate immune system rapidly forms the first line of defence against microorganisms. An important initial step is the recognition of bacterial components by pattern recognition receptors (PRR). These are specialized surface, endosomal or cytoplasmic receptors that can bind pathogen-associated molecular patterns (PAMPs), structures that repeat in many different microbes, thus allowing recognition of many pathogens with a limited number of antigen receptors. These include the toll like receptors (TLR), the retinoic acid (RIG) like receptors, the Nod like receptors (NLR) or aim2-like receptors (ALR) that are involved in forming inflammasomes (35). *Lm* offers a panoply of PRR ligands like flaggelin, lipoteichoic acid, peptidoglycan, lipoproteins or its DNA. The main receptor recognizing *Lm*, thereby inducing IFNβ production, resides in the cytosol. Cytosolic Listeria receptors are among the family of DNA receptors (36) or cyclic di-nucleotide receptors such as STING (37, 38). As a consequence of receptor binding, signal cascades lead to the activation of the S/T kinase TBK1 and the subsequent phosphorylation and activation of transcription factor IRF3 (Fig. 4). IRF3 is a rate-limiting component of an enhancosome, forming at the promoter of type I interferon genes, particularly the IFNβ gene (39). Compared to other Listeria strains, the LO28 strain used in our study is a particular potent inducer of IFN-I because of its superior ability to stimulate the IRF3 pathway (40).

6. Interferons

Interferons were first described in 1957 by Isaacs and Lindenmann as antiviral substances released by virus-infected cells (41). More than 50 years later we know that interferons are in fact released in response to infection with all types of intracellular pathogens (42). They are immunological key regulators which comprise three structurally and functionally interleukin-10-related subtypes of the larger class of type II cytokines (43). Today we know three families of IFNs which all share transcription factors and signalling pathways but bind to specific receptors (Fig.3).

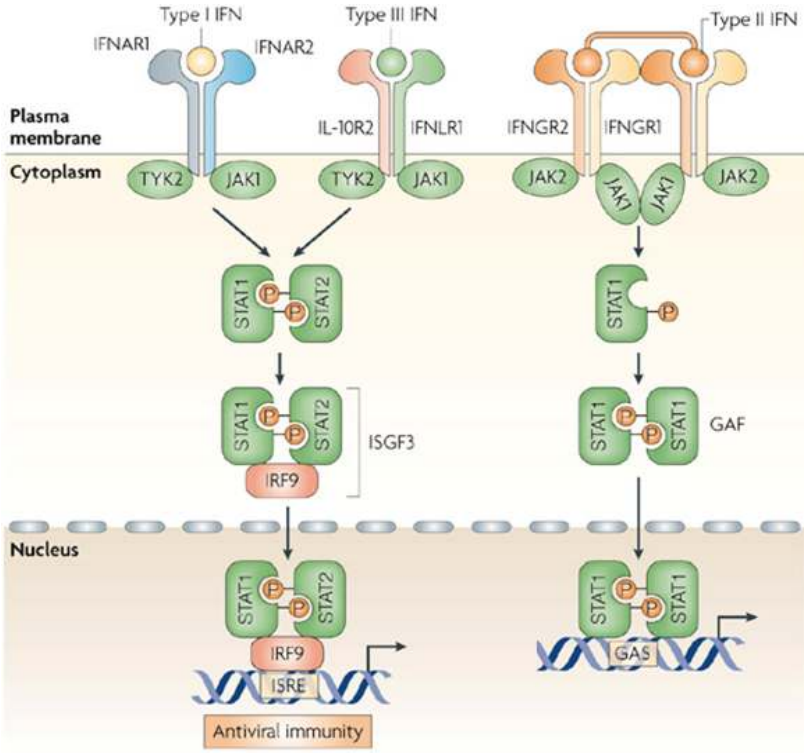

Figure 3 | Signal transduction by interferons. IFN-I bind to their respective receptor (comprising IFNAR1 and IFNAR2 chains) which induces a conformational change that leads to the activation of receptor-associated Janus tyrosine kinases (JAKs). Signal transducers and activators of transcription (STATs) are recruited to the phosphorylated receptor complex via their SH2 domains and are phosphorylated by the JAKs. Tyrosine phosphorylation leads to heterodimerisation of STAT1 and STAT2 and their translocation to the cell nucleus. Together with the interferon regulatory factor 9 (IRF9) the interferon stimulated gene factor 3 complex (ISGF3) is formed and binds to the interferon stimulated response element (ISRE), thus stimulating expression of antiviral and immunoregulatory genes. In contrast, IFN-II bind to a receptor comprising IFNGR1 and IFNGR2 chains. Signalling by this receptor complex leads to the formation of STAT1 homodimers and to the expression of genes with gamma interferon activation sequences (GAS) within their promoters. Upon IFN-III binding, the receptor formed by IFNLR and IL-10R2 chains stimulates the same signal transduction as IFN-I. Therefore, mice lacking the IRF9 transcription factor do not respond to IFN-I and IFN-III. From: (44)

6.1 Type I interferons (IFN-I)

IFN-I comprise about 20 members including 13 IFNα subtypes and one IFNβ subtype, as well as IFNε, IFNκ and IFNω. Classically, these cytokines have potent antiviral and anti-proliferative activity but only IFNα and IFNβ are important in bacterial defences (45). Almost every cell type has the ability to respond to or to produce IFN-I (42). While IFN-I are generally associated with viral clearance their predominant effect on the immune response to non-viral

pathogens and their beneficial or detrimental character strongly depend on the particular pathogen (46).

IFN-I bind to their type-I interferon receptor, comprising the IFNAR1 and IFNAR2 chains (Fig.3). Signal transduction via phosphorylation of the receptor associated Janus kinases, JAK1 and TYK2, leads to the recruitment of the transcription factors STAT1 and STAT2. After tyrosine phosphorylation of these signal transducers and activators of transcription, STAT1/2 heterodimers are formed which migrate to the cell nucleus and, together with the interferon regulatory factor 9 (IRF9), form the trimeric complex called interferon stimulated gene factor 3 (ISGF3). ISGF3 triggers the expression of several antiviral and immunoregulatory genes by binding the interferon response element (ISRE) (46) (Fig.4)

6.2 Type II interferons (IFN-II)

IFNγ is the only IFN-II and primarily used by the immune system to fight intracellular bacteria. It is secreted by specialized immune cells such as T-cells and NK-cells, however, its receptor can be expressed on various cell types, although to different extents (47). IFNγ is a main activator of macrophages that forms the link between innate and adaptive immunity and it is also important for triggering antigen-processing and -presentation (48). IFNγ-deficient mice are highly susceptible to infection with *Lm* and other intracellular bacteria. The receptor for IFNγ comprises of the IFNγ receptor chain 1 (IFNGR1) and IFNGR2 which are associated with JAK1 and JAK2 Janus kinases. Phosphorylation upon receptor binding triggers STAT1 homodimer formation and consequent expression of interferon gamma response elements (GAS) (Fig.3).

6.3 Type III interferons (IFN-III)

The three different IFN-III members IL-29 (IFN-λ1), IL-28A (IFN-λ2) and IL-28B (IFNλ3) bind to their receptor consisting of IFN-λR1 and IL-10R2 chains to stimulate the same signalling pathway as IFN-I. Contrasting the IFN-I receptor however, the IFN-III receptor is expressed exclusively on epithelial layers particularly of the GI-tract or the lung and, in humans but not in mice, the liver (49) (Fig.3). Generally they are considered to support the response of IFN-I at sites of frequent environmental or microbial exposure. Speculatively, this is the reason why they are also expressed in livers as this organ is often challenged by intestine- or blood-borne pathogens (50).

The focus of this thesis lies on the importance of IFN-I, hallmark cytokines of innate responses to pathogens, for immunity to *Lm*. Therefore, innate responses of mice lacking the interferon I receptor (IFNAR1) were compared to those of Wt controls. A number of reports

document that IFN-I increase the susceptibility of mice after systemic infection with *Lm* (51, 52). Here, we specifically investigate the role of type I interferons after intragastric infection and provide the first evidence that the adverse or beneficial role of a cytokine varies with the route of infection and that IFN-I are not harmful when infection with *Lm* occurs via the natural route. Additional data with IRF9-/- mice suggest an impact of the epithelial response to IFN-III on the innate response to *Lm* administered via the gastrointestinal route.

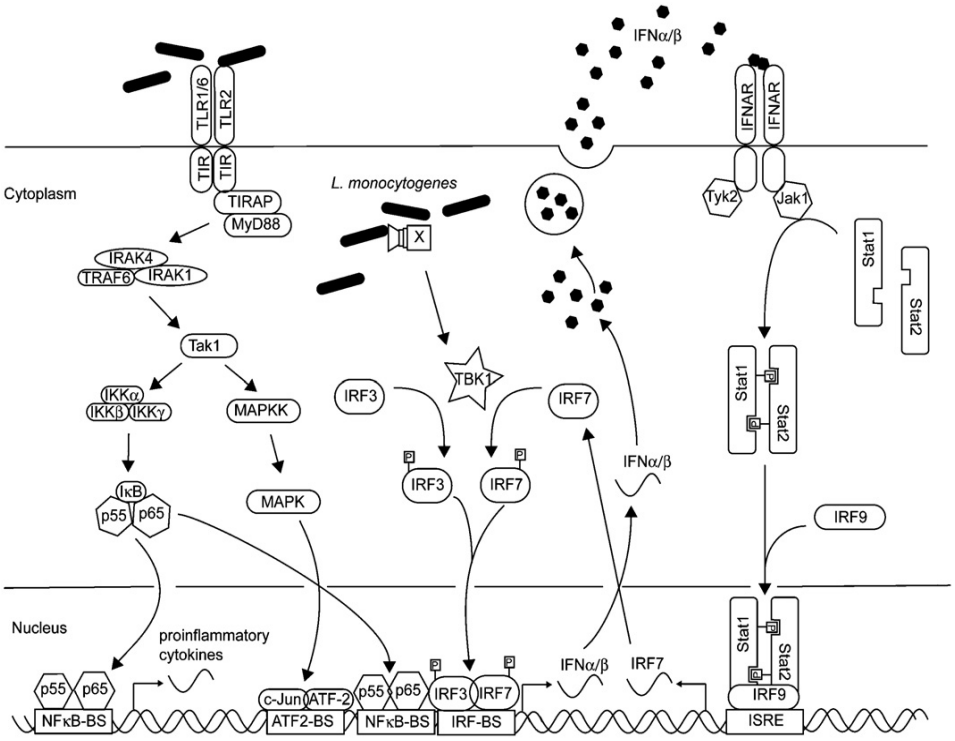

Figure 4 | Induction of IFN-I by *Listeria monocytogenes*. Recognition of *Lm* is mainly by a cytosolic receptor (X, see text) leading to the activation of the TANK-binding kinase 1 (TBK1) or the closely related IKKε that phosphorylate the interferon regulatory factor (IRF3). IRF3 is a transcription factor (TF) for the IFNβ gene. In addition, listerial membrane components can stimulate TLRs to activate MAPK and NFκB pathways and the expression of proinflammatory genes. NFκB and c-Jun/ATF-2 also exhibit transcriptional binding sites within the IFNβ promoter. Together with IRF3 they form the IFNβ enhancosome. IFNβ molecules are secreted and bind their specific receptor (IFNAR). Upon contact with the secreted ligand, the IFNAR1 and 2 subunits undergo a conformational change leading to auto-phosphorylation of the receptor associated tyrosine-kinases (TYK2 and JAK1). This creates a docking site for STAT1/STAT2 which, upon phosphorylation, form STAT1/STAT2 heterodimers. Together with IRF9, they form the interferon-stimulated gene factor 3 complex (ISGF3). This TF induces the expression of antimicrobial genes and of the transcription factor IRF7. In case bacterial stimulation of cytosolic signalling persists, TBK1/IKKε phosphorylate IRF7 to drive the expression of IFNα and IFNβ which then results in a feed-forward amplification of IFN-synthesis and the IFN-I response. IFNAR-/- or IRF9-/- mice are unable to induce this amplification loop. From: (53)

Results

Status: Submitted manuscript

Experimental contribution: EK: 50%
VM: 50%

- bacterial load and survival assays together with EK
- cytokine analysis together with EK
- RNA analysis together with EK
- FACS analysis with IR
- Histology and Immunohistochemistry

Manuscript

Route of infection determines the impact of type I interferons on innate immunity to *Listeria monocytogenes*

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Running title: Intragastric Listeria infections

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This work was supported by the Austrian Science Fund (grant P20522-B05 to TD).

Abstract

Listeria monocytogenes is a food-borne pathogen causing mild to life threatening disease in humans (1). Ingestion of contaminated food delivers the pathogen to the gastrointestinal tract, where it crosses the epithelial barrier and spreads to internal organs. Type I interferons (IFN-I) are produced during infection and decrease host resistance after systemic delivery of *L. monocytogenes*. Here we show that mice benefit from IFN-I production following infection with *L. monocytogenes* via the gastrointestinal route. Intragastric infection lead to increased lethality of IFN-I receptor-deficient (Ifnar1-/-) animals and to higher bacterial numbers in liver and spleen. Compared to infection from the peritoneum, bacteria infecting via the intestinal tract localized more often to periportal and pericentral regions of the liver and less frequently to the margins of liver lobes. Vigorous replication of intestine-borne *L. monocytogenes* in the livers of Ifnar1-/- mice 48h post infection was accompanied by the formation of large inflammatory infiltrates in this organ and massive death of surrounding hepatocytes. This was not observed in Ifnar1-/- mice after intraperitoneal infection. The inflammatory response to infection is shaped by alterations in splenic cytokine production, particularly IFN-γ, which differs after intragastric versus intraperitoneal infection. Taken together, our data provide the first evidence that the adverse or beneficial role of a cytokine varies with the route of infection and that IFN-I are not harmful when infection with *L. monocytogenes* occurs via the natural route.

Introduction

Listeria monocytogenes (Lm) is a food-borne pathogen causing potentially life threatening disease in immunocompromised humans (1). Lm's ability to cross epithelial barriers including the intestinal epithelium, the placental and the blood brain barrier is a prerequisite for systemic dissemination and its sequels such as sepsis, fetal abortion and encephalitis. The natural route of infection is via ingestion of contaminated food and although the course of infection is in most cases very mild, manifested disease has one of the highest mortality rates among food-borne diseases. Lm uses a set of virulence factors to successfully invade hosts. These include internalins that allow attaching to, and subsequently invade various different cell types. Internalins (Inl) A and B are instrumental for the invasion of epithelial cells and hepatocytes (2).

Type I interferons (IFN-I) are cytokines essential for the establishment of innate antiviral immunity. Their role in bacterial infections varies between protective or detrimental, depending on the pathogen and conditions of infection (3). Mice lacking the IFN-I receptor show increased resistance to infection with Lm compared to Wt mice (4-6). Therefore, IFN-I are thought to decrease the ability of mice to combat infection. The harmful effects of IFN-I have been assigned to different aspects of the immune response. The death of effector cells such as T-cells and macrophages increases with IFN-I signalling during Lm infection (6, 7). As a consequence of the increased uptake of apoptotic cells interleukin 10 (IL10) is produced which hampers protective immune responses (8). In line with this, IL10-/- mice are more resistant to Lm infection than Wt mice (9). In addition, IFN-I may also interfere with IFNγ dependent macrophage activation by decreasing cell surface expression of the IFNGR (10).

The data describing the adverse effect of IFN-I on the course of Lm infection stem from systemic infection models, like intravenous or intraperitoneal infection. Immune reactions to intragastric Lm infections, the natural route of infection, have not been studied intensively due to the limitations of the murine system, namely low infection efficiency and receptor incompatibility. The latter results mainly from the inability of murine E-cadherin on epithelial surfaces to interact with the bacterial InlA (11, 12).

Here we show for the first time the effect of IFN-I when infection with Lm occurs via the natural route. Our experiments were carried out with a mouse adapted Lm strain which allows robust and efficient infection of mice via intragastric gavage. We clearly demonstrate that the route of infection matters for the impact of IFN-I on innate resistance, as mice benefit from IFN-I after intragastric infection. Differences in splenic cytokine production, inflammatory cell recruitment and hepatoxicity are suggested to underlie the infection route - dependent impact of IFN-I on the outcome of infection with Lm.

Materials and methods

Mice, bacteria

C57BL/6N (Wt) and Ifnar1-/- (B6.129P2-IfnaR1^{tm1} (13)) mice were housed under SPF conditions. Animal experiments were discussed and approved by the University of Veterinary Medicine Vienna institutional ethics committee and carried out in accordance with protocols approved by the Austrian law (BMWF-68.205/0204-C/GT/2007; BMWF-68.205/0210-II/10b/2009, BMWF-68.205/0243-II/3b/2011). As a prerequisite for constructing the mouse-adapted *Listeria monocytogenes* LO28InlA$^{S192N/Y369S}$ strain we prepared an InlA knockout in LO28wt using the pMAD vector (14) and the following primers for amplification of the upstream and downstream region of the InlA gene from genomic LO28 DNA: InlA_A 5` CAT GGT CGA CGG CAG TCC GCG ATT TAA TGG AAG T 3`, InlA_B 5` CAT GGG ATC CCC TAA TCT ATC CGC CTG AAG CGT TGT 3` InlA_C 5` CAT GGG ATC CGG GAA TTC AGC CAG CAC AAC AAG T 3´ and InlA_D 5` CTG CCA TGG AGG TTT AGG TGC AGT TAT CCG CGT 3`. For genomic integration we used the protocol described in (15). We transformed the LO28InlA knockout strain with the pAUL-A InlA $^{S192N/Y369S}$ -InlB construct kindly provided by WD Schubert (Molecular Host-Pathogen Interactions, Division of Structural Biology, Helmholtz Centre for Infection Research, Braunschweig, Germany (16)) and obtained genomic integration as described in (17). Here the abbreviation Lm for experiments always refers to LO28InlA*.

Infection of mice, determination of bacterial organ loads

Bacteria were prepared for infection as described previously (18). For infection, Lm LO28InlA* were washed, diluted to the respective concentration with PBS (Sigma) and injected intraperitoneally (i.p.) or intravenously (i.v.) into 8- to 10-week-old, sex and age matched C57BL/6N (Wt) and Ifnar1-/- (B6.129P2-IfnaR1^{tm1} (13)) mice. For intragastric infection mice were infected as described (19). Briefly, mice were starved over night and orally gavaged with 15mg $CaCO_3$ in 200µl PBS (50mg/ml) succeeded by the respective dose of Lm in 200µl of PBS. The infectious dose was controlled by plating serial dilutions on Oxford agar plates. The survival of mice was monitored for 10 days and data were displayed as Kaplan-Meier plots. For determination of bacterial loads (colony forming units, CFU) of livers, spleens, mesenteric lymph

nodes (MLN) and Peyer's patches (PP), mice were sacrificed at the indicated time points, organs isolated and homogenized in PBS. Serial dilutions of the homogenates were plated on BHI plates and incubated at 37°C for 24h.

RNA isolation, cDNA synthesis and RT-qPCR

Organs of respective animals were isolated and stored at -80°C until further use. For RNA preparation organs were homogenized in RA1 buffer of the NucleoSpin II RNA isolation kit (Macherey and Nagel) and processed according to protocol. cDNA was prepared as described (20). The RT-qPCRs were run on an Eppendorf cycler. After correction for the housekeeping gene GAPDH, every sample was calculated to the mean of Wt mRNA levels or as a ratio to uninfected samples. Sequences of primers are listed in table I.

IL6	for	TAGTCCTTCCTACCCCAATTTCC
	rev	TTGGTCCTTAGCCACTCCTTC
IL10	for	GGTTGCCAAGCCTTATCGGA
	rev	ACCTGCTCCACTGCCTTGCT
Mx2	for	CCAGTTCCTCTCAGTCCCAAGATT
	rev	TACTGGATGATCAAGGGAACGTGG
MCP1	for	CTTCTGGGCCTGCTGTTCA
	rev	CCAGCCTACTCATTGGGATCA
GAPDH	for	CATGGCCTTCCGTGTTCCTA
	rev	GCGGCACGTCAGATCCA
IFNβ	for	TCAGAATGAGTGGTGGTTGC
	rev	GACCTTTCAAATGCAGTAGATTCA
TNFα	for	CAAAATTCGAGTGACAAGCCTG
	rev	GAGATCCATGCCGTTGGC
IFNγ	for	ATGAACGCTACACACTGCATC
	rev	CCATCCTTTTGCCAGTTCCTC

Table 1: **Primer sequences of cytokines and chemokines used for RT-qPCR.**

Serum cytokine analysis

For cytokine analysis mice were bled via the retro-orbital sinuses and serum was prepared and stored at -80°C. The indicated cytokines were measured using the FlowCytomix kits (eBioscience) in 25µl of serum (IFNγ, MCP-1, IL-6).

Cell culture

Bone marrow derived cells were isolated and grown as described (18). Briefly, bone marrow was isolated from femurs of 6–8 week old mice. For differentiation of bone marrow derived macrophages, cells were grown in DMEM (Gibco, Invitrogen) in the presence of 10% FCS (Gibco, Invitrogen) and L-cell derived CSF-1 as described (18). The cultures contained >99% F4/80+ cells. Bone marrow derived dendritic cells (mDC) were obtained by culture of bone marrow in DMEM (Gibco, Invitrogen), supplemented with 10% FCS (Gibco, Invitrogen) and X-6310 derived GM-CSF as described (18). mDC cultures contained virtually no F4/80+ cells and the purity of CD11c+/CD11b+ cells was between 60 and 70%. Cells were infected with Lm at a MOI of 10 for 1h, extensively washed and resuspended in PBS for injection into mice.

Histology

Mouse organs were fixed with 4% paraformaldehyde over night, paraffin embedded and 3µm sections were prepared using a microtome. Hematoxyline and eosin staining (H&E) were performed using standard protocols. For TdT-mediated dUTP nick end labelling (TUNEL), sections of liver and spleens were stained as described before (21). For Gr-1 staining liver sections were blocked for endogenous peroxidase activity in 50% methanol with 3% H2O2 and boiled for 30’ in 10mM sodium citrate antigen unmasking solution. After cooling down for 30’, the samples were blocked with 5% normal goat serum to reduce background staining. Primary Gr-1 antibody (BD Pharmingen) was applied overnight at 4°C and before adding AEC+ high sensitivity chromogen substrate (Dako) the sections were incubated with biotinylated rabbit α rat IgG (1:250 in PBS) for 30’ at RT following incubation with ABC reagent (Vector) for 30’ at RT. To stain for Lm in infected tissue, thin sections were incubated with 500 µg/ml pronase (Roche) for 10’ at 37°C, washed with PBS containing 0,05% Tween (PBS-T) and blocked with 5% normal goat serum for 30’ at RT. Next, primary Listeria antibody (1:100) (Abcam) was applied for 1h at RT, washed with

PBS-T following incubation with 3 drops of HRP rabbit/mouse polymer (Dako) for 30' at RT. After washing, Lm was visualized by AEC+ high sensitivity chromogen substrate and the cells counterstained with haematoxylin.

Flow cytometry

For isolation of non-parenchymal cells, mice were sacrificed and the liver was perfused immediately with liver perfusion medium (Gibco, Invitrogen) via the portal vein for 3' at a speed of 8ml/min followed by liver digest medium (Gibco, Invitrogen) for 5'. The liver was removed, minced with scissors in 15ml ice-cold DMEM+10%FCS+penicillin/streptomycin and filtered through a 70µm cell strainer. To collect the non-parenchymal cell enriched supernatant the suspension was centrifuged for 5' at 50g. Next, non-parenchymal cells were harvested by centrifugation for 5' at 300g and washed. After red blood cell lysis the cells were blocked with anti mouse CD16/32 and stained for Ly6G (BD-Pharmingen), CD11b, Ly6C, F4/80, CD45 and eFluor fixable viability dye (eBioscience). Flow cytometry was performed using BD FACSAria.

Statistical analysis

Bacterial loads of organs were compared using the Mann-Whitney test and indicating the Median. mRNA expression data were analysed with the Students t Test and indicating the mean. For both the GraphPad Prism (Graphpad) was used.

Results

IFN-I signalling increases resistance to Listeria monocytogenes contracted via the intragastric route. To assess whether the impact of IFN-I on innate immunity to Lm is determined by the infection route, we administered Lm to Wt and Ifnar1-/- mice both by intragastric gavage (i.g.) and intraperitoneal injection (i.p.). These experiments were performed with a mouse-adapted, mutated LO28 strain of Lm, LO28InlA* (see materials and methods). This strain expresses an InlA mutant with increased affinity to murine E-cadherin (16). Compared to LO28 Wt, intragastric administration of LO28InlA* produced a roughly 5-fold higher bacterial burden in the liver and about 3-fold higher burden in spleen 48h after infection (Fig. S1). This difference is similar to or larger than that reported for the mouse-adapted EGD strain (16, 22). By contrast, infection rates were virtually indistinguishable 48h after i.p. infection.

In keeping with previous reports, a drastic difference in the bacterial load was observed in livers and spleens of Wt and Ifnar1-/- mice 72h after i.p. infection (Figs. 1A, B). When mice were infected i.g., bacterial loads at day three were similar or even slightly elevated in Ifnar1-/- spleens and livers (Figs. 1C, D). To monitor survival we infected Ifnar1-/- and control mice i.g. with a high dose of Lm. IFN-I signalling protected mice from lethal infection with Lm, as Ifnar1-/- mice were the only animals to succumb to infection (Fig. 1E). This is in striking contrast to the results reported after i.p. or intravenous (i.v.) infections (6).

The bacterial burden of Lm-infected animals is routinely assessed at day three after inoculation. However, the organ loads shown in figure 1 insufficiently explain the increased mortality and poorer condition of i.g.-infected Ifnar1-/- mice. To determine the time point at which IFN-I exert their protective effects, we monitored bacterial replication between 24 and 72h after infection (Fig. 2). We detected reduced or equal amounts of bacteria in livers and spleens of Ifnar1-/- mice compared to Wt mice 24h after infection. 48h after i.g. infection, Ifnar1-/- mice showed higher numbers of bacteria in these organs, with the most striking difference seen in the liver (Figs. 2A, B) whereas at day three after infection the bacterial loads in Ifnar1-/- mice were comparable to Wt levels (Figs. 1A, B). In striking contrast to these results, i.p. infected Ifnar1-/- mice had lower amounts of Lm in liver and spleen at both 48h (Figs. 2C, D) and 72h (Figs. 1C, D)

after infection. 24h after i.p. infection with Lm the organ load was similar in Ifnar1-/- and Wt mice (18).

The detrimental or beneficial effect of IFN-I is not determined by the size of the L. monocytogenes inoculum. I.p. or i.v. injections of bacteria deliver the entire inoculum directly to internal organs. By contrast, i.g. infection might result in a more gradual release of bacteria from the intestinal tract, hence a lower primary infectious dose for internal organs. To examine whether the amount of the primary inoculum delivered to target organs influences the effect of IFN-I, we infected Ifnar1-/- and control animals with 10^2 Lm i.v. Figure 3A shows that even at this very low dose Ifnar1-/- mice showed an increased ability to prevent Lm replication, similar to mice infected with 100-fold more bacteria. Therefore, systemic delivery rather than inoculum size seems to be the main determinant for the effect of IFN-I. Alternatively the difference between i.p. and i.g. infection might arise from the mode of dissemination. According to previous reports (e.g. (23)), Lm traversing the intestinal epithelium are thought to be taken up and spread throughout the host organism via phagocytic cells residing in mucosal lymphoid organs such as macrophages or DC. We tried to mimic this situation by injecting various amounts of *in vitro* Lm-infected macrophages or dendritic cells i.v. into Wt and Ifnar1-/- mice. 40-50% of these cells harboured bacteria (data not shown). Figure 3B demonstrates that intracellular delivery of Lm resulted in enhanced bacterial clearance by Ifnar1-/- compared to Wt mice. Therefore, intracellular dissemination cannot per se explain how the infection route shapes the impact of IFN-I on antibacterial innate immunity.

IFN-I do not inhibit invasion of the gut mucosa or of mucosa associated lymphoid tissue. We tested the hypothesis that the beneficial effect of IFN-I on i.g.-infected mice might result from a decreased rate of intestinal invasion. This is suggested by older reports that epithelial cells treated with IFN-I show increased resistance against invasion by enteropathogens (24, 25). To examine the uptake of Lm by intestinal tissue or the gut-associated lymphoid tissue we first performed immunohistochemistry on the intestinal mucosa 48h after i.g. infection. Visualization of Lm with a specific antiserum demonstrated the presence of bacteria in mucosal tissue. Very low numbers of Lm were found in epithelial cells, the vast majority had crossed the epithelial barrier to reside in the underlying mucosa (Fig. 4A). No differences between Wt and Ifnar1-/- mice were noted (Fig. 4A, upper panels). Similarly, infection of Peyer's patches or

mesenteric lymph nodes did not reveal an effect of IFN-I early after invasion (Fig. 4A, lower panels, Figs. 4B, C). Together the data suggest that there is little measurable effect of IFN-I on intestinal invasion by Lm. Profiling cytokine mRNA production strengthened the notion that IFN-I have little impact on early events after intestinal invasion. Apart from IFN-β, which is amplified by a positive feedback loop involving IFN-I (3), and the IFN-I inducible Mx gene, there was very little impact of Ifnar1 deletion on cytokine expression in PP (Fig. 4D). 48h post infection IFN-γ and MCP-1 production were significantly reduced. At this time the infection is systemic and, as shown below, the control of the immune response most likely dominated by splenic cytokine production.

Absence of a type I IFN response exacerbates inflammatory pathology in livers of mice infected via the gastrointestinal route. Analysis of the bacterial organ burden during the course of infection demonstrated a pronounced peak of multiplication of Lm in the livers of Ifnar1-/- mice infected i.g. for 48h. Mice begin to die shortly after this period. Furthermore, our recent demonstration that liver damage is closely correlated to the lethality of infection (21) suggests that IFN-I may reduce the severity of liver pathology in i.g.-infected mice. Livers were therefore subjected to histological, immunohistochemical and flow cytometric examination.

To assess whether the route of infection alters the predominant localization of Lm, livers 48h after i.g. or i.p. infection were subjected to immunohistochemistry with anti-Listeria serum. Bacteria localized to inflammatory infiltrates. Quantitative evaluation revealed a larger fraction of Lm in periportal or pericentral areas after i.g. infection, whereas Lm in i.p.-infected mice showed increased localization at the margins of liver lobes (Fig. 5A).

In accordance with the bacterial loads determined in figure 2, H&E staining demonstrated an increased number of small inflammatory infiltrates 24h after infection in Wt compared to Ifnar1-/- livers (Fig. 5B). By contrast, more and much larger infiltrates were observed in Ifnar1-/- mice compared to their Wt controls 48h after infection. Likewise, Ifnar1-/- mice infected i.p. did not display a similarly high number or similarly large size of inflammatory liver infiltrates (data not shown). Closer inspection of the inflammatory infiltrate showed that they contained a large number of $Gr1^+$ cells (neutrophils and inflammatory monocytes) and that they were the predominant sites of infection (Figs. 5C, D). Strikingly, the infiltrates as well as the surrounding

hepatic tissue contained a large number of TUNEL-positive dying or dead cells (Fig. 5E) which is consistent with reports that Lm kills infected hepatocytes, macrophages, or dendritic cells (26).

We proceeded to determine the innate effector cell populations present in the fraction of non-parenchymal liver cells (NPC) 48h after i.p. or i.g. infection. Ifnar1-/- livers contained significantly less neutrophils after i.p. injection of Lm and the difference was larger after i.g. infection (Fig. 6A). Ifnar1-/- livers from i.g.-infected mice also contained slightly less F4/80+ macrophages that include a large fraction of the resident Kupffer cells (Fig. 6B). Notably, i.g. infection of Ifnar1-/- mice significantly reduced the fraction of inflammatory monocytes compared to Wt, whereas both genotypes contained equal numbers of this cell type following infection through the peritoneum (Fig. 6C).

In mice infected through systemic routes, IFN-I strongly enhance the death of apoptotic lymphocytes. To assess whether this activity differs after gastrointestinal infection, in situ TUNEL staining of infected splenic tissue was performed 48h post infection (right panels of Fig. 7) and further compared to listeria-staining of serial sections (left panels of Fig. 7) . Consistent with published data (5, 21) the absence of Ifnar1 strongly reduced the number of apoptotic cells in extra-follicular areas of the white pulp after i.p. infection (upper right panels of Fig. 7). Spleens after i.g. infection contained less apoptotic cells in correlation with roughly 10-fold less bacteria (lower right panels of Fig. 7, Fig. 2). In spite of an increase in bacterial numbers relative to Wt controls (lower left panel of Fig. 7, Fig. 2), Ifnar1-/- spleens contained fewer TUNEL+ cells. Thus, the inhibitory activity of IFN-I on splenocyte death appears to be independent of the infection route.

IFN-I accelerate and increase proinflammatory cytokine activity after intragastric *Listeria monocytogenes* infection. Our analysis of liver inflammation suggests it contributes to, or reflects the different impact of IFN-I on mice infected through enteral or parenteral routes. However, production of cytokines that regulate inflammation and immunity is to a large extent an attribute of leukocytes residing in the blood or in lymphoid organs. Therefore, we determined cytokine mRNA expression in the spleen.

Splenic IFNβ mRNA was expressed up to 72h after i.g. application of Lm. In accordance with expectations, the IFN-I induced gene Mx2 was expressed in infected spleens up to 72h in

Wt, but was strongly reduced in Ifnar1-/- mice (Fig. 8A). Among the pro-inflammatory cytokines and chemokines tested, IL-6 and MCP-1 were decreased early after infection. Notably, this effect was not observed at a similar time after i.p. infection. Of further interest, early splenic IFN-γ production was increased in Ifnar1-/- mice compared to Wt after i.p. infection, but not after infection through the gastrointestinal tract. Early TNF-α production was reduced in Ifnar1-/- mice infected through the intestinal tract and even more strongly after i.p. infection. Compared to Wt mice, immunesuppressive IL-10 was elevated in Ifnar1-/- 72h after i.g. infection, but reduced after i.p. infection. The data suggest that splenic cytokine production is an important determinant of the different impact of IFN-I after enteral and parenteral infection routes. Due to the overwhelming importance of IFN-γ for innate resistance to Lm (27, 28), we consider the delayed and lower production of this cytokine after i.g. infection versus i.p. infection the most striking result. It reflects the detrimental increase of hepatic Lm between 24 and 48h after i.g. infection. To determine whether systemic IFN-γ production corresponds to splenic mRNA, serum IFN-γ was measured and found to correlate well with the levels of splenic IFN-γ mRNA (Fig. 8B).

Discussion

Lm is a widely studied pathogen that has been instrumental in advancing the knowledge about innate responses to intracellular bacteria. However, the vast majority of data about immunity to Lm stems from animals infected via the intraperitoneal or intravenous route. Comparably little knowledge has been obtained about infection through the intestinal tract which represents the natural entry route in humans. The incompatibility of the InlA/ E-cadherin interaction in murine hosts, required for efficient invasion of epithelial cells, posed an obstacle to murine models of gastrointestinal infection. To overcome this limitation, we followed the approach of Wollert *et al* (16) to construct a Lm strain expressing a mutant, 'murinized' InlA gene which interacts with mouse E-cadherin and therefore improves the invasive capacity of Lm. We applied this approach to Lm strain LO28, a potent inducer of IFN-I *in vitro* and *in vivo* (29). E-cadherin is located at the basolateral side of epithelial cells, making it less accessible to bacteria in the gut lumen. Pentecost and colleagues have shown that at sites of epithelial cell turnover E-cadherin becomes accessible to Lm (30). InlB also contributes to the initial uptake into host epithelia (31, 32). Finally, the major virulence factor Listeriolysin O (LLO), a member of the bacterial hemolysin family, promotes the uptake of Lm into hepatocytes (26).

The results of our study clearly show that the route of uptake, hence the initial interactions of Lm with the host, determine the innate antibacterial response. Strikingly, the impact of IFN-I synthesis changes from being adverse (4-6) to being beneficial when i.p. and i.g. routes of infection are compared. The possible mechanisms of these detrimental actions are increased cell death upon IFN-I signalling of lymphocytes and macrophages and unresponsiveness to IFN-γ (33, 34). Following systemic delivery, IFN-I increase production of immune-suppressive IL-10 and restrain TNF-α producing cells in the spleen, thereby limiting a protective inflammatory response (4, 8). Here we show that critical cytokines are controlled by the type I IFN system. These include the protective cytokines TNF-α, IL-6 and IFN-γ and the anti-inflammatory IL-10 in addition to chemokines such as MCP-1 that regulate the recruitment of myeloid cells (9, 28, 35-37). Unlike early TNF-α synthesis that requires IFN-I after both i.p. and i.g. infection, the production of IL-6 and MCP-1 during the initial 24h of infection is reduced specifically when bacteria enter their host via the intestine. Most importantly, increased IFN-γ production in absence of type I IFN

responsiveness was delayed and less pronounced after i.g. compared to i.p. infection. Thus, reduced synthesis of protective cytokines during the early phase of infection provides a likely explanation for the different impact of IFN-I on mice infected via enteral or parenteral routes. This explanation is in accordance with the timing of bacterial replication, which is strongly accelerated in i.g.-infected Ifnar1-/- mice between 24 and 48h. The increased IFN-γ synthesis at 48h explains why a significant fraction of Ifnar1-/- mice proceeds to clear bacteria with equal efficiency as their Wt counterparts and survives. We hypothesize that those Ifnar1-/- mice that die from i.g. infection are unable to cope with the damage inflicted by the large Lm burden between 24 and 48h. Unexpectedly, intestinal invasion and replication in the intestinal mucosa or the mucosa-associated lymphoid tissue demonstrated little control by IFN-I. In contrast, the liver, most likely the most rapidly infected internal organ, revealed striking differences comparing intragastric or intraperitoneal infection routes, most obviously the vigorous replication of Lm between 24 and 48h and the correspondingly stronger inflammatory response. Our recent report clearly demonstrated a close correlation between the lethality of Lm infection and the extent of liver damage (21). It appears likely, therefore, that the hepatic response to infection is a major determinant for the beneficial or adverse effects of IFN-I on Lm infection. Contrasting the spleen, the liver is not itself a site of IFN-I synthesis during Lm infection and it responds poorly to IFN-I (18). This supports our conclusion that the innate hepatic response is critically influenced by IFN-I-regulated splenic cytokine synthesis.

Our data further suggest that in addition to cytokines, the extent of liver inflammation may be influenced by different bacterial entry points after intestinal or intraperitoneal infection routes. Lm arriving from the intestinal tract localizes mostly to periportal or pericentral areas and the inflammatory response is directed to these regions. This suggests that important Lm entry routes are the portal blood stream or, following systemic dissemination, the central veins, with little contribution of peritoneal invasion and direct infection of liver lobes. By contrast, a larger fraction of intraperitoneally administered Lm may choose direct intraperitoneal access to the margins of liver lobules rather than systemic dissemination through blood or lymphatic vessels. Speculatively, the location of replicating Lm may influence the speed with which an inflammatory infiltrate is formed and regulated by blood-borne cytokines and chemokines.

One very obvious cause for liver damage noted in our experiments was the death of cells both within the inflammatory infiltrate and the surrounding hepatic tissue. Cell death may be a direct consequence of intracellular Lm in macrophages, neutrophils or hepatocytes (38-40) or an indirect consequence of inflammation. Liver infiltrates formed more frequently in i.g.-infected Ifnar1-/-mice and developed to significantly larger sizes, thus causing a much larger fraction of hepatic tissue to die. Livers of Ifnar1-deficient mice contained a smaller fraction of inflammatory monocytes, important antibacterial effector cells (41), specifically following infection via the gastrointestinal route. This may reflect the reduced synthesis of chemokines and contribute to the enhanced multiplication of Lm in the liver.

Apoptosis of splenic lymphocytes is thought to be an important cause for the adverse consequence of IFN-I production after i.p. infection. While spleens from i.g.-infected mice contained less apoptotic cells compared to i.p.-infected controls, absence of the IFNAR reduced the number of dying or dead splenocytes despite a higher bacterial burden. This suggests that the enhancement of splenocyte death by IFN-I is established independently of the route of infection. Our data suggest that the beneficial effect of IFN-I on the innate response to gastrointestinal infection results from ensuring the rapid upregulation of critical protective cytokines that limit hepatic bacterial replication and inflammation. Within the IFN-I-regulated cytokine milieu IFN-γ suffices for survival, but becomes limiting in the early phase of i.g. infection if IFN-I cannot ensure production of other protective proinflammatory cytokines/chemokines. In contrast, i.p.-infected mice benefit from an early boost of IFN-γ production in absence of an IFN-I response. The cellular and molecular mechanisms underlying the infection route-dependent impact of IFN-I on the timing and intensity of IFN-γ production will be investigated in future studies.

Acknowlegments

We thank WD Schubert (Molecular Host-Pathogen Interactions, Division of Structural Biology, Helmholtz Centre for Infection Research, Braunschweig, Germany) for providing the pAUL-A InlA-InlB construct.

References

1. **Vazquez-Boland, J. A., M. Kuhn, P. Berche, T. Chakraborty, G. Dominguez-Bernal, W. Goebel, B. Gonzalez-Zorn, J. Wehland, and J. Kreft.** 2001. Listeria pathogenesis and molecular virulence determinants. *Clin Microbiol Rev* 14:584-640.

2. **Hamon, M., H. Bierne, and P. Cossart.** 2006. Listeria monocytogenes: a multifaceted model. *Nat Rev Microbiol* 4:423-434.

3. **Decker, T., M. Muller, and S. Stockinger.** 2005. The yin and yang of type I interferon activity in bacterial infection. *Nat Rev Immunol* 5:675-687.

4. **Auerbuch, V., D. G. Brockstedt, N. Meyer-Morse, M. O'Riordan, and D. A. Portnoy.** 2004. Mice lacking the type I interferon receptor are resistant to Listeria monocytogenes. *J Exp Med* 200:527-533.

5. **Carrero, J. A., B. Calderon, and E. R. Unanue.** 2004. Type I interferon sensitizes lymphocytes to apoptosis and reduces resistance to Listeria infection. *J Exp Med* 200:535-540.

6. **O'Connell, R. M., S. K. Saha, S. A. Vaidya, K. W. Bruhn, G. A. Miranda, B. Zarnegar, A. K. Perry, B. O. Nguyen, T. F. Lane, T. Taniguchi, J. F. Miller, and G. Cheng.** 2004. Type I interferon production enhances susceptibility to Listeria monocytogenes infection. *J Exp Med* 200:437-445.

7. **Zwaferink, H., S. Stockinger, P. Hazemi, R. Lemmens-Gruber, and T. Decker.** 2008. IFN-beta increases listeriolysin O-induced membrane permeabilization and death of macrophages. *J Immunol* 180:4116-4123.

8. **Carrero, J. A., B. Calderon, and E. R. Unanue.** 2006. Lymphocytes are detrimental during the early innate immune response against Listeria monocytogenes. *J Exp Med* 203:933-940.

9. **Dai, W. J., G. Kohler, and F. Brombacher.** 1997. Both innate and acquired immunity to Listeria monocytogenes infection are increased in IL-10-deficient mice. *J Immunol* 158:2259-2267.

10. **Rayamajhi, M., J. Humann, K. Penheiter, K. Andreasen, and L. L. Lenz.** 2010. Induction of IFN-alphabeta enables Listeria monocytogenes to suppress macrophage activation by IFN-gamma. *J Exp Med* 207:327-337.

11. **Czuprynski, C. J., N. G. Faith, and H. Steinberg.** 2003. A/J mice are susceptible and C57BL/6 mice are resistant to Listeria monocytogenes infection by intragastric inoculation. *Infect Immun* 71:682-689.

12. **Cossart, P.** Illuminating the landscape of host-pathogen interactions with the bacterium Listeria monocytogenes. *Proc Natl Acad Sci U S A* 108:19484-19491.

13. **Muller, U., U. Steinhoff, L. F. Reis, S. Hemmi, J. Pavlovic, R. M. Zinkernagel, and M. Aguet.** 1994. Functional role of type I and type II interferons in antiviral defense. *Science* 264:1918-1921.

14. **Arnaud, M., A. Chastanet, and M. Debarbouille.** 2004. New vector for efficient allelic replacement in naturally nontransformable, low-GC-content, gram-positive bacteria. *Appl Environ Microbiol* 70:6887-6891.

15. **Chakraborty, T., M. Leimeister-Wachter, E. Domann, M. Hartl, W. Goebel, T. Nichterlein, and S. Notermans.** 1992. Coordinate regulation of virulence genes in Listeria monocytogenes requires the product of the prfA gene. *J Bacteriol* 174:568-574.

16. **Wollert, T., B. Pasche, M. Rochon, S. Deppenmeier, J. van den Heuvel, A. D. Gruber, D. W. Heinz, A. Lengeling, and W. D. Schubert.** 2007. Extending the host range of Listeria monocytogenes by rational protein design. *Cell* 129:891-902.

17. **Lingnau, A., E. Domann, M. Hudel, M. Bock, T. Nichterlein, J. Wehland, and T. Chakraborty.** 1995. Expression of the Listeria monocytogenes EGD inlA and inlB genes, whose products mediate bacterial entry into tissue culture cell lines, by PrfA-dependent and -independent mechanisms. *Infect Immun* 63:3896-3903.

18. **Stockinger, S., R. Kastner, E. Kernbauer, A. Pilz, S. Westermayer, B. Reutterer, D. Soulat, G. Stengl, C. Vogl, T. Frenz, Z. Waibler, T. Taniguchi, T. Rulicke, U. Kalinke, M. Muller, and T. Decker.** 2009. Characterization of the interferon-producing cell in mice infected with Listeria monocytogenes. *PLoS Pathog* 5:e1000355.

19. **Cabanes, D., M. Lecuit, and P. Cossart.** 2008. Animal models of Listeria infection. *Curr Protoc Microbiol* Chapter 9:Unit9B 1.

20. **Stockinger, S., B. Reutterer, B. Schaljo, C. Schellack, S. Brunner, T. Materna, M. Yamamoto, S. Akira, T. Taniguchi, P. J. Murray, M. Muller, and T. Decker.** 2004. IFN regulatory factor 3-dependent induction of type I IFNs by intracellular bacteria is mediated by a TLR- and Nod2-independent mechanism. *J Immunol* 173:7416-7425.

21. **Kernbauer, E., V. Maier, D. Stoiber, B. Strobl, C. Schneckenleithner, V. Sexl, U. Reichart, B. Reizis, U. Kalinke, A. Jamieson, M. Muller, and T. Decker.** Conditional Stat1 Ablation Reveals the Importance of Interferon Signaling for Immunity to Listeria monocytogenes Infection. *PLoS Pathog* 8:e1002763.

22. **Monk, I. R., P. G. Casey, C. Hill, and C. G. Gahan.** Directed evolution and targeted mutagenesis to murinize Listeria monocytogenes internalin A for enhanced infectivity in the murine oral infection model. *BMC Microbiol* 10:318.

23. **Pron, B., C. Boumaila, F. Jaubert, P. Berche, G. Milon, F. Geissmann, and J. L. Gaillard.** 2001. Dendritic cells are early cellular targets of Listeria monocytogenes after intestinal delivery and are involved in bacterial spread in the host. *Cell Microbiol* 3:331-340.

24. **Niesel, D. W., C. B. Hess, Y. J. Cho, K. D. Klimpel, and G. R. Klimpel.** 1986. Natural and recombinant interferons inhibit epithelial cell invasion by Shigella spp. *Infect Immun* 52:828-833.

25. **Bukholm, G., B. P. Berdal, C. Haug, and M. Degre.** 1984. Mouse fibroblast interferon modifies Salmonella typhimurium infection in infant mice. *Infect Immun* 45:62-66.

26. **Vadia, S., E. Arnett, A. C. Haghighat, E. M. Wilson-Kubalek, R. K. Tweten, and S. Seveau.** The Pore-Forming Toxin Listeriolysin O Mediates a Novel Entry Pathway of L. monocytogenes into Human Hepatocytes. *PLoS Pathog* 7:e1002356.

27. **Huang, S., W. Hendriks, A. Althage, S. Hemmi, H. Bluethmann, R. Kamijo, J. Vilcek, R. M. Zinkernagel, and M. Aguet.** 1993. Immune response in mice that lack the interferon-gamma receptor. *Science* 259:1742-1745.

28. **Harty, J. T., and M. J. Bevan.** 1995. Specific immunity to Listeria monocytogenes in the absence of IFN gamma. *Immunity* 3:109-117.

29. **Reutterer, B., S. Stockinger, A. Pilz, D. Soulat, R. Kastner, S. Westermayer, T. Rulicke, M. Muller, and T. Decker.** 2008. Type I IFN are host modulators of strain-specific Listeria monocytogenes virulence. *Cell Microbiol* 10:1116-1129.

30. **Pentecost, M., G. Otto, J. A. Theriot, and M. R. Amieva.** 2006. Listeria monocytogenes invades the epithelial junctions at sites of cell extrusion. *PLoS Pathog* 2:e3.

31. **Chiba, S., T. Nagai, T. Hayashi, Y. Baba, S. Nagai, and S. Koyasu.** Listerial invasion protein internalin B promotes entry into ileal Peyer's patches in vivo. *Microbiol Immunol* 55:123-129.

32. **Pentecost, M., J. Kumaran, P. Ghosh, and M. R. Amieva.** Listeria monocytogenes internalin B activates junctional endocytosis to accelerate intestinal invasion. *PLoS Pathog* 6:e1000900.

33. **Rayamajhi, M., J. Humann, S. Kearney, K. K. Hill, and L. L. Lenz.** Antagonistic crosstalk between type I and II interferons and increased host susceptibility to bacterial infections. *Virulence* 1:418-422.

34. **Carrero, J. A., and E. R. Unanue.** 2007. Impact of lymphocyte apoptosis on the innate immune stages of infection. *Immunol Res* 38:333-341.

35. **Pfeffer, K., T. Matsuyama, T. M. Kundig, A. Wakeham, K. Kishihara, A. Shahinian, K. Wiegmann, P. S. Ohashi, M. Kronke, and T. W. Mak.** 1993. Mice deficient for the 55 kd tumor necrosis factor receptor are resistant to endotoxic shock, yet succumb to L. monocytogenes infection. *Cell* 73:457-467.

36. **Dalrymple, S. A., L. A. Lucian, R. Slattery, T. McNeil, D. M. Aud, S. Fuchino, F. Lee, and R. Murray.** 1995. Interleukin-6-deficient mice are highly susceptible to Listeria monocytogenes infection: correlation with inefficient neutrophilia. *Infect Immun* 63:2262-2268.

37. **Pietras, E. M., L. S. Miller, C. T. Johnson, R. M. O'Connell, P. W. Dempsey, and G. Cheng.** A MyD88-dependent IFNgammaR-CCR2 signaling circuit is required for mobilization of monocytes and host defense against systemic bacterial challenge. *Cell Res* 21:1068-1079.

38. **Stockinger, S., T. Materna, D. Stoiber, L. Bayr, R. Steinborn, T. Kolbe, H. Unger, T. Chakraborty, D. E. Levy, M. Muller, and T. Decker.** 2002. Production of type I IFN sensitizes macrophages to cell death induced by Listeria monocytogenes. *J Immunol* 169:6522-6529.

39. **Navarini, A. A., M. Recher, K. S. Lang, P. Georgiev, S. Meury, A. Bergthaler, L. Flatz, J. Bille, R. Landmann, B. Odermatt, H. Hengartner, and R. M. Zinkernagel.** 2006. Increased susceptibility to bacterial superinfection as a consequence of innate antiviral responses. *Proc Natl Acad Sci U S A* 103:15535-15539.

40. **Rogers, H. W., M. P. Callery, B. Deck, and E. R. Unanue.** 1996. Listeria monocytogenes induces apoptosis of infected hepatocytes. *J Immunol* 156:679-684.

41. **Pamer, E. G.** 2004. Immune responses to Listeria monocytogenes. *Nat Rev Immunol* 4:812-823.

Figures and Figure legends

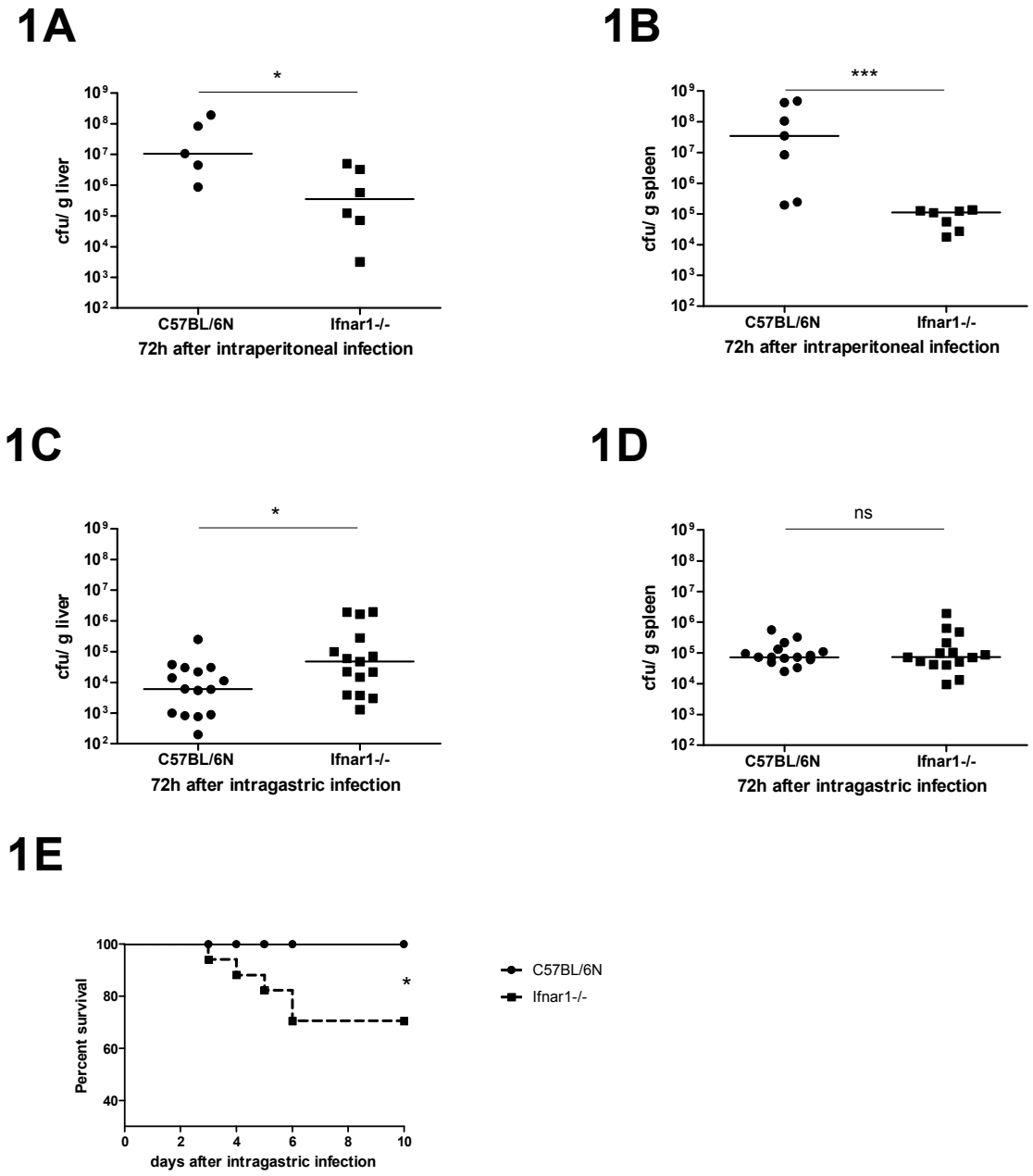


Figure 1: **IFN-I increase host resistance after intragastric infection with *Listeria monocytogenes*.** C57BL/6N Wt and Ifnar1-/- mice were infected with Lm strain LO28InlA*. A, B. Numbers of bacteria in livers (A) and spleens (B) were determined by CFU assay 72h after intraperitoneal (i.p.) infection with 1x10^6 Lm. C, D; Bacterial loads of livers (C) and spleens (D) were examined by CFU assay 72h after intragastric gavage (i.g.) with 5x10^9 Lm. Plots indicate the Median of bacterial counts. E; 14 mice per group were infected i.g. with 5x10^9 Lm LO28InlA* and survival was monitored over ten days.

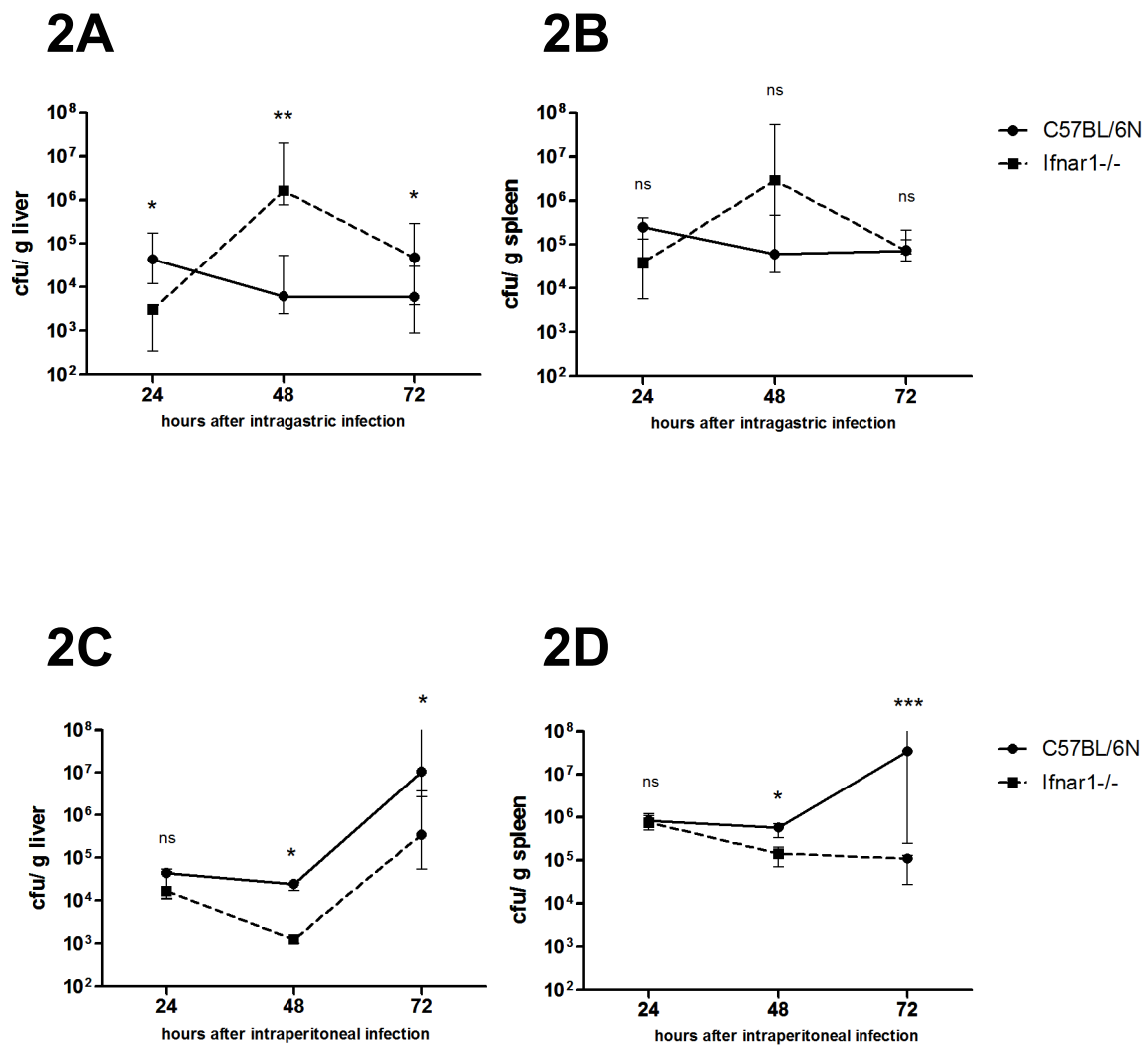


Figure 2: **Bacterial burden after various periods of intragastric infections of Wt and Ifnar1-/- mice with *Listeria monocytogenes*.** A-B. C57BL/6N Wt and Ifnar1-/- mice were infected with 5x10^9 CFU of strain LO28InlA* by intragastric gavage (i.g.) and bacterial loads of livers (A) and spleens (B) were monitored over three days by CFU assay. C, D, bacterial burden of livers (C) and spleens (D) was determined by CFU assay over three days after i.p. infection of C57BL/6N Wt and Ifnar1-/- mice with strain LO28InlA*.

For i.g. experiments at least 7 mice per genotype and for i.p. experiments at least 5 mice per genotype were used for each time point. Standard variations indicate the median with interquartile range.

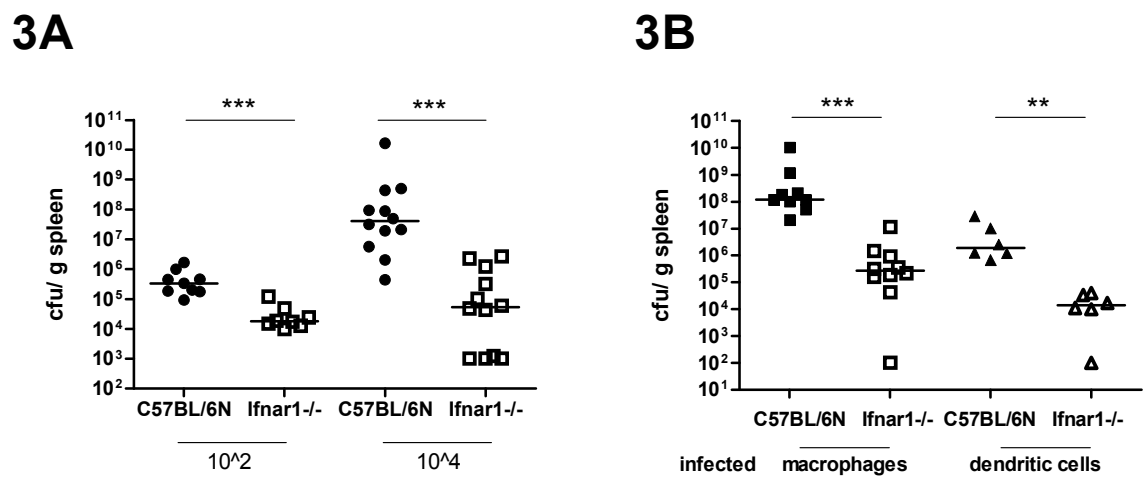
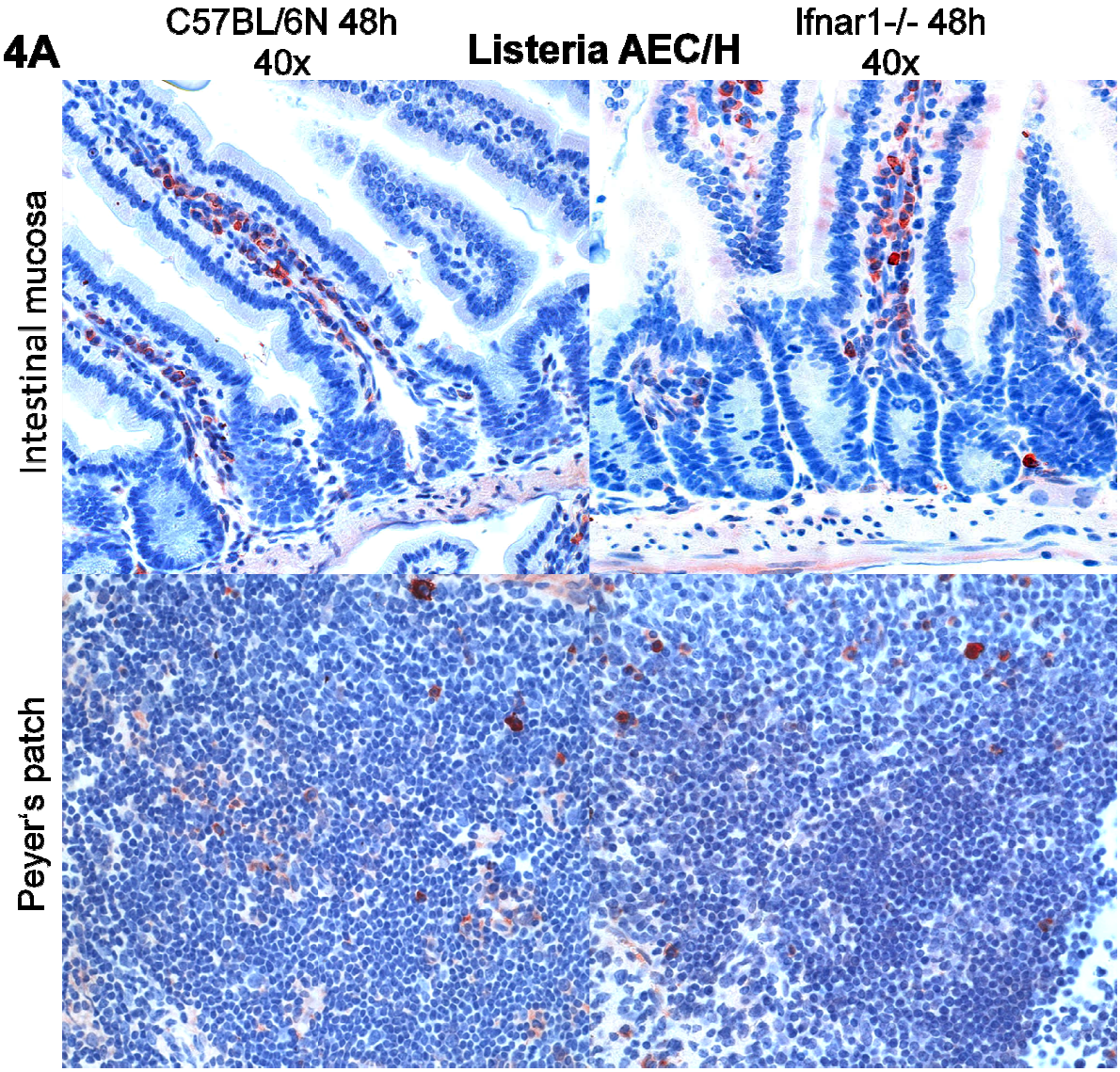
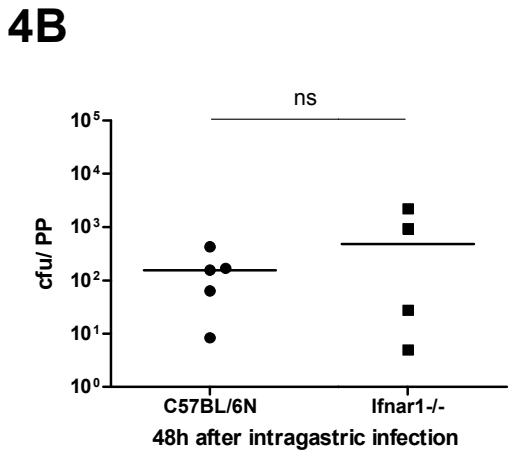
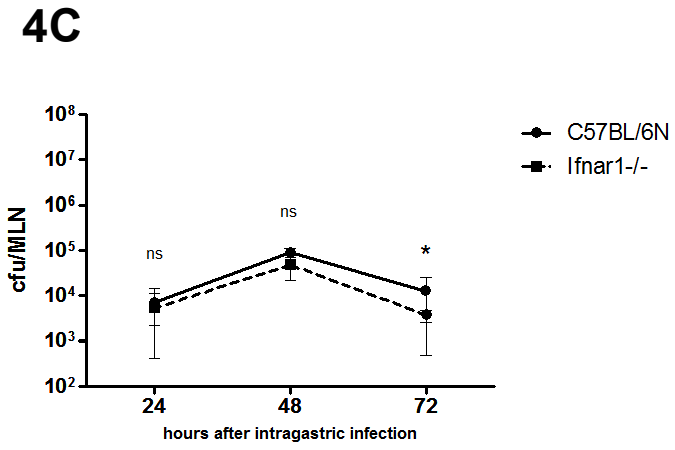


Figure 3: **Low infectious doses or dissemination via infected cells do not alter the adverse effect of IFN-I after systemic infection with *Listeria monocytogenes*.** A. Doses of 10^2 and 10^4 Lm were injected intravenously (i.v.) into C57BL/6N Wt and Ifnar1-/- mice and bacterial loads in spleens were determined by CFU assay 72h after infection. B. Wt bone marrow-derived macrophages or myeloid dendritic cells were infected *in vitro* with a MOI of 10 for 1h and then, 10^4 of the respective cell type was injected i.v. into C57BL/6N Wt and Ifnar1-/- mice. Bacterial loads in the spleen were measured by CFU assay 72h after infection.


4A
C57BL/6N 48h
40x
Listeria AEC/H
Ifnar1-/- 48h
40x
Intestinal mucosa
Peyer's patch


4B
ns
cfu/ PP
C57BL/6N
Ifnar1-/-
48h after intragastric infection


4C
cfu/MLN
ns
ns
*
24
48
72
hours after intragastric infection
C57BL/6N
Ifnar1-/-

4D mRNA

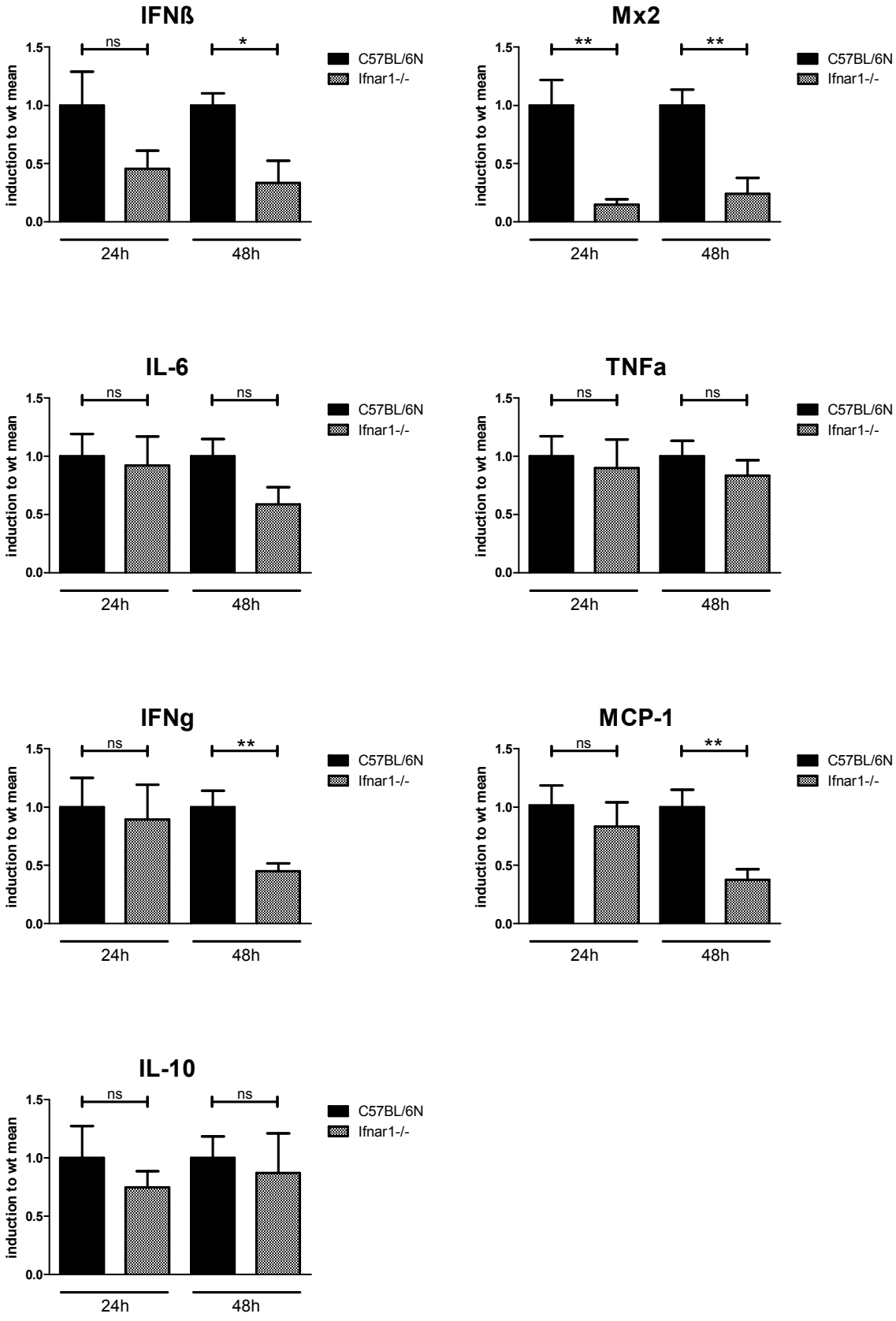
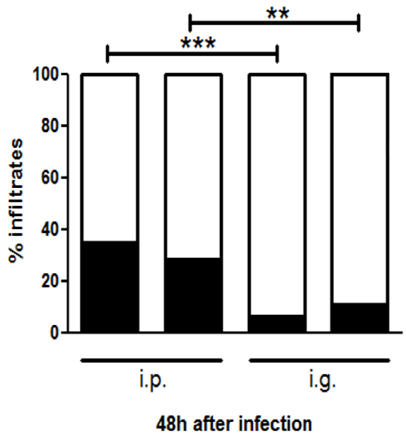

IFNß
Mx2
IL-6
TNFa
IFNg
MCP-1
IL-10
induction to wt mean
1.5
1.0
0.5
0.0
ns
*
**
24h
48h
C57BL/6N
Ifnar1-/-

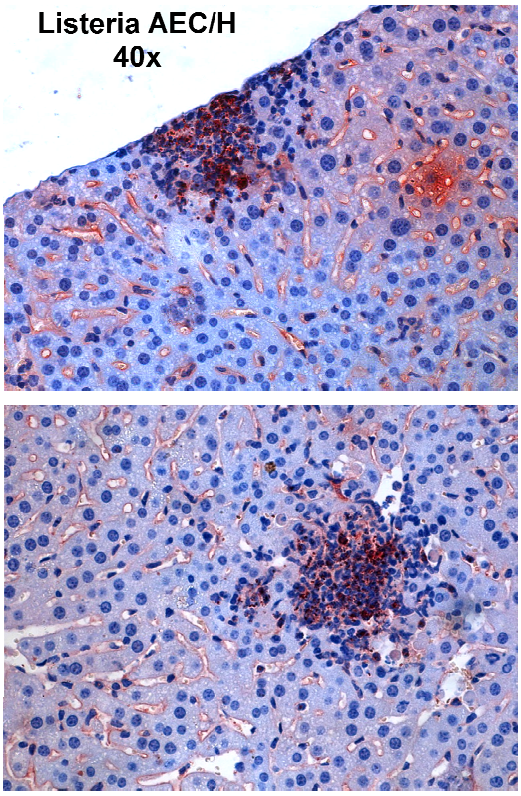
Figure 4. **Localization and replication of *Listeria monocytogenes* in the intestinal tract.** A, upper panels. Anti-Listeria serum was used to detect Lm in the intestinal mucosa from C57BL/6N Wt or Ifnar1-/- mice 48h post infection. Listeria infection, in both Wt and Ifnar1-/- mice occurred mostly in mucosal tissue beneath the epithelial layer. A, lower panels. Anti-Listeria serum was used to detect Lm in Wt or Ifnar1-/- Peyer's patches (PP) 48h post infection. B, C; Bacterial numbers in Peyer's patches (B) at day 2 or mesenteric lymph nodes (MLNs, C) over three days, determined by CFU assay. D. Analysis of Peyer's patch-mRNAs by qPCR at the indicated times after infection. All experiments were performed with C57BL/6N Wt and Ifnar1-/- mice infected with a dose of 5x10^9 CFU of the LO28InlA* strain by intragastric gavage (i.g.).

5A

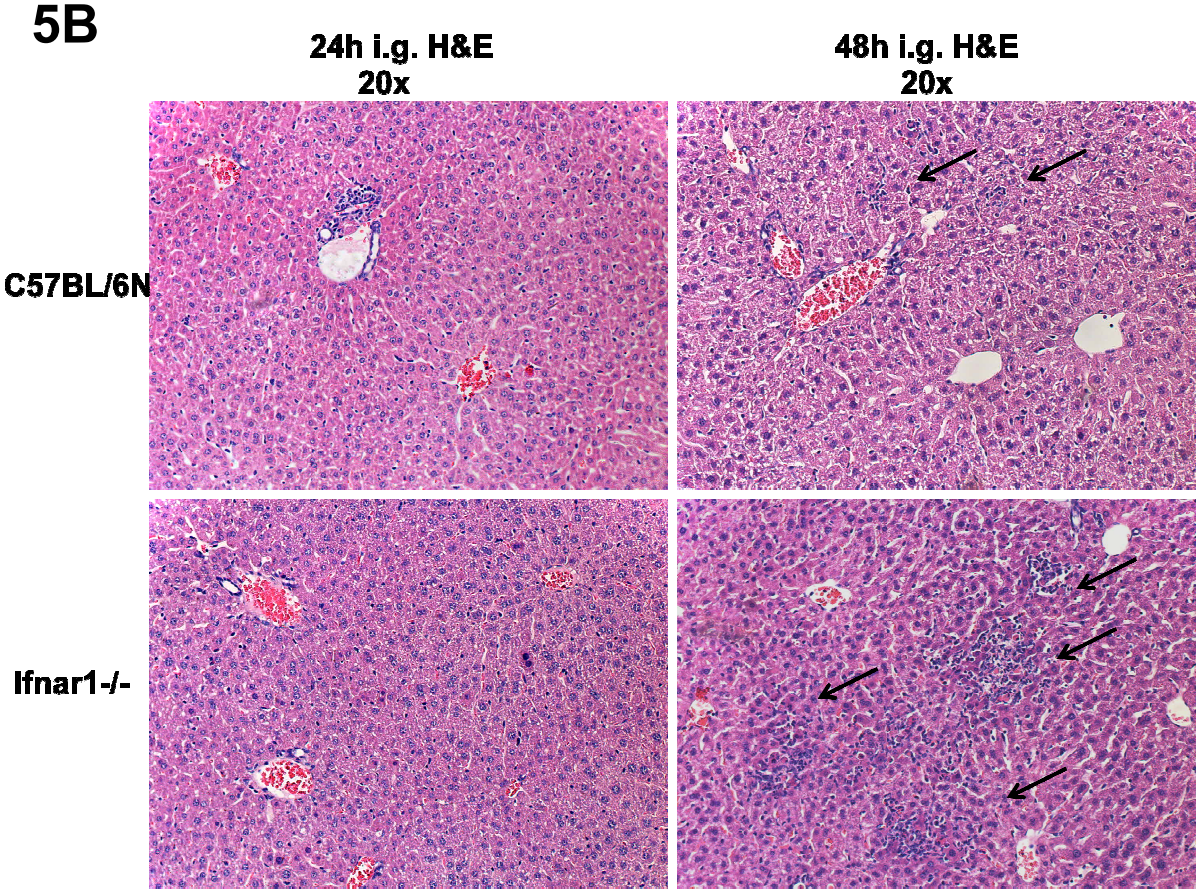


inflammatoryinfiltates at the margins of liver lobs

Inflammatory infiltrates periportal and pericentral

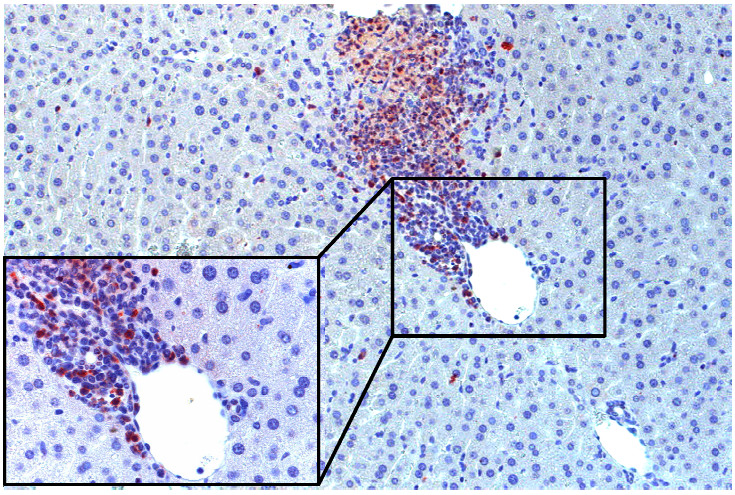


5B



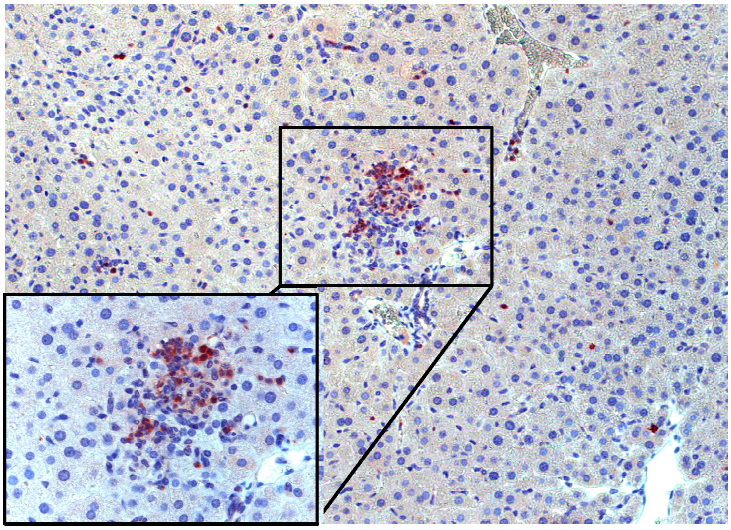
5C

Ifnar1-/-
Gr1 AEC/H 48h
40x/63x



5D

C57BL/6N
Gr1 AEC/H 48h
40x/63x



5E

Ifnar1-/-
Tunel AEC/H 48h
40x/63x

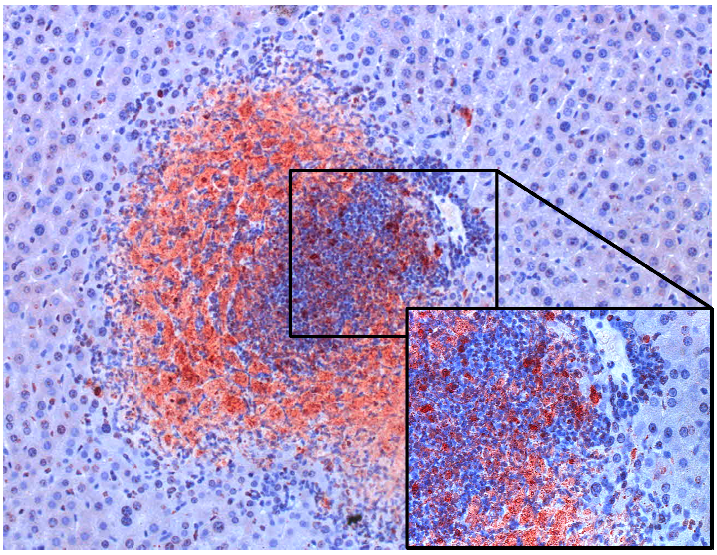


Figure 5: **Localization of inflammatory infiltrates in livers of i.p. vs i.g. infected mice**. A. Localization of bacteria-containing infiltrates to the margins of the liver lobes (black) or to the periportal or pericentral region (white). The graph indicates relative numbers determined in five C57BL/6N Wt and Ifnar1-/- mice 48h post i.p. or i.g. infection. The anti-Listeria staining in the right panel indicates marginal and periportal infiltrates from a representative Wt sample 48h after infection. B. Histochemical analysis of hematoxylin-stained liver sections obtained 24h or 48h after i.g. administration of strain LO28InlA* to C57BL/6N Wt (upper panels) or Ifnar1-/- mice (lower panels). C-D. Histochemical analysis of Gr-1- stained liver sections obtained 48h after i.g. administration of strain LO28InlA* to C57BL/6N Wt (D) or Ifnar1-/- mice (C). E. TUNEL staining of infected liver sections obtained 48h after administration of strain LO28InlA* to Ifnar1-/- mice.

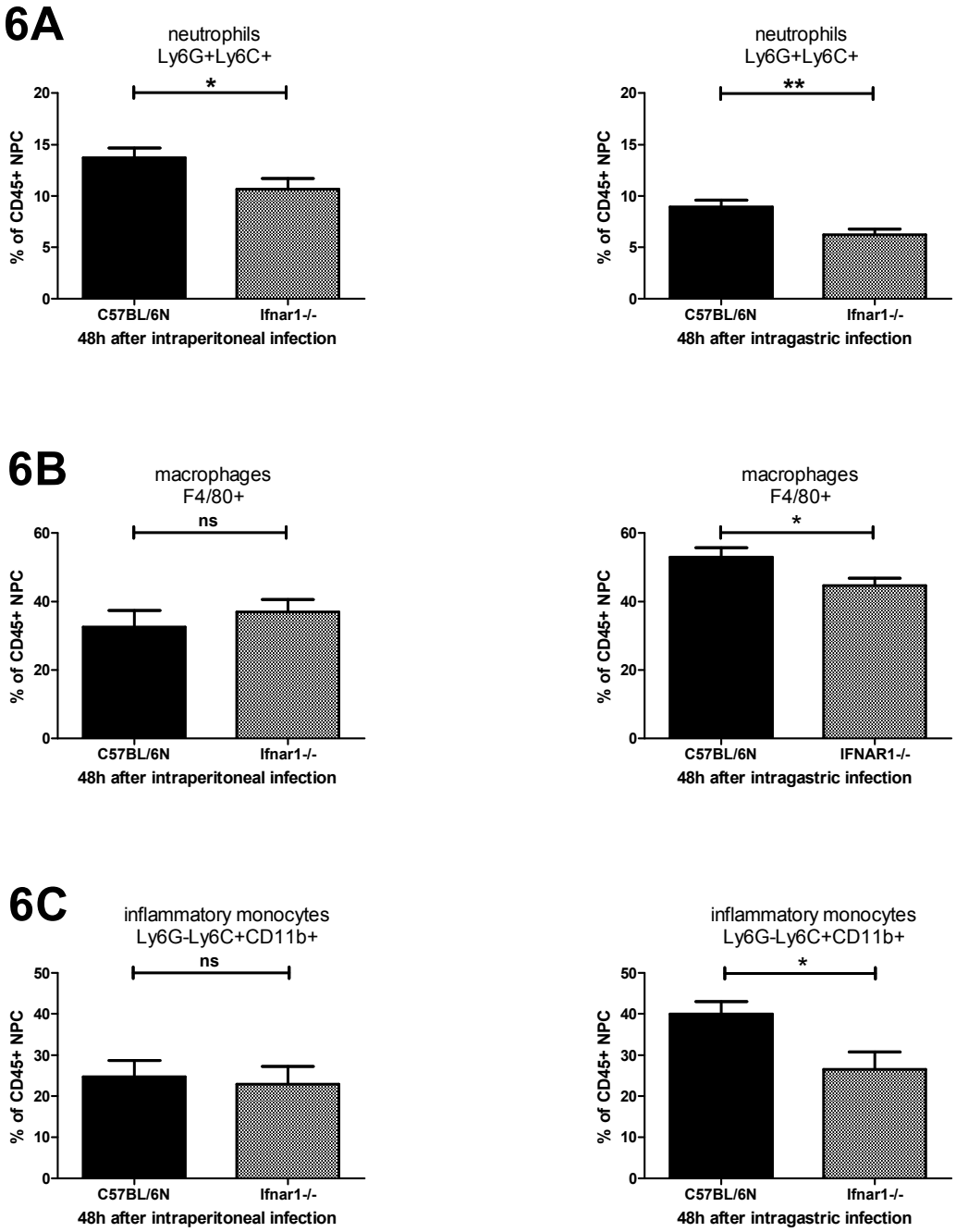


Figure 6: **Comparison of the CD45+ fraction of non-parenchymal liver cells (NPC) of i.g.- and i.p.-infected C57BL/6N Wt and Ifnar1-/- mice.** A-C. Livers from infected mice were analyzed for neutrophils (A), macrophages (B) and inflammatory monocytes (C) using the indicated markers 48h post infection. The data are representative of three different experiments with four mice in each group. I.p. infections were performed with doses of 1x10^6 CFU and i.g. infections with 5x10^9 CFU of LO28InlA*.

7

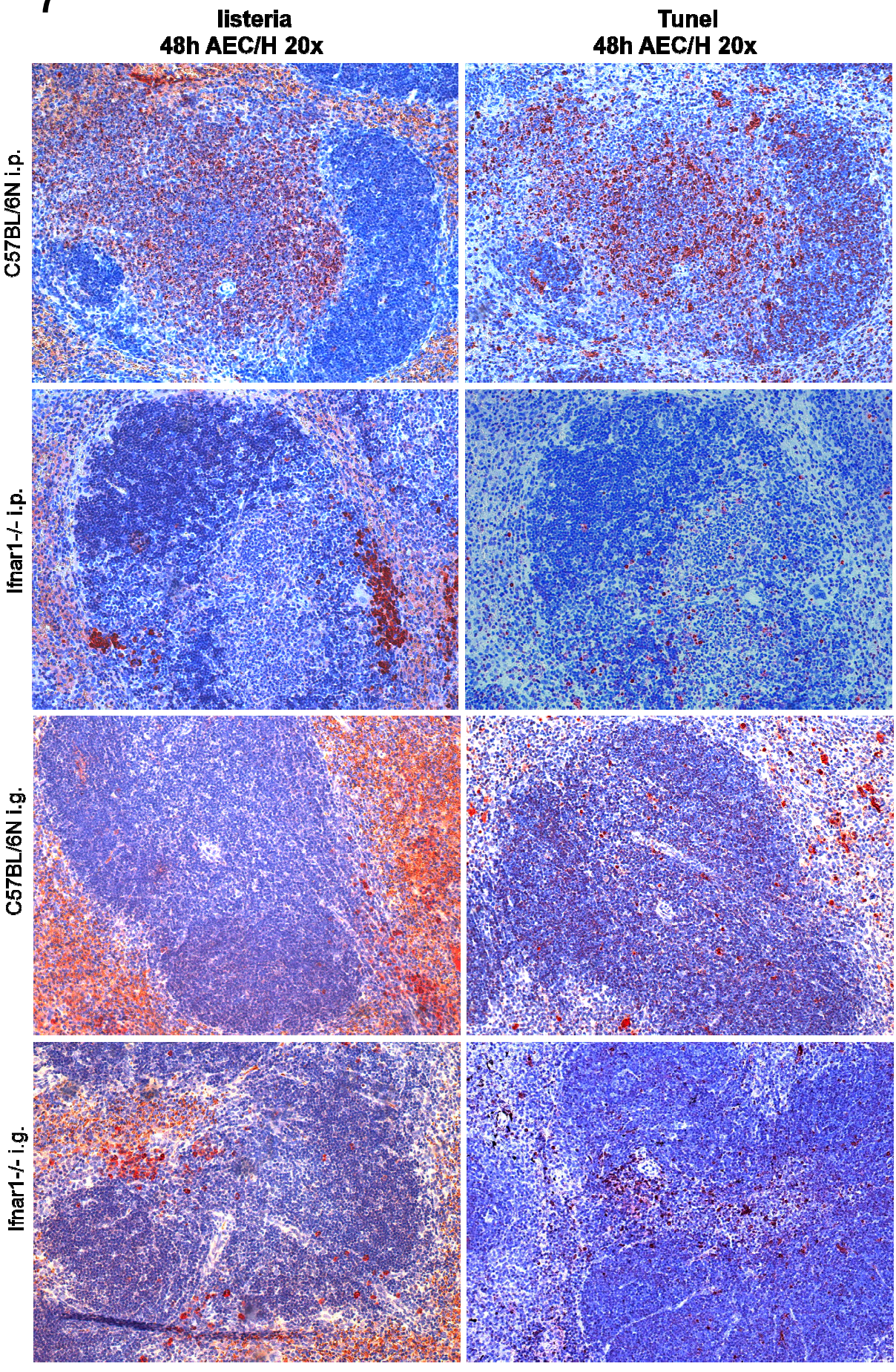
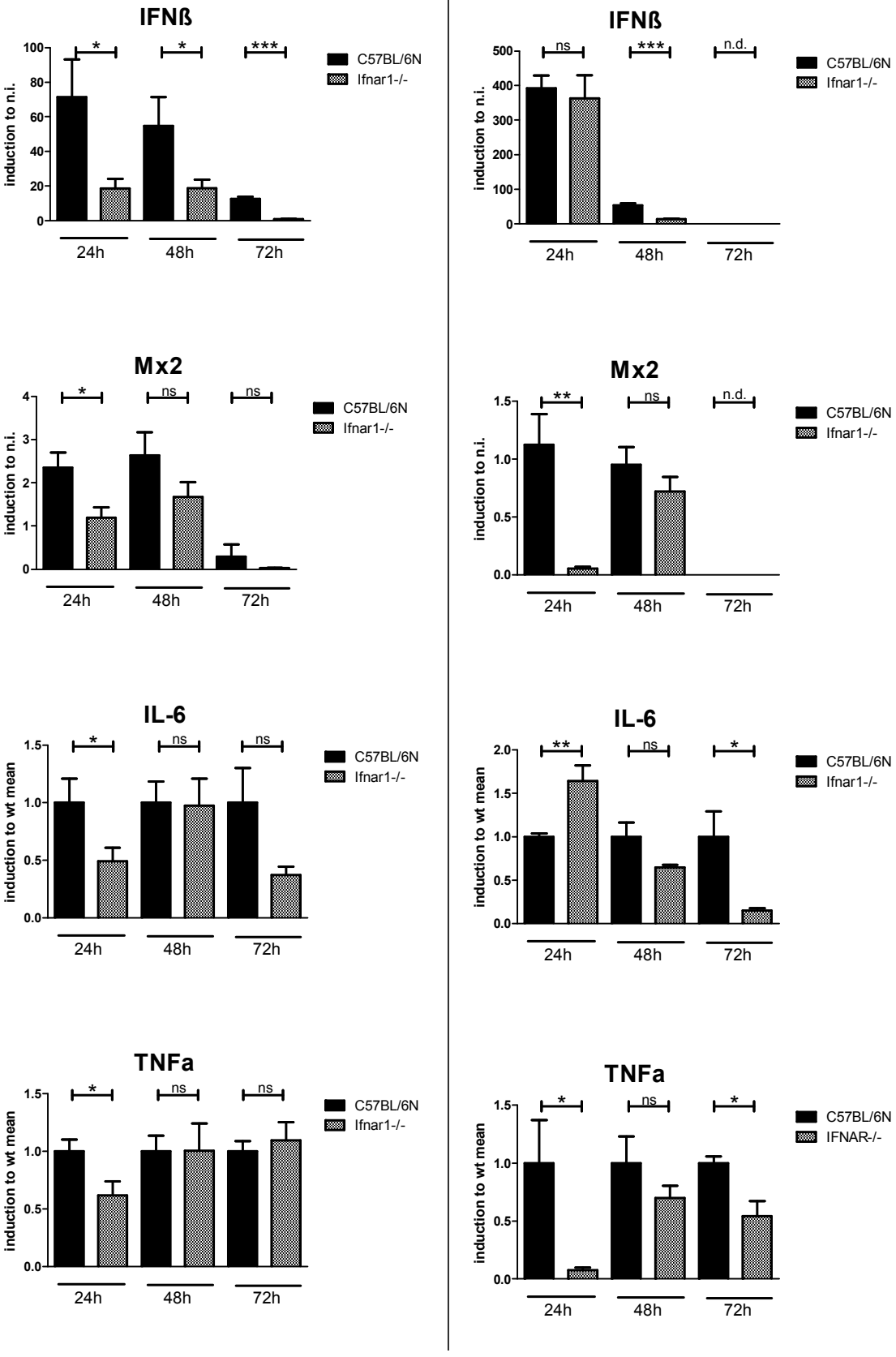
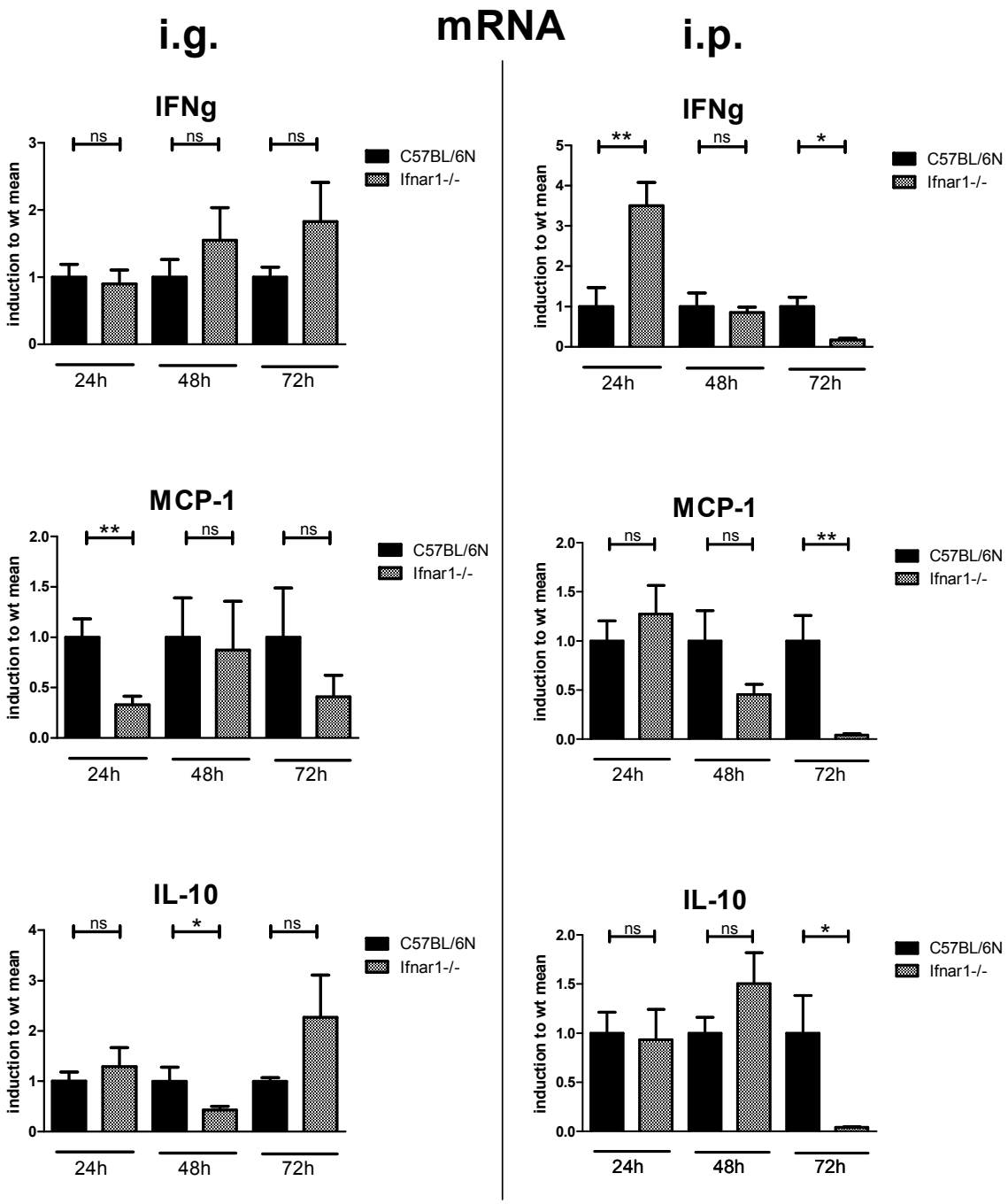

listeria
48h AEC/H 20x
Tunel
48h AEC/H 20x
C57BL/6N i.p.
Ifnar1-/- i.p.
C57BL/6N i.g.
Ifnar1-/- i.g.

Figure 7: **Listeria and TUNEL staining of spleens from i.p.- or i.g.- infected C57BL/6N Wt and Ifnar1-/- mice.** Representative samples obtained 48h after i.p. administration of 1x10^6 CFU of strain LO28InlA* (upper panels) and i.g. administration of 5x10^9 LO28InlA* (lower panels) to C57BL/6N Wt or Ifnar1-/- mice.

8A **i.g.** **mRNA** **i.p.**


IFNß
induction to n.i.
100
80
60
40
20
0
*
*
***
24h
48h
72h
C57BL/6N
Ifnar1-/-
IFNß
induction to n.i.
500
400
300
200
100
0
ns
***
n.d.
24h
48h
72h
C57BL/6N
Ifnar1-/-
Mx2
induction to n.i.
4
3
2
1
0
*
ns
ns
24h
48h
72h
C57BL/6N
Ifnar1-/-
Mx2
induction to n.i.
1.5
1.0
0.5
0.0
**
ns
n.d.
24h
48h
72h
C57BL/6N
Ifnar1-/-
IL-6
induction to wt mean
1.5
1.0
0.5
0.0
*
ns
ns
24h
48h
72h
C57BL/6N
Ifnar1-/-
IL-6
induction to wt mean
2.0
1.5
1.0
0.5
0.0
**
ns
*
24h
48h
72h
C57BL/6N
Ifnar1-/-
TNFa
induction to wt mean
1.5
1.0
0.5
0.0
*
ns
ns
24h
48h
72h
C57BL/6N
Ifnar1-/-
TNFa
induction to wt mean
1.5
1.0
0.5
0.0
*
ns
*
24h
48h
72h
C57BL/6N
IFNAR-/-


i.g.
mRNA
i.p.
IFNg
induction to wt mean
ns
ns
ns
C57BL/6N
Ifnar1-/-
24h
48h
72h
IFNg
induction to wt mean
**
ns
*
C57BL/6N
Ifnar1-/-
24h
48h
72h
MCP-1
induction to wt mean
**
ns
ns
C57BL/6N
Ifnar1-/-
24h
48h
72h
MCP-1
induction to wt mean
ns
ns
**
C57BL/6N
Ifnar1-/-
24h
48h
72h
IL-10
induction to wt mean
ns
*
ns
C57BL/6N
Ifnar1-/-
24h
48h
72h
IL-10
induction to wt mean
ns
ns
*
C57BL/6N
Ifnar1-/-
24h
48h
72h

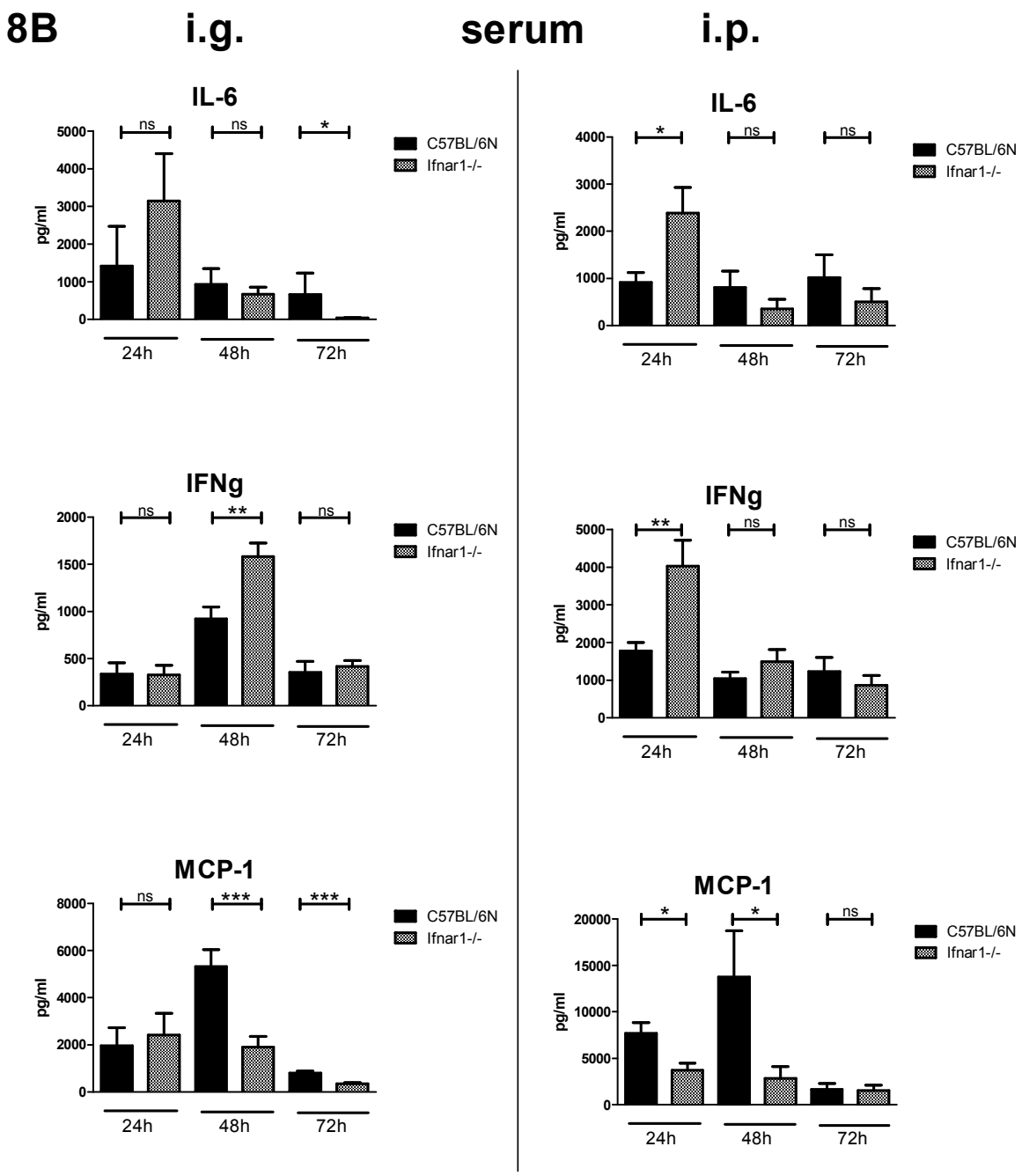


Figure 8: **Delayed cytokine response in Ifnar1-/- compared to Wt mice after intragastric infection with *Listeria monocytogenes*.** A. Cytokine mRNA expression in the spleens of C57BL/6N Wt (solid bars) or Ifnar1-/- mice (hatched bars) 24h, 48h and 72h after infection with 5x10^9 CFU of strain LO28InlA* by intragastric gavage (i.g.), or 1x10^6 LO28InlA* by intraperitoneal infection (i.p.) was determined by qPCR. mRNAs were normalized to the GAPDH housekeeping control and are displayed as the ratio obtained from non-infected (n.i.) and infected samples or as the ratio between expression in Wt and Ifnar1-/- animals. B. Serum IFNγ levels of 10- 15 C57BL/6N Wt and Ifnar1-/- mice infected i.g. for 24h, 48h or 72h with 5x10^9

CFU of strain LO28InlA* by intragastric gavage (i.g.), or with 1x10^6 CFU LO28InlA* by intraperitoneal injection were determined using a flow cytometry-based bead array.

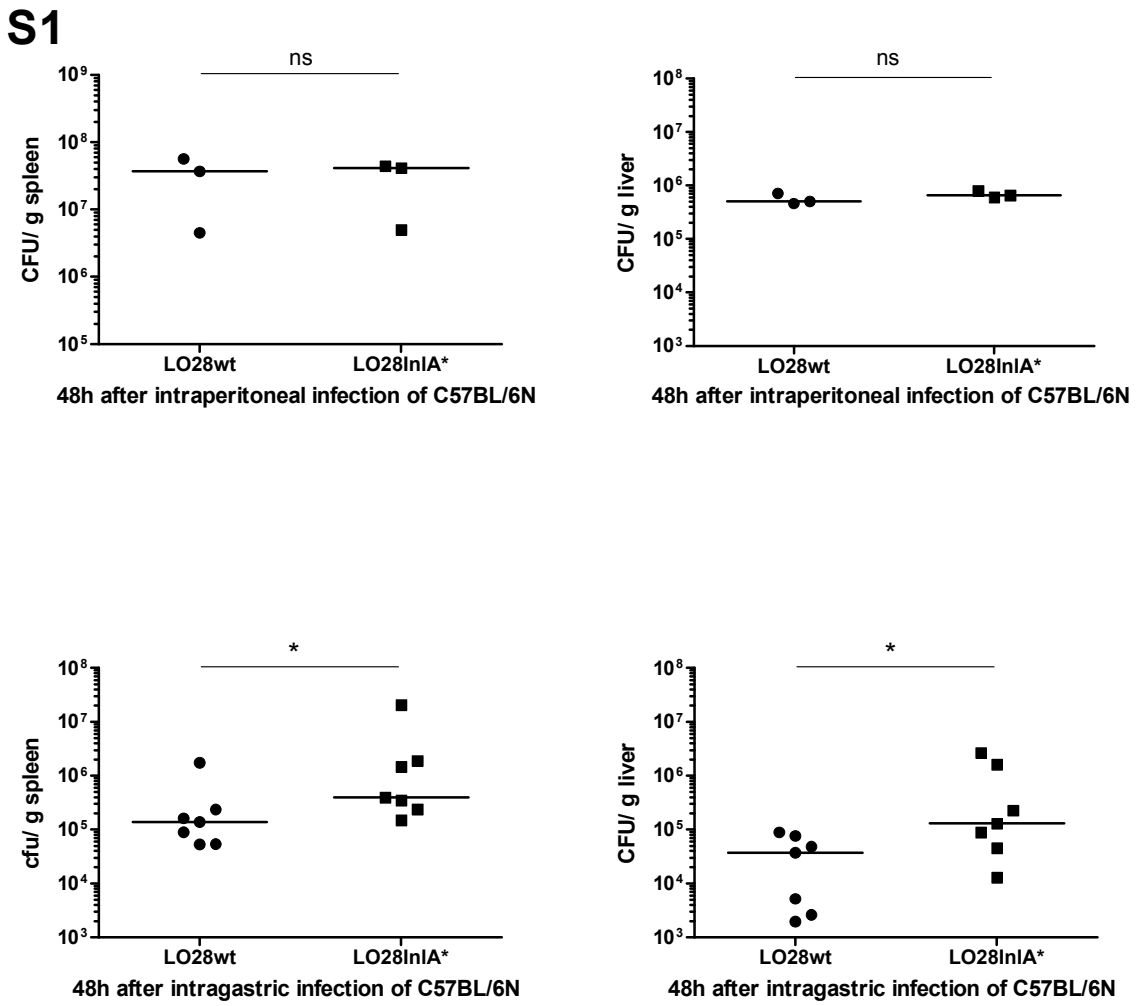


Figure S1: **Comparison of the LO28wt and LO28InlA*.** C57BL/6N Wt mice were infected with Lm strain LO28wt or LO28InlA*. Numbers of bacteria in spleens (upper left panel) and livers (upper right panel) were determined by CFU assay 48h after intraperitoneal (i.p.) infection with 1x10^6 Lm. Bacterial loads of spleens (lower left panel) and livers (lower right panel) were examined by CFU assay 48h after intragastric gavage (i.g.) with 5x10^9 Lm. Plots indicate the Median of bacterial counts.

Additional Data

The role of Type III interferons after intragastric infection with *Lm*

In the first part of my thesis I focused on the expression of IFN-I after oral infection of *Lm* in C57BL/6N Wt and Ifnar1-/- mice. However, the data shows little measurable effects of IFN-I in the intestinal epithelia and gut-associated-lymphoid tissue. Recently, type III interferons (IFN-III) (see 6.3.) received considerable attention because of their ability to enhance or complete the function of IFN-I especially in epithelial tissue and hepatocytes (50, 54, 55). Here, I show data of experiments performed in IRF9-/- mice which, unlike Ifnar1-/- mice, are unable to respond to IFN-III.

IFN-III accelerate and increase proinflammatory cytokines in serum after intragastric Lm infection

To characterize the impact of IFN-III during listeriosis, we used mice incapable to synthesise the transcription factor IRF9. We first monitored the CFUs 48h after intragastric infection. Although the severity of infection was generally milder in these two consolidated experiments, IRF9-/- mice showed significantly higher bacterial burden than Wt mice. Organ loads of Ifnar1-/- were in between Wt and IRF9-/- mice, highlighting the additional benefits of IFN-III.

Relative numbers of bacteria in infected organs correlated well with MCP-1, MCP-3, IL-6 and RANTES protein levels in the serum 48h after infection which indicates a related mechanism of delayed immune response as observed in Ifnar1-/- mice. However, other cytokines were not directly correlated to the severity of infection in the investigated genotypes. IL-12p70 and IL-22 were low or not detectable in Wt and IRF9-/- whereas they were found significantly enhanced in Ifnar1-/-. IL-12p70 promotes NK activation and Th1-cell differentiation, both resulting in increased IFNγ synthesis. Consistently, the production of IFNγ was strongly decreased in IRF9-/- compared to Wt mice. IL-22 is mainly produced by activated NK-cells, T-cells and innate lymphoid cells (ILC) but the receptor is mainly found on fibroblasts, epithelial cells and hepatocytes (56). Speculatively, IFNλ is responsible for the activation of NK cells and T-cells, but this effect is less pronounced in Wt mice due to a lower bacterial burden. MIP-1ß (CCL4) is a chemo-attractant for NK-cells and inflammatory monocytes. Compared to Wt controls it was reduced to the same extent in Ifnar1-/- and IRF9-/- mice, indicating that its synthesis is controlled mainly by IFN-I.

Definitive proof for the influence of IFN-III on infected epithelia, will require analyses of infection kinetics as well as experiments in mice lacking the IFN-λ- receptor.

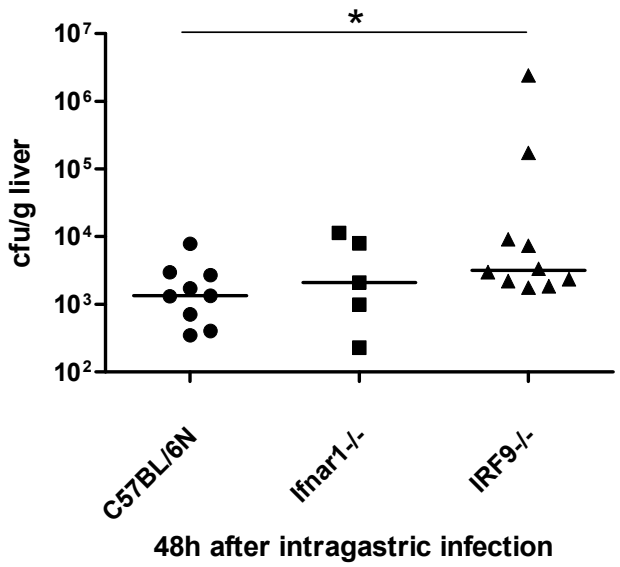


Figure 5 | IFN-III increase host resistance after intragastric infection with *Lm.* C57BL/6N Wt and Ifnar1-/- mice were infected with the strain LO28InlA* by intragastric gavage with 5x10^9 bacteria and bacterial loads were examined by CFU assay 48h after infection.

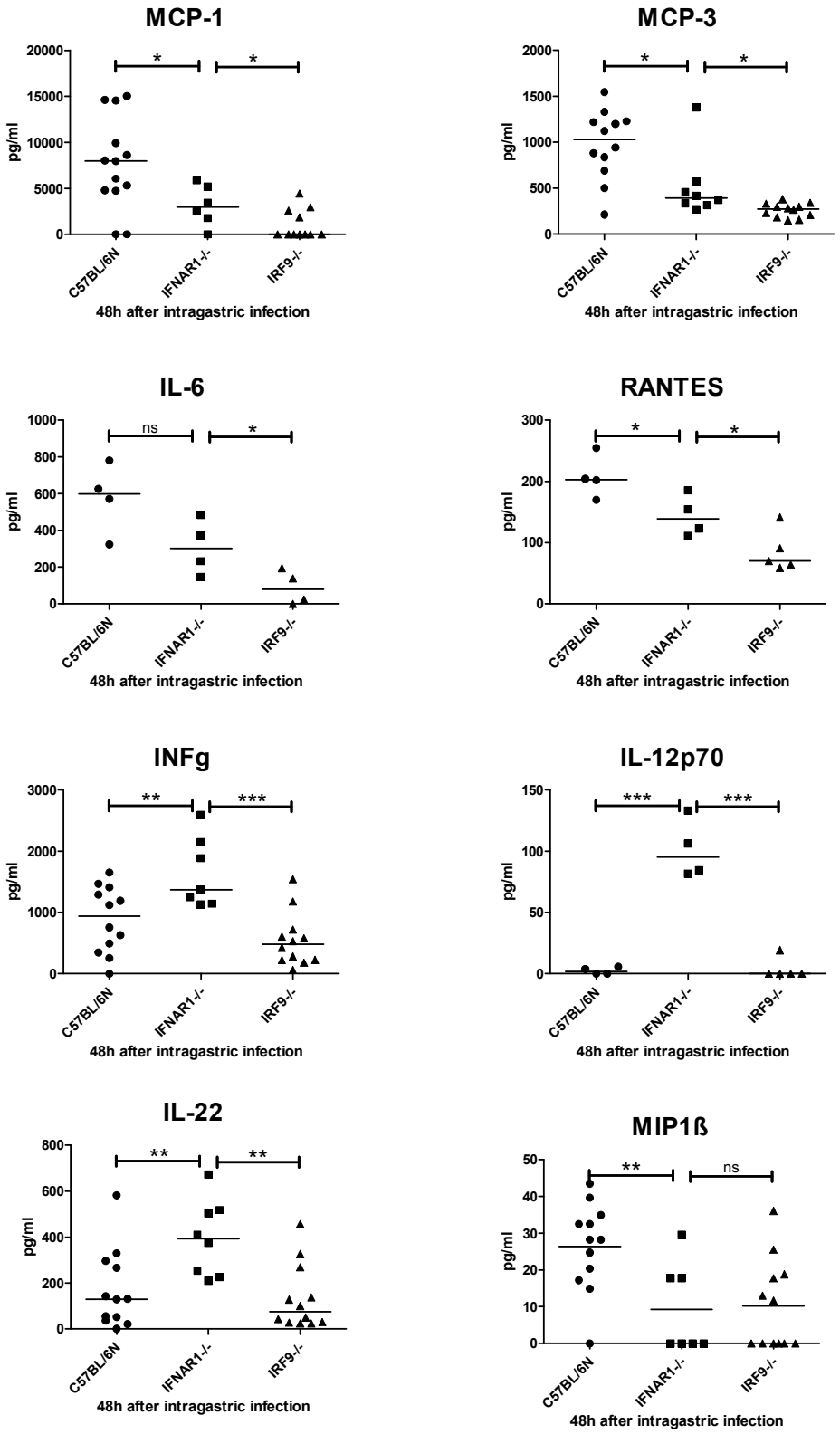


Figure 6 | Cytokine synthesis in response to *Lm* in Wt, Ifnar1-/- and IRF9-/- mice after i.g. infection. Serum cytokines were determined by a bead-based array 48h after infection of C57BL/6N Wt (dots), Ifnar1-/- (squares) and IRF9-/- (triangles) with strain LO28InlA* by intragastric gavage. Experiments were performed twice. Graphs indicate median values.

Discussion

Type I interferons, originally identified by Isaacs and Lindenmann for their antiviral properties, are major components of the early innate immune response (41). Since then, a large body of work showed that these proteins are among the first cytokines that respond to various pathogens like bacteria, parasites, fungi and also tumors (45). Although the impact of IFN-I in viral infection has been shown to be exclusively protective, their role in bacterial infections is less well defined (46). In this case it can either be adverse or beneficial but no separation between gram-positive, gram-negative, intracellular or extracellular bacteria is obvious; indicating more complicate scenarios.

My thesis shows investigations addressing the role of IFN-I in infections with the Gram-positive intracellular pathogen *Listeria monocytogenes* in Wt- and Ifnar1-/- mice. The latter animals lack the receptor for IFN-I and are therefore unable to induce the feed forward amplification loop of IFN-I synthesis or to respond to IFN-I. We reproduced the sensitizing impact of IFN-I after intraperitoneal administration of *Lm in vivo*. On the contrary, we could show that mice benefit from IFN-I production when infection occurred via the gastrointestinal tract which is the natural route of *Lm*. This opened a great opportunity to study the reason behind the diverse action of IFN-I in anti-listerial immunity. In principle, there are two different options for this dual effect: Either the pathogen encounters a different cell type or the same cell type responds differently in the intestine or the peritoneal cavity. We soon observed decreased mRNA levels of IL-6, IFNγ and TNFα in spleens of Ifnar1-/- mice within the first 24h after oral inoculation and concluded that this is probably due to the reduced MCP-1 levels and delayed effector cell recruitment. Surprisingly, there are also decreased serum levels of MCP-1 in Ifnar1-/- mice infected via the peritoneal cavity, indicating that controlling the synthesis of chemokines for the recruitment of inflammatory cell populations is a general feature of IFN-I.

Among the leading chemo-attracted cells in listerial infections are neutrophils. We observed faster but smaller infiltrates containing Gr1+ cells in livers of Wt mice and delayed but huge infiltration in Ifnar1-/- mice until day two post infection. However, a large number of cells within the biggest infiltrates were Gr1-, possibly DC, NK-cells or T-cells (14). The Gr1+ marker is expressed on inflammatory monocytes which are quickly recruited by proinflammatory cytokines and chemokines released by neutrophils and other cells participating in the inflammatory reaction. With the use of immunohistochemistry we could not unequivocally identify the cell types involved. However, FACS analysis of non-parenchymal liver cells revealed that in both infection routes Ifnar1-/- mice had significantly decreased neutrophils, but the genotype difference was stronger in i.g.-infected mice. Furthermore, Ifnar1-/- mice contained significantly fewer inflammatory monocytes and F4/80+ cells after i.g., but not after i.p. infection. Again this strengthened the

hypothesis of delayed immune response in i.g. infection- fatal for the host; an advantage to take root for *Lm*.

Together the data argue in favour of a critical role of IFN-I in controlling the synthesis of proinflammatory chemokines and cytokines. Type I IFN may contribute to a cytokine storm after systemic administration of *Lm* and Ifnar1-/- mice are protected. However, this protection is not seen after infection via the natural route where cytokines may be more gradually released and do not accumulate to severely harmful or lethal concentrations. Low dose i.v. infection experiments did not support this assumption as systemic IFN-I were still detrimental. This leaves the possibility that the IFN-I response of different cell types at the primary site of infection determines the adverse or beneficial effect. Thus, the speed and intensity of inflammation ensuing in the early phase of infection may be controlled by cells showing various aspects of cell type specificity in their IFN-I response.

To obtain further information about the immune response at the site of infection we monitored invasion of the murinized LO28InlA* strain by immunohistochemistry of the gut. Interestingly, there were no bacteria attached or inside the epithelium. The vast majority resided underneath the epithelium in the GALT or in follicle associated lymphoid tissue. Listeria occurred in patches rather than being equally distributed. Maybe they simply entered at sites lacking epithelial integrity due to the extrusion of cells (13) (9). Peyer's patches were found infected, but neither histopathology nor CFU assay of PP revealed significant differences between Wt and Ifnar1-/- mice at the site of infection. Judging from my data, the liver appears to much more reflect the impact of genotype and infection route on the severity of infection. The reasons underlying the critical importance of the liver as a determinant of the course of infection will be subject to future investigations.

Innate responses of the intestinal epithelium are regulated by IFN-I as well as IFN-III. Examination of IRF9-/- mice suggested that type III interferons may indeed counteract intestinal colonization by *Lm*. This subtype of the interferon family has first been described in 1993 and relatively little is known besides their impact on viral invasion of respiratory and intestinal epithelia (55, 57). Our data show higher bacterial burden in the liver of IRF9-/- mice compared to Ifnar1-/- mice. Since mouse livers don't express receptors for IFN-III, increased amounts of *Lm* in this organ are likely to reflect IFN-III activity at an earlier step of infection, most likely colonization of the gut. The change in the immune response resulting from the combined unresponsiveness to IFN-I and IFN-III results in alterations of serum cytokine levels in IRF9-/- compared to Ifnar1-/- mice. Among these are IL-12p70, IL-22 and IFNγ. Interestingly, these cytokines are involved in T-cell and NK-cell activation and appear to be reduced in IRF9-/- mice but elevated in Ifnar1-/- mice.

Besides the possible impact of interferons at the site of infection, the dissemination routes to target organs might have an outstanding role. Besides systemic spread via infected monocytes,

bacteria may also choose a direct route to the liver via the portal vein. This assumption is strengthened by our data showing that pathogen loads and inflammation of the liver more accurately reflect the severity of infection than the spleen. Initially, infiltrates were often found in periportal areas. As infection progressed, more and more inflammatory infiltrates localized to areas surrounding the central veins. By contrast, this was much less the case in i.p. infections, where the bacterium may choose direct access from the peritoneal cavity beside systemic dissemination. Speculatively, the origin of replicating *Lm* from either the intestine or the peritoneal cavity may tip the balance between the two infection routes. *Lm* might upregulate different transcriptional profiles or simply take advantage of an immunetolerant organ, like the liver or a tightly regulated tissue, like the mucosa.

In the end, the question of IFN-I as beneficial or detrimental cytokines will keep us busy for some time. The data shown here push IFN-I again towards the group of “good” cytokines and encourage us to investigate the cell types responsible for their production and/ or response. Furthermore, comparing i.g. and i.p. or i.v. infection routes with *Lm* offers a good tool to study the interplay of cell types and organs in the development of protective adaptive immunity. In the age of conditional knock out mice we have a unique opportunity to investigate cell type specific IFN-I responses. The variation of beneficial or detrimental roles of this cytokine with the route of infection should also encourage us to consider the importance of administration in the context of other pathogens, especially if results are used for pharmacological interventions on humans rather than for basic research and cell signalling studies.

References

1. **Neutra, M. R., N. J. Mantis, and J. P. Kraehenbuhl.** 2001. Collaboration of epithelial cells with organized mucosal lymphoid tissues. *Nat Immunol* 2:1004-1009.

2. **Rescigno, M., M. Urbano, B. Valzasina, M. Francolini, G. Rotta, R. Bonasio, F. Granucci, J. P. Kraehenbuhl, and P. Ricciardi-Castagnoli.** 2001. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol* 2:361-367.

3. **Schulz, O., E. Jaensson, E. K. Persson, X. Liu, T. Worbs, W. W. Agace, and O. Pabst.** 2009. Intestinal CD103+, but not CX3CR1+, antigen sampling cells migrate in lymph and serve classical dendritic cell functions. *J Exp Med* 206:3101-3114.

4. **Artis, D.** 2008. Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. *Nat Rev Immunol* 8:411-420.

5. **Gellin, B. G., and C. V. Broome.** 1989. Listeriosis. *JAMA* 261:1313-1320.

6. **Cossart, P., J. Pizarro-Cerda, and M. Lecuit.** 2003. Invasion of mammalian cells by Listeria monocytogenes: functional mimicry to subvert cellular functions. *Trends Cell Biol* 13:23-31.

7. **Stark, G. R.** 2007. How cells respond to interferons revisited: from early history to current complexity. *Cytokine Growth Factor Rev* 18:419-423.

8. **Lecuit, M., S. Dramsi, C. Gottardi, M. Fedor-Chaiken, B. Gumbiner, and P. Cossart.** 1999. A single amino acid in E-cadherin responsible for host specificity towards the human pathogen Listeria monocytogenes. *EMBO J* 18:3956-3963.

9. **Pentecost, M., G. Otto, J. A. Theriot, and M. R. Amieva.** 2006. Listeria monocytogenes invades the epithelial junctions at sites of cell extrusion. *PLoS Pathog* 2:e3.

10. **Nikitas, G., C. Deschamps, O. Disson, T. Niault, P. Cossart, and M. Lecuit.** Transcytosis of Listeria monocytogenes across the intestinal barrier upon specific targeting of goblet cell accessible E-cadherin. *J Exp Med* 208:2263-2277.

11. **Marco, A. J., J. Altimira, N. Prats, S. Lopez, L. Dominguez, M. Domingo, and V. Briones. 1997.** Penetration of Listeria monocytogenes in mice infected by the oral route. *Microb Pathog* 23:255-263.

12. **Pron, B., C. Boumaila, F. Jaubert, S. Sarnacki, J. P. Monnet, P. Berche, and J. L. Gaillard.** 1998. Comprehensive study of the intestinal stage of listeriosis in a rat ligated ileal loop system. *Infect Immun* 66:747-755.

13. **Daniels, J. J., I. B. Autenrieth, and W. Goebel.** 2000. Interaction of Listeria monocytogenes with the intestinal epithelium. *FEMS Microbiol Lett* 190:323-328.

14. **Pamer, E. G.** 2004. Immune responses to Listeria monocytogenes. *Nat Rev Immunol* 4:812-823.

15. **Havell, E. A., G. R. Beretich, Jr., and P. B. Carter.** 1999. The mucosal phase of Listeria infection. *Immunobiology* 201:164-177.

16. **Jensen, V. B., J. T. Harty, and B. D. Jones.** 1998. Interactions of the invasive pathogens Salmonella typhimurium, Listeria monocytogenes, and Shigella flexneri with M cells and murine Peyer's patches. *Infect Immun* 66:3758-3766.

17. **Autenrieth, I. B., and R. Firsching.** 1996. Penetration of M cells and destruction of Peyer's patches by Yersinia enterocolitica: an ultrastructural and histological study. *J Med Microbiol* 44:285-294.

18. **Gaillard, J. L., P. Berche, and P. Sansonetti.** 1986. Transposon mutagenesis as a tool to study the role of hemolysin in the virulence of Listeria monocytogenes. *Infect Immun* 52:50-55.

19. **Krawczyk-Balska, A., and J. Bielecki.** 2005. Listeria monocytogenes listeriolysin O and phosphatidylinositol-specific phospholipase C affect adherence to epithelial cells. *Can J Microbiol* 51:745-751.

20. **Vadia, S., E. Arnett, A. C. Haghighat, E. M. Wilson-Kubalek, R. K. Tweten, and S. Seveau.** The pore-forming toxin listeriolysin O mediates a novel entry pathway of L. monocytogenes into human hepatocytes. *PLoS Pathog* 7:e1002356.

21. **Wollert, T., B. Pasche, M. Rochon, S. Deppenmeier, J. van den Heuvel, A. D. Gruber, D. W. Heinz, A. Lengeling, and W. D. Schubert.** 2007. Extending the host range of Listeria monocytogenes by rational protein design. *Cell* 129:891-902.

22. **Pron, B., C. Boumaila, F. Jaubert, P. Berche, G. Milon, F. Geissmann, and J. L. Gaillard.** 2001. Dendritic cells are early cellular targets of Listeria monocytogenes after intestinal delivery and are involved in bacterial spread in the host. *Cell Microbiol* 3:331-340.

23. **Rivollier, A., J. He, A. Kole, V. Valatas, and B. L. Kelsall.** Inflammation switches the differentiation program of Ly6Chi monocytes from antiinflammatory macrophages to inflammatory dendritic cells in the colon. *J Exp Med* 209:139-155.

24. **Geissmann, F., S. Jung, and D. R. Littman.** 2003. Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity* 19:71-82.

25. **Drevets, D. A., T. A. Jelinek, and N. E. Freitag.** 2001. Listeria monocytogenes-infected phagocytes can initiate central nervous system infection in mice. *Infect Immun* 69:1344-1350.

26. **Aoshi, T., J. A. Carrero, V. Konjufca, Y. Koide, E. R. Unanue, and M. J. Miller.** 2009. The cellular niche of Listeria monocytogenes infection changes rapidly in the spleen. *Eur J Immunol* 39:417-425.

27. **Unanue, E. R.** 1997. Inter-relationship among macrophages, natural killer cells and neutrophils in early stages of Listeria resistance. *Curr Opin Immunol* 9:35-43.

28. **Melton-Witt, J. A., S. M. Rafelski, D. A. Portnoy, and A. I. Bakardjiev.** Oral infection with signature-tagged Listeria monocytogenes reveals organ-specific growth and dissemination routes in guinea pigs. *Infect Immun* 80:720-732.

29. **Czuprynski, C. J., N. G. Faith, and H. Steinberg.** 2003. A/J mice are susceptible and C57BL/6 mice are resistant to Listeria monocytogenes infection by intragastric inoculation. *Infect Immun* 71:682-689.

30. **Protzer, U., M. K. Maini, and P. A. Knolle.** Living in the liver: hepatic infections. *Nat Rev Immunol* 12:201-213.

31. **Kinoshita, M., T. Uchida, A. Sato, M. Nakashima, H. Nakashima, S. Shono, Y. Habu, H. Miyazaki, S. Hiroi, and S. Seki.** Characterization of two F4/80-positive Kupffer cell subsets by their function and phenotype in mice. *J Hepatol* 53:903-910.

32. **Gregory, S. H., L. P. Cousens, N. van Rooijen, E. A. Dopp, T. M. Carlos, and E. J. Wing.** 2002. Complementary adhesion molecules promote neutrophil-Kupffer cell interaction and the elimination of bacteria taken up by the liver. *J Immunol* 168:308-315.

33. **Mackaness, G. B.** 1962. Cellular resistance to infection. *J Exp Med* 116:381-406.

34. **Wing, E. J., and S. H. Gregory.** 2000. From hot dogs to CD8+ T cells: Listeria monocytogenes. *Trans Am Clin Climatol Assoc* 111:76-83; discussion 84.

35. **Kawai, T., and S. Akira.** Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity* 34:637-650.

36. **Hornung, V., and E. Latz.** Intracellular DNA recognition. *Nat Rev Immunol* 10:123-130.

37. **Sauer, J. D., K. Sotelo-Troha, J. von Moltke, K. M. Monroe, C. S. Rae, S. W. Brubaker, M. Hyodo, Y. Hayakawa, J. J. Woodward, D. A. Portnoy, and R. E. Vance.** The N-ethyl-N-nitrosourea-induced Goldenticket mouse mutant reveals an essential function of Sting in the in vivo interferon response to Listeria monocytogenes and cyclic dinucleotides. *Infect Immun* 79:688-694.

38. **Burdette, D. L., K. M. Monroe, K. Sotelo-Troha, J. S. Iwig, B. Eckert, M. Hyodo, Y. Hayakawa, and R. E. Vance.** STING is a direct innate immune sensor of cyclic di-GMP. *Nature* 478:515-518.

39. **Panne, D.** 2008. The enhanceosome. *Curr Opin Struct Biol* 18:236-242.

40. **Reutterer, B., S. Stockinger, A. Pilz, D. Soulat, R. Kastner, S. Westermayer, T. Rulicke, M. Muller, and T. Decker.** 2008. Type I IFN are host modulators of strain-specific Listeria monocytogenes virulence. *Cell Microbiol* 10:1116-1129.

41. **Isaacs, A., and J. Lindenmann.** 1957. Virus interference. I. The interferon. *Proc R Soc Lond B Biol Sci* 147:258-267.

42. **Borden, E. C., G. C. Sen, G. Uze, R. H. Silverman, R. M. Ransohoff, G. R. Foster, and G. R. Stark.** 2007. Interferons at age 50: past, current and future impact on biomedicine. *Nat Rev Drug Discov* 6:975-990.

43. **Renauld, J. C.** 2003. Class II cytokine receptors and their ligands: key antiviral and inflammatory modulators. *Nat Rev Immunol* 3:667-676.

44. **Sadler, A. J., and B. R. Williams.** 2008. Interferon-inducible antiviral effectors. *Nat Rev Immunol* 8:559-568.

45. **Bogdan, C., J. Mattner, and U. Schleicher.** 2004. The role of type I interferons in non-viral infections. *Immunol Rev* 202:33-48.

46. **Decker, T., M. Muller, and S. Stockinger.** 2005. The yin and yang of type I interferon activity in bacterial infection. *Nat Rev Immunol* 5:675-687.

47. **Young, H. A., and J. H. Bream.** 2007. IFN-gamma: recent advances in understanding regulation of expression, biological functions, and clinical applications. *Curr Top Microbiol Immunol* 316:97-117.

48. **Schroder, K., P. J. Hertzog, T. Ravasi, and D. A. Hume.** 2004. Interferon-gamma: an overview of signals, mechanisms and functions. *J Leukoc Biol* 75:163-189.

49. **Mordstein, M., E. Neugebauer, V. Ditt, B. Jessen, T. Rieger, V. Falcone, F. Sorgeloos, S. Ehl, D. Mayer, G. Kochs, M. Schwemmle, S. Gunther, C. Drosten, T. Michiels, and P. Staeheli.** Lambda interferon renders epithelial cells of the respiratory and gastrointestinal tracts resistant to viral infections. *J Virol* 84:5670-5677.

50. **Doyle, S. E., H. Schreckhise, K. Khuu-Duong, K. Henderson, R. Rosler, H. Storey, L. Yao, H. Liu, F. Barahmand-pour, P. Sivakumar, C. Chan, C. Birks, D. Foster, C. H. Clegg, P. Wietzke-Braun, S. Mihm, and K. M. Klucher.** 2006. Interleukin-29 uses a type 1 interferon-like program to promote antiviral responses in human hepatocytes. *Hepatology* 44:896-906.

51. **Auerbuch, V., D. G. Brockstedt, N. Meyer-Morse, M. O'Riordan, and D. A. Portnoy.** 2004. Mice lacking the type I interferon receptor are resistant to Listeria monocytogenes. *J Exp Med* 200:527-533.

52. **O'Connell, R. M., S. K. Saha, S. A. Vaidya, K. W. Bruhn, G. A. Miranda, B. Zarnegar, A. K. Perry, B. O. Nguyen, T. F. Lane, T. Taniguchi, J. F. Miller, and G. Cheng.** 2004. Type I interferon production enhances susceptibility to Listeria monocytogenes infection. *J Exp Med* 200:437-445.

53. **Stockinger, S., and T. Decker.** 2008. Novel functions of type I interferons revealed by infection studies with Listeria monocytogenes. *Immunobiology* 213:889-897.

54. **Witte, K., E. Witte, R. Sabat, and K. Wolk.** IL-28A, IL-28B, and IL-29: promising cytokines with type I interferon-like properties. *Cytokine Growth Factor Rev* 21:237-251.

55. **Sommereyns, C., S. Paul, P. Staeheli, and T. Michiels.** 2008. IFN-lambda (IFN-lambda) is expressed in a tissue-dependent fashion and primarily acts on epithelial cells in vivo. *PLoS Pathog* 4:e1000017.

56. **Sonnenberg, G. F., L. A. Fouser, and D. Artis.** Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. *Nat Immunol* 12:383-390.

57. **Bierne, H., L. Travier, T. Mahlakoiv, L. Tailleux, A. Subtil, A. Lebreton, A. Paliwal, B. Gicquel, P. Staeheli, M. Lecuit, and P. Cossart.** Activation of type III interferon genes by pathogenic bacteria in infected epithelial cells and mouse placenta. *PLoS One* 7:e39080.

Acknowledgement

I would like to thank Thomas Decker for all the important skills I could learn in his lab and for being a great supervisor during my Master thesis. Further, I want to express my gratitude to Elisabeth Kernbauer for introducing me to this topic and for the good and successful time we shared. I would like to acknowledge my lab members Isabella Rauch, Andrea Majoros, Birgit Rapp, Sebastian Wienerroither, Amanda Jamieson as well as my fellow students Ekaterini Platanitis, Pia Gamradt, Tatjana Hirschmugl and Iris Steinparzer for the great working atmosphere.

I would like to thank my family, who is and was always my emotional backbone. Finally, I sincerely thank Philipp Freimann who kept on encouraging me and for being my supportive force during the last years.

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"Conditional Stat1 ablation reveals the importance of interferon signalling for immunity to Listeria monocytogenes infection." PLoS Pathogens 2012