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THE ROLE OF INTERFERON BETA (IFN-β) IN THE PATHOGENESIS OF INFECTION CAUSED BY *STREPTOCOCCUS SUIS* SEROTYPE 2

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RÉSUMÉ

Streptococcus suis serotype 2, est un agent pathogène porcin important et un agent zoonotique émergent de septicémie et de méningite. La connaissance des réponses immunitaires de l'hôte envers *S. suis*, et les stratégies utilisées par ce pathogène pour subversion de ces réponses sont rares. L'augmentation de la gravité des infections à *S. suis* chez l'homme souligne le besoin critique de mieux comprendre les interactions entre *S. suis* et le système immunitaire pour générer une réponse immunitaire efficace contre ce pathogène. Les cellules dendritiques (DC) et les macrophages (Mθ) sont de puissantes cellules présentant les antigènes. Une fois activés, ils produisent des médiateurs inflammatoires, à leur intérieur, l'interféron-β (IFN-β), l'un des membres les plus importants de la famille d'interféron de type I (IFN-I) qui, bien qu'également est associé à un rôle antiviral, les dernières études ont montré un rôle de confusion dans les infections bactériennes. Ainsi, l'objectif principal du projet était d'étudier le rôle de l'IFN-β dans la pathogenèse de l'infection causée par *S. suis* serotype 2.

Pour obtenir une meilleure connaissance de la source d'IFN-β induite par *S. suis* serotype 2, ainsi que des récepteurs cellulaires et des voies responsables de l'activation cellulaire et de la production ultérieure de cette cytokine, nous avons étudié l'induction *in vitro* d'IFN-β par les DC dérivés de la moelle osseuse murine et Mθ activés par des souches de sérotype 2 de *S. suis* de milieux différents et de virulence, et nous avons évalué le rôle de différents récepteurs et voies cellulaires dans l'induction de l'IFN-β par l'agent pathogène. Les DC ont résulté en une source beaucoup plus importante d'IFN-β que Mθ et la souche moins virulente testée était la plus internalisée et la principale souche inductrice d'IFN-β, ce qui suggère une production d'IFN-β dépendante de l'internalisation et un rôle protecteur de cette cytokine. La production d'IFN-β a montré qu'il dépendait fortement des voies de signalisation dépendantes de MyD88, et les récepteurs les plus impliqués sont le récepteur endossomal Toll-like (TLR) 7 et TLR9. Enfin, l'infection *in vivo* a démontré que l'IFN-β joue un rôle protecteur dans la maladie causée par *S. suis*.

Dans l'ensemble, ces résultats fournissent une connaissance plus approfondie des mécanismes et de la capacité d'induction de l'IFN-β par différentes souches de *S. suis* serotype 2, ainsi que le rôle de cette cytokine lors de l'infection par ce pathogène.

Mots-clés: *Streptococcus suis* serotype 2; Interféron de type I; Cellules dendritiques; Inflammation; virulence; IFN-β

SUMMARY

Streptococcus suis serotype 2 is an important swine pathogen and an emerging zoonotic agent of septicemia and meningitis. Knowledge of host immune responses towards *S. suis,* and strategies used by this pathogen for subversion of these responses is scarce. An increased severity of *S. suis* infections in humans underscores the critical need for a better understanding of the interactions between *S. suis* and the immune system in order to propose more effective therapeutic strategies against this pathogen. Dendritic cells (DCs) and macrophages (Mθ) are powerful antigen-presenting cells. Once activated, they produce inflammatory mediators, within them interferon-β (IFN-β), one of the most important member of the type I interferon (IFN-I) family which although is normally associated with an anti-viral role, latest studies have indicated a confounding role of IFN-β in bacterial infections. Thus, the main objective of the project was to study the role of IFN-β in the pathogenesis of the infection caused by *S. suis* serotype 2.

To obtain a better knowledge about the source of IFN-β induced by *S. suis* serotype 2, as well as the cellular receptors and pathways responsible for cell activation and subsequent production of this cytokine, we studied the *in vitro* induction of IFN-β by murine bone marrow-derived DCs and Mθ activated by strains of *S. suis* serotype 2 from different backgrounds and virulence, and we assessed the role of different cell receptors and pathways in the induction of IFN-β by the pathogen. DCs resulted to be a much more important source of IFN-β than Mθ, and the less virulent strain tested was the most internalized and the main IFN-β inducing strain, which suggests an internalization-dependent IFN-β production and a protective role of this cytokine. IFN-β production has shown to strongly rely on MyD88-dependent signaling pathways, and the most implicated receptors are the endosomal Toll-like receptor (TLR) 7 and TLR9. Finally, the *in vivo* infection has demonstrated that IFN-β plays a protective role in the disease caused by *S. suis*.

Overall, these results provide a deeper knowledge of the mechanisms and the capacity of induction of IFN-β by different strains of *S. suis* serotype 2, as well as the role of this cytokine during infection with this pathogen.

Keywords: *Streptococcus suis* serotype 2; type I interferon; dendritic cells; inflammation; virulence; IFN-β

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LIST OF ACRONYMS AND ABBREVIATIONS

APC: Antigen presenting cell

BBB: Blood brain barrier

BMEC: Brain microvascular endothelial cells

CARD: Caspase activation recruitment domain

CCL: Chemokine (C-C motif) ligand

CD: Cluster of differentiation

CFU: Colony-forming unit

CLR: C-type lectin receptor

CNS: Central nervous system

CPEC: Choroid plexus epithelial cells

CpG: Cytosine-phosphate-Guanosine

CPS: Capsular polysaccharide

CSF: Cerebrospinal fluid

CXCL: Chemokine (C-X-C motif) ligand

CytD: Cytochalasin D

DC: Bone marrow-derived Dendritic Cell

DNA: Deoxyribonucleic acid

Dpp: Dipeptidylpeptidase

ECM: Extracellular matrix

EF: Extracellular factor

Fbp: Fibronectin-binding protein

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

GAS: Group A *Streptococcus*

GBS: Group B *Streptococcus*

GM-CSF: Granulocyte macrophage colony-stimulating factor

IFN: Interferon

IFNAR: Interferon-α/β receptor

IFNγR: Interferon-γ receptor

Ig: Immunoglobulin

IKK: IκB kinase

IL: Interleukin

iNOS: Inductible Nitric Oxide Synthase

IRF: Interferon Regulatory Factor

ISGF: Interferon-stimulated gene factor

JAK: Janus kinase

KO: Knock-out

LLO: Listeriolysin O

LTA: Lipoteichoic acid

MAVS: Mitochondrial antiviral-signaling protein

MCP-1: Monocyte Chemoattractant protein-1

M-CSF: Macrophage colony-stimulating factor

MDAs: Melanoma differentiation antigens

MLST: Multilocus sequence typing

Mθ: Macrophages

MOI: Multiplicity of infection

MRP: Muramidase-released protein

MyD88: Myeloid Differentation Primary Response Protein-88

NF-κB: Nuclear factor-κβ

NK: Natural killer cell

NLR: NOD-like receptor

NO: Nitric oxide

PAMP: Pathogen-associated molecular pattern

PBMC: Peripheral blood mononuclear cell

PGN: Peptidoglycan

p.i.: Post-infection

PLP: Myelin proteolipid protein

PMN: Polymorphonuclear cell

PRR: Pattern recognition receptor

RecN: Recombination/repair protein

RLR: Retinoic acid-inducible gene (RIG)-I like receptor

RNA: Ribonucleic acid

RT-qPCR: Quantitative Reverse Transcription Polymerase Chain Reaction

SLSS: Septic-like shock syndrome

SLY: Suilysin

SodA : Superoxide dismutase A

SspA: Surface-associated subtilisine-like protease

ST: Sequence type

STAT: Signal transducer and activator of transcription

STING: Stimulator of interferon genes

STSLS: Streptococcal toxic shock-like syndrome

TBK1: TANK-binding kinase 1

TGF-β: Transforming growth factor-β

THA: Todd Hewitt broth agar

THB: Todd Hewitt broth

Th1: T helper cell type 1

TICAM: TIR-domain containing adaptor molecule

TIRAP: TIR-containing adaptor protein

TLR: Toll-like receptor

TNF: Tumoral necrosis factor

TRIF: TIR-domain-containing adaptor-inducing interferon-β

TyK: Tyrosine kinase

WT: Wild-type

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I - INTRODUCTION

S. suis, an encapsulated Gram-positive bacterium, is an important porcine bacterial pathogen and zoonotic agent responsible for sepsis and meningitis. In fact, *S. suis* has become the leading cause of adult meningitis in Vietnam, the second leading cause in Thailand, and the third leading cause in Hong Kong. The clinical characteristics typical of acute meningitis in humans caused by *S. suis* changed after the 2005 outbreak in the Chinese province of Sichuan. That was characterized as streptococcal toxic shock-like syndrome (STSLS), similar to that usually associated with *Streptococcus pyogenes* (125).

To date, 35 serotypes based on the capsular polysaccharide (CPS) composition have been described, the serotype 2 being the most commonly isolated from diseased animals and humans in many countries. Data obtained by multilocus sequence typing (MLST) have showed that different serotype 2 STs predominate in different regions of the world. The ST1 is mostly associated with disease in pigs and humans in Europe, Asia and Argentina. The ST7, responsible for the 1998 and 2005 epidemics, is mostly endemic to mainland China. North American cases vary from those in Eurasia, with most strains being either ST25 or ST28 (125).

In recent years, an increasing number of studies were performed to understand the pathogenesis of the *S. suis* infection, however, further investigation is needed to clearly elucidate the molecular mechanisms of its pathogenesis. Similarly, the mechanisms involved in the host immune response to *S. suis* as well as those used by *S. suis* to subvert this response remains poorly characterized. However, several virulence factors have been proposed to be involved in the pathogenesis of *S. suis* infection. Among them, the CPS, which presents an anti-phagocytic role, has been demonstrated as a critical virulence factor and its structure has been described (149). In addition, a hemolysin (suilysin - SLY) has been characterized and described to be involved in the modulation of *S. suis* interactions with host cells, such as endothelial cells, epithelial cells, neutrophils, dendritic cells (DCs) and macrophages (Mθ) (149). Regarding the survival in blood and dissemination, the resistance to phagocytosis require modification of the cell wall peptidoglycan by means of *N*-deacetylation. A well encapsulated mutant strain devoid of the deacetylase peptidoglycan*N*–acetylglucosamine (PgdA), responsible for this modification, showed impaired resistance to neutrophil killing and was severely attenuated in murine and porcine infection models (149, 170). Similarly, d-alanylation of the *S. suis* lipoteichoic acid (LTA) plays a major role in survival of this pathogen (171).

Inflammation is a protective response of the body to ensure removal of detrimental stimuli, as well as a healing process for repairing damaged tissue (239). Germline-encoded pattern recognition receptors (PRR) are responsible for sensing the presence of microorganisms by recognizing structures conserved among microbial species, which are called pathogen-associated molecular patterns (PAMPs). Different classes of PRR families have been identified, such as transmembrane and endosomal proteins: the Toll-Like Receptors (TLRs), as well as cytoplasmic proteins such as the Retinoic acid-inducible gene (RIG)-I-like receptors (RLRs) and NOD-like receptors (NLRs). These PRRs are expressed in macrophages, neutrophiles and dendritic cells but also in various nonprofessional immune cells. The sensing of PAMPs by PRRs upregulates the transcription of genes involved in inflammatory responses. These genes encode pro-inflammatory cytokines, type I IFNs, chemokines and antimicrobial proteins, proteins involved in the modulation of PRR signaling, and many uncharacterized proteins (240).

Focusing on interferons, these are a family of cytokines which act early in the innate immune response and are very well known for inducing an antiviral activity in infected cells. In addition to this antiviral activity, they play a role in regulating the immune response (12). Among the three distinct interferon families, the type I IFN (IFN-I) family is a multi-gene cytokine family, being IFNα and IFNβ (IFNα/β) the best-defined and most broadly expressed ones. IFN-I have numerous additional functions not only during the viral, but also in bacterial infections. The outcome of its response during infectious diseases is highly context-dependent. Different conditions induced during specific infections modulate when and where IFN-I signals are delivered, as well as the signalling pathways that are triggered downstream of the type I IFN receptor (IFNAR) (19). Recents *in vivo* studies with extracellular bacteria have shown contradictory results, since in some cases type I interferon have played a protective role, but in other cases the role was detrimental (44-52, 255). In relation with the source of IFN-I, particularly IFN-β, previous studies have compared the role of antigen presenting cells, such as DCs and Mθ, after the infection with different streptococci (83, 85).

Not much data are available about the interplay between *S. suis* infection and IFN-β. A study has shown that after an *in vivo* infection with strains of *S. suis* serotype 2 of different backgrounds and virulence, the least virulent strain tested resulted to be the main IFN-β inducing strain (247).

Thus, the scatter and lack of data regarding the ability of different strains of *S. suis* serotype 2 to induce IFN-β have motivated this work. Moreover, the host source of IFN-β induced by *S. suis* is unknown. Furthermore, the cellular receptors and pathways responsible for cell activation and subsequent production of IFN-β by *S. suis* have barely been studied, and the role of this cytokine in infections caused by *S. suis*, is not known.

Based on the observations mentioned above, we hypothesized that IFN-β plays a protective role in infections caused by *S. suis* serotype 2. Based on the aforementioned effect of North American strains, it is further hypothesized that the increased production of this type I interferon by them would be responsible, at least in part, of their lower virulence.

The general objective of this thesis was to study the role of IFN-β in infections caused by *S. suis* serotype 2. Moreover, three specific objectives were proposed:

1. Study the *in vitro* induction of IFN-β in murine bone marrow-derived dendritic cells and macrophages activated by different well-encapsulated *S. suis* serotype 2 strains.

2. Assess the role of different cell receptors and pathways in the induction of IFN-β by *S. suis* serotype 2.

3. Study the role of IFN-β during *in vivo* infections with *S. suis* serotype 2.

In this research, we have demonstrated that DCs are an important source of *S. suis*-induced IFN-β, which occurs mainly through a MyD88 pathway and requires bacterial internalization. With the less virulent strain, a greater internalization and higher IFN-β production was observed. In addition, a protective role of type I interferon was described during an *in vivo* infection by *S. suis*. Thus, our findings have provided a novel knowledge of the mechanisms and the capacity of induction of IFN-β by different strains of *Streptococcus suis* serotype 2, and shed a light on the role of this cytokine during the infection with this pathogen.

II - SCIENTIFIC LITTERATURE REVIEW

1. *S. suis*

1.1 Introduction: History and epidemiology

Streptococcus suis is a pathogen in pigs that can cause severe systemic infection in humans and was first reported by veterinarians in 1954, after outbreaks of meningitis, septicemia, and purulent arthritis occured among piglets (1). Among the 35 serotypes based on capsular antigens that have been described, the type 2 is the most frequently isolated from diseased pigs in most countries (2, 3) and is also considered a zoonotic agent. First reported in Denmark in 1968 (4), human infections have been documented in several European and Asian countries as well as in North and South America, Australia and New Zealand (5,6). In western countries, *S. suis* infections in humans have most often been restricted to workers in close contact with pigs or swine byproducts. However, in southeast and east Asia, also affects the general population and it represents a significant public health concern (5).

In 2005, a human and pig outbreak (molecularly demonstrated as being caused by the same *S. suis* strain) in the Sichuan province of China resulted in 204 human cases and 38 deaths, a mortality rate that was higher than expected; the outbreak was characterized by the unexpectedly high percentage of patients developing streptococcal toxic shock-like syndrome (STSLS - unexpected because *S. suis* was not considered to be cause of that until then), shorter incubation period, rapid disease progression, and high mortality rate (1,5). A subsequent report from Thailand indicated that *S. suis* meningitis was far from being a rare sporadic disease in the area, with a series of 66 cases in the period 2005–2007 showing the traditional disease characteristics (86). Even more surprisingly, this pathogen was convincingly demonstrated to be the commonest cause of bacterial meningitis in humans older than 14 years of age in two different cohort studies from Vietnam in 2011 and 2012 (87, 88), and the third most common cause of community-acquired bacterial meningitis in Hong Kong, indicating that, at least for Southeast Asia, the disease is not as sporadic as was previously considered (7-11).

S. suis can be isolated from other animals, such as ruminants, cats, dogs, deer, and horses, and is believed to be a commensal in the intestinal flora (89). Healthy pigs can carry multiple serotypes in their nasal cavities, tonsils, and upper respiratory, genital, and alimentary tracts (89–91).

1.2 Disease and clinical manifestations

1.2.1 Pigs

With almost 100% of pig farms worldwide having carrier animals, *S. suis* is one of the most important bacterial pig pathogens. Transmission among animals is considered to be mainly through the respiratory route (92). There are many descriptions of the pathological and histopathological lesions in infected pigs (89). The most common gross lesions are the congestion of the meninges, lymph nodes and lungs, and the most common histopathological findings are located within the choroidal plexus. Evidence of encephalitis, oedema, and congestion of the brain may be present. In the central nervous system (CNS), lesions associated with meningitis and choroiditis may be observed, including oedema of the leptomeninges and the dura mater, hyperaemic meningeal blood vessels, and an increased quantity of cerebrospinal fluid (CSF). The most characteristic histopathological lesion of acute *S. suis* meningitis is a diffuse neutrophilic infiltrate (94, 95). Nevertheless, in peracute cases of infection, pigs are often found dead with no premonitory signs of disease (93). Presumptive diagnosis in pigs is usually based on clinical signs and macroscopic lesions. Confirmation of the infection is a mandatory requirement and must be achieved by isolation and characterization of the pathogen (89).

1.2.2 Humans

Human infections with *S. suis* are most frequently manifested as purulent meningitis, but reports of septic shock with multiple organ failure, endocarditis, pneumonia, arthritis, and peritonitis have also been reported. Differences in clinical signs among patients infected have been observed. In the acute form of meningitis, symptoms include high fever, headache, chills, nausea, vomiting, and vertigo, followed by one or more of the following: hearing loss, walking ataxia, coma, neck stiffness, petechia, articular pain, peripheral and facial paralysis, severe myalgia, ecchymosis, rashes, and rhabdomyolysis (94, 96-99). In the acute form of toxic septic shock, besides high fever, chills, headache, vomiting, vertigo, and abdominal pain, other clinical signs were also observed, such as hypotension, tachycardia, liver dysfunction, subcutaneous haemorrhage, disseminated intravascular coagulation, acute renal failure, and acute

respiratory distress syndrome (95, 97, 98, 100). Hearing loss is the most common sequela after recovery from purulent meningitis, whereas death often follows septic shock.

An increased amount of CSF has also been reported in human meningitis cases by lumbar puncture (94, 95). Obtaining this fluid is important because it is an important element in the diagnosis of neurological diseases, such as meningeal syndromes, subarachnoid hemorrhages, cerebrospinal tumors, etc.

1.3 Pathogen characteristics

1.3.1 Characteristics of *S .suis*, including serotype, serotype distribution

S. suis is a Gram-positive facultative anaerobe, coccoid or ovoid, and occurs as single cells, in pairs, or in short chains (101). Based on the CPS, 35 serotypes have been identified (types 1–34 and 1/2) (125) but serotypes 32 and 34 have since been proven to be *Streptococcus orisratti* (102). Recent analyses of genes encoding manganese-dependent superoxide dismutase (*sodA*) and the recombination/repair protein (*recN*) indicated that the serotypes 20, 22, 26, and 33 need to be taxonomically removed from *S. suis* (249), and serotypes 20, 22, and 26 were proposed to be a novel species, *Streptococcus parasuis* (250). Serotype 2 is most commonly associated with diseases in pigs and human beings, and is the most frequently reported serotype worldwide (3, 103, 104). The natural habitat is the upper respiratory tract, specially tonsils and nasal cavities, as well as the genital and alimentary tracts of pigs (105, 106). It colonises the palatine tonsils of clinically ill and apparently healthy pigs, and is usually transmitted nasally or orally (107). In relation with the resistence to enviromental conditions, it can survive for 10 min at 60°C, 2 h at 50°C, and 6 weeks in carcasses at 10°C. At 0°C, can survive for 1 month in dust and for over 3 months in faeces, whereas at 25°C, it can survive for 24 h in dust and for 8 days in faeces. However, it can be killed easily with 5% bleach at 1:799 dilution (108).

S. suis is sensitive to antibiotics, including penicillin, ceftriaxone, cephalosporin, ampicillin, and amoxicillin. Penicillin G is commonly used to treat or control infections but penicillin-resistant strains have been isolated (109,110), and strains highly resistant to other commonly used antibiotics have also been reported (111).

The genome, which has been completely sequenced, contains 20074917 bp (112). Although the functions of 20–30% of the genes are unknown, many genes that may play a part in the pathogenesis of the infection have been studied, including polysaccharide production, capsular transport, iron-restriction factors, suilysin, virulence-associated proteins, various enzymes, arginine deiminase system, and IgG binding proteins (113-118).

S. suis is able to grow in anaerobic or aerobic conditions, but not in 6,5% NaCl solution (119). Colonies are small (0,5–1,0 mm diameter), greyish or transparent, and slightly mucoid. They produce narrow zones of α-haemolysis on sheep blood agar plates and β- haemolysis on horse blood agar plates (89). Presumptive identification based on four biochemical tests (Voges-Proskauer, salicin, trehalose, and 6,5% NaCl) can be successful for almost all capsular types (124). Recently, Ishida *et al*. developed a novel PCR method targeting the recombination/repair protein (*recN*) gene of *S. suis*, which corresponds to the current reclassification of this bacterium. *recN* PCR using *S. suis* reference strains could discriminate *S. suis* from those that should not been included in *S. suis* (251). Regarding the serotyping, molecular assays by PCR amplification of serotype specific *cps* genes does not require antisera and is an attractive alternative to the current agglutination and co-agglutination tests. However, it is known that the *cps* gene clusters of serotype 1 and serotype 14, and of serotype 2 and serotype 1/2 are very similar with the nucleotide sequence of the *wzy* genes being nearly identical in these two pairs of serotypes. Therefore, the mPCR assays cannot discriminate these two pairs of serotypes. Because of that serotype 1 and 14, and serotype 2 and 1/2 will require the use of serotype specific antisera (252). Recently, was developed a pipeline which permits *in silico* serotype determination from whole-genome sequencing (WGS) short-read data that can readily identify *S. suis* serotypes. Indeed, it can discriminate between serotype 1 and 14, and between serotype 1 and 1/2, which solves a three-decade longstanding *S. suis* typing issue (272).

Globally, the predominant serotypes isolated from clinical cases in pigs are, in decreasing order, serotypes 2, 9, 3, 1/2 and 7. However, there is a clear geographical effect on the distribution of serotypes and these figures are influenced by the number of published studies. Almost 70% of studies on worldwide isolates recovered from diseased pigs are from North America. In fact, 97% of North American data are from Canada and the rest from the United States, with no data from Mexico. Serotype 2 is the most prevalent in Canada, while in the United States, it is serotype 3. Both serotypes are

the two most prevalent serotypes isolated from clinical pig cases in North America with 24.3% and 21.0% of prevalence, respectively, followed by serotypes 1/2, 8 and 7. Similar distributions may be explained by a fluid movement of animals between the two countries (125).

In South America, only two studies have been published, both from Brazil, which report serotype 2 as being the most prevalent with a mean of 57.6% of cases, followed by, in decreasing order of prevalence, serotypes 1/2, 14, 7 and 9. In Asia, the most prevalent serotypes in infected pigs are, in decreasing order, serotypes 2, 3, 4, 7 and 8 (125).

Important pig producing European countries, such as Denmark, Belgium, France, Germany, Italy and the United Kingdom, have not recently reported the distribution of serotypes recovered from clinical cases in pigs (3, 127). Before the year 2000, serotype 2 was the most common serotype recovered in Italy, France and Spain, whereas serotype 9 was more frequently found in the Netherlands, Germany and Belgium (128). The only two countries with more recent data are Spain and the Netherlands. In the first one, serotype 2 is no longer the most prevalent, but the second behind serotype 9, followed by serotypes 7, 8 and 3 (129-131). In the Netherlands, between 2002 and 2007, serotype 9 was still the most prevalent, followed by serotypes 2, 7, 1 and 4 (132). Moreover, in 2013, was reported the first case of *S. suis* serotype 9 human infection in Thailand (273). As such, there is an urgent need to evaluate fresh data on the prevalence of *S. suis* in clinical isolates from pigs in Europe where many countries are among the most important pig producers in the world (125).

1.3.2 Data obtained by multilocus sequence typing (MLST): Sequence type (ST), distribution of serotype 2

Different serotype 2 sequence types predominate in different regions of the world. The ST1 is mostly associated with disease in pigs and humans in Europe (though the ST20 is important in the Netherlands), Asia (Cambodia, mainland China, Hong Kong, Japan, Thailand and Vietnam) and Argentina. The ST7, responsible for the 1998 and 2005 epidemics, is mostly endemic to mainland China. North American cases vary from those in Eurasia, with most strains being either ST25 or ST28, which were also recovered in Thailand and Japan, respectively. Finally, the ST101 to ST104 are endemic to Thailand and appear to be more commonly isolated from human cases, especially the

ST104. So, we can observe that the current distribution of the *S. suis* serotype 2 STs greatly varies throughout the world, though data have only been available for a little over a decade, from only a few countries and mostly only for the serotype 2 (133).

In North America, the majority of MLST studies conducted on *S. suis* strains isolated from diseased pigs have been serotype 2. It was determined that 44% of North American strains are ST25, 51% ST28 and 5% are ST1. In Canada, the proportions of ST25 and ST28 are 54% and 46%, respectively, but in the United States, 75% of strains were shown to be ST28 and only 10% ST25, while the remaining 15% are ST1 (133). As with North American strains, the majority of European studies have been done using serotype 2. Most of these have demonstrated that ST1 is predominately isolated from diseased pigs in the Netherlands, Spain and the United Kingdom (132, 134, 135). King *et al*. had already associated the serotype 2 ST1 strains with invasive infections (134). Nevertheless, many strains of serotype 9, have also been recovered from diseased pigs and typed (132, 135). In the Netherlands and Spain, serotype 9 isolates were identified as belonging to the ST16, where they represent 43% of strains in the Netherlands (132). Unlike some countries in Europe, where the serotype 9 is as important as the serotype 2, most strains isolated from diseased pigs in Asia are serotype 2, and it represents 90% of cases in mainland China (136). However, there are relatively few reports of isolation from diseased pigs in Asia. Of these serotype 2 cases, the predominant STs are ST1, ST7 and ST28. In mainland China, Chen *et al*. demonstrated that 22% of serotype 2 strains are ST1 and 77% are ST7. The few ST28 strains recovered in that country were mostly associated with cases of pneumonia (137). With respect to Japan, ST1 and ST28 strains isolated from cases of endocarditis in diseased pigs account for 8% and 76% of serotype 2 strains, respectively (138).

Regarding human cases, globally, ST1 strains have been described as mostly responsible for *S. suis* serotype 2 infections, particularly in South America, Europe and Asia, but also one case in North America (10, 132, 139-142). Nevertheless, multiple other STs have been described worldwide, though these appear to be endemic to certain geographical regions. For example, the ST20 is important in the Netherlands and France but not in the rest of Europe (132). ST7 strains, the ones responsible for the 1998 and 2005 Chinese epidemics were isolated from human patients only in mainland China and Hong Kong (141, 142). ST25 and ST28 strains have been particularly associated with human cases in North America and Japan, respectively (141, 143, 144). In Thailand, ST1 and ST104 strains are predominant, causing mainly meningitis and non-meningitis

cases, respectively (140, 143, 145). A few cases of ST25, ST28, ST101, ST102 and ST103 have also been described (140).

Although *S. suis* serotype 14 infections are less frequent in humans than serotype 2 cases (2% of all the serotype-confirmed cases) the number appears to be increasing. The ST105 is prevalent in Southeast Asia, especially in Vietnam and Thailand. Only three human *S. suis* cases, other than those caused by serotypes 2 and 14, have been typed by MLST, and were described as newly identified STs. Those are serotypes 5, 16 and 24, identified as ST181, ST106 and ST221, respectively (146, 147), but no data have yet been published on the possible presence of these three newly identified STs for human isolates in diseased pigs.

1.3.3 Virulence

1.3.3.1 Virulence factors

Identification of *S. suis* virulence factors was limited from the lack of clear definition of 'virulence'. Different studies have defined field strains as virulent or avirulent based on the clinical condition of the animal (or human being) from which the strains were isolated, or based on the presence of previously described and nonuniversal virulence-associated proteins or, using different experimental infection models. For instance, if strains were isolated from the clinically healthy animals they were arbitrarily considered avirulent whereas the ones isolated from diseased animals/human beings gives strains arbitrarily considered as virulent. However, it is known that the presence of some proposed virulence factors does not necessarily define the strain virulence potential, because its absence is not a sufficient condition for classifying the strain as avirulent. Moreover, the virulence of serotype 2 strains recovered in Europe and Asia from diseased piglets seems to be higher than that observed in strains from North America (133, 148).

Although the actual early mechanisms used by *S. suis* to colonize the host are poorly known, it was reported that the pathogen may survive in swine tonsils for long periods of time. However, it is still unknown how *S. suis* manages to cross the first natural line of the host defense and initiates the disease. The most accepted hypothesis is that it breaches the mucosal epithelium in the upper respiratory tract of pigs (106). In humans, *S. suis* may interact with epithelial cells either at the epidermal surface or in the intestine

(5, 6, 146). Adhesins at the surface of *S. suis* appear to be hindered by the CPS, as suggested by the fact that *S. suis* CPS deficient mutants adhered better than the encapsulated parental strain to porcine (LLC-PK1 and PK15), canine (MDCK) and human (A549 and HeLa) cell lines. Thus, we can hypothesize that *S. suis* downregulates expression of CPS during the early steps of the infection in response to signals from the environment, resulting in a better interplay between bacterial adhesins and host receptors (149, 274).

S. suis interacts with components of the extracellular matrix (ECM) such as fibronectin and plasminogen (150). The fibronectin-binding protein Fbps was shown to bind human fibronectin and fibrinogen *in vitro*. Infection of pigs with a *fbps* mutant strain showed that Fbps is not required for colonization of the tonsils but it may play a role in colonization of specific organs, including CNS (151, 190). *S. suis* enolase which binds plasminogen and fibronectin, is highly expressed *in vivo*, inducing the production of antibodies in infected pigs, although its potential being used as a protective antigen remains controversial (152, 153). In addition, antibodies against enolase decrease adhesion and invasion of porcine brain microvascular endothelial cells (BMEC) (191). A dipeptidylpeptidase DppIV was also shown to interact with human fibronectin, and the virulence of a *dppIV*-deficient mutant was greatly attenuated (154). Binding of *S. suis* to collagen has also been reported (150), and a mutant strain defective in a putative collagenase was impaired in survival after experimental inoculation of pigs (126). Similarly, a mutant strain devoid of the housekeeping sortase *SrtA* showed weaker adherence to ECM proteins, suggesting that peptidoglycan- anchored, LPXTG motif-containing adhesins are also important for interactions of this pathogen with ECM proteins. The same was observed with BMEC (155).

Other proteins were identified as adhesins, including a 39-kDa glyceraldehyde- 3-phosphate dehydrogenase (GAPDH) (156,157). Two studies showed reduced adherence of *S. suis* to porcine tracheal rings and HEp-2 cells when cells were preincubated with recombinant *S. suis* GAPDH (157,158). The pathogen upregulates the expression of the gene encoding this protein *in vivo* in different porcine organs (159). An enzyme with 6-phosphogluconate- dehydrogenase activity, a bifunctional amylopullulanase, as well as a glutamine synthetase, were shown to contribute to *S. suis* adherence to epithelial cells (160,161). Mutants devoid of the two-component regulatory system CiaRH or the orphan transcriptional regulators RevSC21 and CovR were also impaired in adherence to epithelial cells (162-164), but the mechanisms have not yet been deciphered.

Epithelial cells invasion may also represent the beginning of systemic dissemination and disease. As in the case of adhesion, only unencapsulated strains seem to be able to invade these cells (165). The disruption, including the disruption to cross the blood–brain barrier (BBB) and the blood– CSF barrier of Choroid plexus epithelial cell (CPEC), is also possible since suilysin (SLY)-positive strains are highly toxic for the cells (106, 193). This 54-kDa hemolysin is a thiol-activated toxin that targets cholesterol in the membrane of eukaryotic cells (166, 167). However, strains not producing SLY are also able to do that (2, 192). But this concern remains still controversial.

Regarding the survival in blood and dissemination of the pathogen, the resistance to phagocytosis require modification of the cell wall peptidoglycan by means of *N*-deacetylation. A well encapsulated mutant strain devoid of the deacetylase PgdA, responsible for this modification, showed impaired resistance to neutrophil killing and was severely attenuated in murine and porcine infection models (170). Similarly, d-alanylation of the *S. suis* lipoteichoic acid (LTA) plays a major role in survival of this pathogen (171), and was shown that mutants impaired in LTA d-alanylation adhered and invaded porcine BMEC lesser than the wildtype strain. Although SLY-negative strains can be virulent and survive in blood, SLY-positive strains, additionally, reduce phagocytosis and killing of *S. suis*. This pathogen is also able to affect neutrophil recruitment by degrading IL-8, presumably by the production of a serine protease (172).

A cell wall-anchored DNAse, specific for single and double-stranded linear DNA is expressed by *S. suis* (173) and it is believed that it plays a role in disruption of neutrophil extracellular traps (174). *S. suis* requires nutrients like trace metals, whose availability within the infected host is relatively low. AdcR, a streptococcal transcription factor homologous to the zinc-uptake regulator Zur, and the ferric uptake regulator Fur, one of the most important transcription factors controlling iron metabolism, are both important for *S. suis* survival *in vivo* (175, 176). Eighteen unique iron restriction induced genes have been identified, including the *cpsA* gene, encoding a putative regulator of CPS biosynthesis and *iri-7*, homolog of *Streptococcus mutans rpgG*, a gene involved in capsule biosynthesis. It was proposed that because the CPS of *S. suis* becomes thicker after growth *in vivo*, where free iron is scarce, an upregulated expression of *cps2A* and *rpgG* under iron starvation might be expected (116). Despite these findings, it has been reported that *S. suis*, which does not secrete siderophores, adapts to iron restricted conditions by a change in its metabolism, replacing iron by

manganese or magnesium (177, 178). Interestingly, the lipoprotein TroA, which is required for *S. suis* growth in environments low in manganese, is crucial for bacterial survival *in vivo* (179). Recently, it was shown that deletion of a lipoprotein involved in zinc uptake - SSU0308 (Lipoprotein 103) - resulted in a mutant strain that was 50-times less virulent than the parental one (180). As mentioned earlier, the CPS constitutes a physical barrier against phagocytosis. However, if internalized, *S. suis* possesses factors that might contribute to the resistance to intracellular killing machinery of phagocytic cells (165). It is worth mentioning the presence of an active superoxide dismutase (SodA), although it is unlikely that SodA produced by *S. suis* type 2 mediates intracellular survival of pathogenic isolates in macrophages (181). In addition, survival of *S. suis* under acidic conditions has been linked to the presence of an arginine deiminase system catalyzing the conversion of arginine to ornithine, ammonia and carbon dioxide (117, 118).

Although rare, septic shock in some cases can be a result of excessive or poorly regulated immune response to the offending organism (182). Are the virulence factors responsible? Lipoproteins present in the cell wall could be, in part, responsible for cell receptor(s) recognition (183). Recently, it has been shown that a putative prolipoprotein diacylglyceryl transferase present in *S. suis* cell wall is required for innate immune activation (184), while suilysin was shown to activate phagocytes and to induce the release of pro-inflammatory cytokines (185,186). In addition, suilysin may cause a release of hemoglobin from red blood cells, which will raise the levels of pro-inflammatory mediators by acting in synergy with *S. suis* cell wall components (187), while a surface-associated subtilisinlike protease (SspA) induces the secretion of different pro-inflammatory cytokines and chemokines by macrophages (188).

Finally, there are some putative virulence factors like a glutamine synthetase (194), a serum opacity-like factor (195), a protein of unknown function encoded by *virA* (196) and a trag factor (197), but the mechanisms by which these factors affect *S. suis* virulence remain obscure. Also, there are proteins whose deletion affect virulence, including the response regulator RevS (198), the autoinducer LuxS (199), the sugar regulator CcpA (200), Rgg-like regulators (201), the orphan transcriptional regulators RevSC21 (162) and CovR (164), as well as the SalK/SalR (176) and CiaRH two-component systems (163). Again, how these regulators influence virulence (and the extent of their regulons) has not yet been elucidated. In addition, other proteins have been suspected to be ‘virulence factors’ since antibodies against them confer protection.

They are HP0245, HP0272 and HP0197, of unknown function (202-205), the surface antigen One (206), and HtpS, a histidine triad protein (207).

1.3.3.2 Difference in virulence of different ST and geographical origin

The virulence markers used in association with STs are the SLY, the Muramidase Released Protein - MRP and the Extracellular Factor - EF. Serotype 2 ST1 strains, mostly associated with disease in pigs and humans in Europe, Asia (Cambodia, mainland China, Hong Kong, Japan, Thailand and Vietnam) and Argentina, have for the most part been genotyped/phenotyped as *sly*+ *mrp*+ *epf*+ / SLY+ MRP+ EF+, regardless of the geographic origin, which is identical to the serotype 2 ST7 strains isolated from diseased pigs and humans in mainland China (133, 135, 137, 138, 141, 235). Nevertheless, important genetic differences vary between ST1 and ST7 strains including the presence of a 89K pathogenicity island in ST7 strains (236). These ST1 complex strains differ from the human serotype 2 ST104 strains of Thailand which are *sly*+ *mrp*- *epf*- and from the human serotype 2 ST20 strains recovered in the Netherlands that were *epf*- (132, 140, 141). Interestingly, a human case from Spain whit the serotype 2 strain was typed as being a ST3, and presented a *mrp* variant (*mrp*)*, which has a higher molecular weight, though being *sly*+ *epf*+ (237, 238).

In Europe, it was determined that strains isolated from diseased pigs belonging to the ST61 (serotype 9) complex differ from the ST1 (serotype 2) complex strains in being *mrp** rather than *mrp*. Particularly in Spain, the endemic ST123 and ST125 (serotype 9) are both *mrp*- and *epf*- (135).

In North America, ST25 strains isolated from diseased pigs were identified as *sly*- *mrp*- *epf*-, while the ST28 isolated from North America, mainland China and Japan are *sly*- *mrp*+ *epf*- or SLY- MRP+ EF- (133, 137, 138).

Although not as widely used, different pili (*srtB, srtC, srtD, srtF* and *srtG*) have also been associated with different STs. It was identified that ST1 strains isolated from both diseased pigs and human cases of serotype 2 infections in Japan and Thailand are *srtBCD*+ and *srtF*+, but *srtG*- (143). North American ST25 strains isolated from diseased pigs and human cases are *srtF*- and *srtG*+ and ST28 strains isolated from diseased pigs and human cases from North America and Japan are *srtF*+ and *srtG*+ (133, 138).

An experimental study performed by Lachance *et al.* compares the innate immune response of the host following an acute infection by three different strains of *S. suis* with different virulence potentials: an intermediately pathogenic ST25 North American strain (89-1591), a highly pathogenic ST1 European strain (P1/7) and the epidemic ST7 Chinese (SC84) strain responsible from outbreaks in China. Authors observed that, during the acute infection, the survival of mice infected with the North American 89-1591 strain was not significantly affected and was similar to that of mock-infected mice. Contrarily, mice infected with the highly virulent Chinese strain had a 50% survival rate at 17 h p.i., while mice infected with the virulent European strain had a 50% survival rate at 44 h p.i. They also observed that cytokines levels, but not bacterial burden, correlate with the degrees of virulence of ST7, ST1 and ST25 strains (247).

1.4 Steps of the pathogenesis

1.4.1 Adherence to, colonization and invasion of epithelial host cells

As mentioned above, adhesins present at the surface of *S. suis* appear to be hindered by the CPS and, it is believed that *S .suis* down regulates their expression during the early steps of the infection in response to signals from the environment, resulting in a better interplay between bacterial adhesins and host receptors (149). Moreover, *S. suis* interacts with components of the ECM such as fibronectin and plasminogen (150). In addition, it is already known that IgA-mediated immunity plays a major role in defense against recurrent mucosal pathogens. It has been reported that *S. suis* produces an IgA1 protease capable of cleaving human IgA1 (168). This protease is highly immunoreactive to convalescent sera, and an isogenic mutant defective in the production of this enzyme showed significantly decreased lethality in pigs (169).

It is believed that nonvirulent strains of *S. suis* can probably adhere and colonize pigs without causing disease. However, since the disease caused by this pathogen is mainly systemic, virulent strains should, after adhesion, either directly invade and translocate, and/or reduce cell viability and increase mucosal barrier permeability (274). Although cell invasion is one of the expected outcomes of bacterial adhesion to cells, the invasion of mucosal epithelial cells by well-encapsulated *S. suis* is still controversial. As in the case of adhesion, only poorly encapsulated strains seem to be able to clearly invade these cells (274). The disruption of epithelial cell is possible, since SLY-positive strains

are toxic for these cells (106) because they attack cholesterol in the membrane of eukaryotic cells (166, 167). However, SLY-negatives are also able to reach the bloodstream and disseminate (2).

1.4.2 Surviving in the bloodstream: innate immune response

It was proposed that *S. suis* may gain entry to the systemic circulation primarily through the palatine tonsils, after adhesion and invasion of epithelial cells and later, through the interaction with cells of the myeloid lineage (222, 223). Once pathogen reaches deep tissues and/or the bloodstream, it is subjected to the action of phagocytic cells of the innate immune system.

1.4.2.1 Phagocytosis: monocytes/macrophages, PMN, dendritic cells (DCs)

Bacterial survival depends on the production of CPS, since it was documented by *in vitro* and *in vivo* experiments thatvCPS protects *S. suis* from neutrophil and monocyte/macrophage-mediated phagocytosis and killing (2). The fine structure of the *S. suis* serotype 2 CPS has recently been solved, indicating the presence of *N*-acetyl-neuraminic acid (sialic acid) residues. It is worth noting that the capsules of the two most important serotypes that cause disease in humans (2 and 14) possess sialic acid (224). Sialic acid has also been implicated in the adherence (without phagocytosis) of *S. suis* to monocytes, suggesting a 'modified Trojan horse' hypothesis, in which the pathogen would travel in the bloodstream externally associated with these phagocytic cells (106). In addition, an effect of molecular mimicry has been suggested (2), based on the fact that the conserved 2–6 linked sialic acid terminal capsular moiety found in serotypes 2 and 14 (225) is similar to sugar epitopes displayed on the surface of all mammalian cells (5). This molecular mimicry could be the reason of the absence of antigen recognition by the immune system of the host (123, 226). Despite the critical role played by the CPS in *S. suis* virulence, some avirulent serotype 2 field strains are well encapsulated (227), indicating that the survival in blood does not rely solely on encapsulation.

Resistance to phagocytosis is multifactorial and requires modification of the cell wall peptidoglycan - *N*-deacetylation- by a deacetylase PgdA (170). A well encapsulated mutant strain devoid of that enzyme showed impaired resistance to neutrophil killing.

Similarly, d-alanylation of the *S. suis* lipoteichoic acid (LTA) plays a role in survival of this pathogen (171). A mutant strain producing LTA devoid of d-alanine residues was more susceptible than the parental strain to the action of cationic antimicrobial peptides and killing by porcine neutrophils. Apart from these major cell wall structures, many surface proteins can induce antibodies that increase the killing of *S. suis* by phagocytes (152, 207, 228, 229). However, the mechanisms of action of these proteins or their role at the bacterial-phagocyte interface are still unknown.

Although SLY-negative strains can be virulent and survive in blood, SLY-positive strains seem to benefit additionally from their toxic effects on monocytes and neutrophils (230, 231).

S. suis is also able to affect neutrophil recruitment by degrading IL-8, because of the production of a serine protease (172). The pathogen requires nutrients including trace metals like zinc and iron, whose availability within the infected host is relatively low, and are important for the survival *in vivo* (175, 176). It has been reported that *S. suis*, is able to adapt to iron-restricted conditions by a change in its metabolism, through replacing iron by manganese or magnesium (177, 178).

As mentioned before, the CPS constitutes a physical barrier against phagocytosis. However, if internalized, *S. suis* possesses factors that might contribute to resist the intracellular killing machinery of phagocytic cells (165), like the arginine deiminase system which catalyzes the conversion of arginine to ornithine, ammonia and carbon dioxide under acidic conditions (117, 118).

1.4.3 Meningitis

1.4.3.1 Blood brain barrier (BBB) penetration

If death from sepsis or toxic shock-like syndrome does not occur and bacteremia remains high, *S. suis* may cause meningitis (148, 212). As a blood-borne pathogen, *S. suis* must cross the BBB and/or the blood–CSF barrier in order to cause CNS infections. The BBB is an anatomical and functional barrier that separates the brain from the intravascular compartment and maintains the homeostasis of the CNS environment (189); its main cellular type is BMEC. *S. suis* survived up to 7 h within porcine BMEC (192), which is a very interesting finding, because a crucial element for

the development of meningitis is the ability of pathogens to cross the BBB as live bacteria (213).

As was mentioned, the CPS of *S. suis* interferes with the process of adhesion/invasion. Although serum components may participate in the interactions between the pathogen and porcine BMECs (192, 214), only fibronectin was shown to play an important role in this process (190). SLY positive strains may also disrupt the BBB through cytotoxic effects, however it was reported that the negative mutants can successfully invade these cells (192).

Another CNS entry portal for *S. suis* can be the blood–CSF barrier. This invasion route is supposed to involve three steps: invasion of porcine CPEC from the basolateral side; transport within membrane-bound endocytic vacuoles to the apical side and exocytosis onto the apical membrane of the blood–CSF barrier (193). The translocation across the blood–CSF barrier activates neutrophils (215) and affects the barrier´s function and integrity, further facilitating trafficking of bacteria and leukocytes. It has been shown that *S. suis* induces CPEC necrosis and the release of pro-inflammatory cytokines and chemokines by human and porcine BMEC, murine microglia and astrocytes (216-219). However, the specific bacterial components responsible for exaggerated inflamatory reactions are not accurately known. In addition to cell wall components, bacterial CPS induces human macrophages to secrete prostaglandin E2 and matrix metalloproteinase 9, which may also be involved in disruption of the BBB (220). Purified SLY has been shown to induce the release of several pro-inflammatory cytokines by human and porcine BMEC (172, 192, 218) and the upregulation of adhesion molecules on human monocytes (221).

1.4.4 Inflammation as a hallmark of *S. suis* infection

1.4.4.1 Systemic

Although activation of the immune system during microbial infection is generally protective, septic shock may result as a consequence of excessive or poorly regulated immune response against the microorganism (182).

1.4.4.1.1 *In vitro* (monocytes/macrophages, PMN, dendritic cells)

In vitro experiments have shown that some pattern recognition receptors (PRR), such as CD14 and Toll like receptor 2 (TLR2), could be responsible for cell activation by *S. suis*, which would lead to release inflammatory compounds (185, 231-233). However, macrophages isolated from TLR2 knockout mice have showed a highly reduced, but not completely abrogated cytokine production. That suggests the involvement of other TLRs in cytokine production (232).

Suilysin was shown to activate phagocytes, induce the release of pro-inflammatory cytokines IL-1β, IL-6 and chemokine IL-8 (185, 186), and it might release hemoglobin from red blood cells, contributing to the increased levels of the pro-inflammatory mediators by acting in synergy with *S. suis* cell wall components (187). Bi *et al.* showed that SLY also induced a strong TNF-α release from human peripheral blood mononuclear cells (PBMCs) and primary peritoneal murine macrophages. Moreover, a long-terme release of that cytokine from PBMCs was observed. In adittion, the authors demonstrated for the first time that SLY activates human PBMCs via TLR4, a receptor that has been reported to recognize the lipopolysaccharides from Gram-negative bacteria (253). In addition, Sspa induces the secretion of different pro-inflamatory cytokines and chemokines by macrophages (188). *S. suis* may modulate the response, and improve its survival, by actively degrading the chemokines and thus delaying recruitment of neutrophils to the site of inflammation (172).

1.4.4.1.2 *In vivo*

It is known that inflammation plays an important role in the pathogenesis of *S .suis*-induced septicemia and meningitis, and that TLRs are critical sensors in detecting infections and initially activate the innate immune system, specially TLR2 which has been implicated as the major PRR for ligands derived from gram-positive bacteria (258). Data from Lachance *et al*. indicated that *in vivo* TLR2-mediated recognition contributes to the severe outcome following infection with *S. suis* ST1 European strain. A signifincantly lower induction of pro-inflammatory cytokines and chemokines in TLR2-/- mice, was confirmed too, but interestingly, inflammatory mediators were significantly reduced but not abolished. These data suggest the contribution of other receptors. The same study showed that TLR2 does not seem to be mainly implicated in

the severe symptoms and lethality associated with the ST7 Chinese strain, since bacterial loads and levels of pro-inflammatory cytokines and chemokines were independent of TLR2. Levels of mRNA and protein of interferon (IFN)- γ were significantly higher in ST7 than ST1-infected mice, confirming previous observations about that the higher mortality observed with the ST7 strain compared to ST1 strain was mainly due to a massive secretion of IFN- γ by Natural killer (NK) cells. Based on *in vitro* studies results, they hypothesized that different bacterial components present in typical *S. suis* strains and those from the epidemic ST7 strain may vary and play a distinct role on cell activation and in the pathogenesis of the systemic inflammatory disease caused by this pathogen (260).

Another *in vivo* study, performed using a *S. suis* serotype 2 ST7 05ZYH33 strain, have showed that the cytokine levels may represent a prognostic indicator of the disease outcome. Massive amounts of cytokines TNF-α, IL-1β, IL-6, IL-10, IFN-γ and TGF-β levels were found in sera of piglets with septic-like shock syndrome (SLSS). Conversely, lower and irregular cytokine expression levels were found in the pigs with prostration syndrome, indicating that a strong cytokine storm caused the SLSS. By this way, cytokine over-expression breaks the immunologic balance between pro and anti-inflammatory actions which can protect hosts against pathogens (211) and is necessary for disease recovery. Moreover, a systemic cytokine storm can produce systemic sepsis and septic shock. In addition, the destructive effects of virulence factors, in the infected hosts ultimately manifest as persistent hypotension, leukocytosis or leucopenia, multiple organ dysfunction or lesions, STSLS or SLSS, coma, and even rapid death (261).

Lachance *et al*. have compared cytokines levels following an *in vivo* infection with different STs of *S. suis* serotype 2, such as the intermediately pathogenic ST25 North American Strain, the highly pathogenic ST1 European strain and the epidemic Chinese ST7 strain (247). They found that cytokine and chemokines levels, but not bacterial burden, correlate with the degrees of virulence of the strains, being that plasma levels of CXCL1, CXCL2, CCL2, CCL3, CCL4, IL-1β, IL-6, TNF and IFN- γ were higher in mice infected with the epidemic Chinese strain of *S. suis*. Other proteins, such as CXCL9 and CXCL10, were produced at high levels following the induction by ST7, and ST25 strains of *S. suis*. It was because the latter strain induces high levels of IFN-β, which can also contribute to the CXCL9 and CXCL10 induction (247).

1.4.4.2 CNS

Meningitis-associated brain injury and neuronal death is also associated with a host reaction to bacterial components (234).

1.4.4.2.1 *In vitro* (astrocytes, glial cells)

Studies have shown that *S. suis* is able to induce the release of pro-inflammatory cytokines and chemokines by human and porcine BMEC, murine microglia and astrocytes. However, the specific bacterial components responsible for the exaggerated inflammatory reactions are not accurately reported. Specifically in microglia cells, *S. suis* induces the release of high levels of TNF-α and the chemoattractant MCP-1, but low levels of IL-β1 even in absence of CPS. In the case of IL-6, it is released after infection only in absence of the CPS. This observation is interesting because it is known that this cytokine is highly secreted in the bloodstream during the septicaemic phase, after infection with wild type encapsulated strain of *S. suis*. Under the same condition, with an unencapsulated strain, was also observed a high activation of inducible nitric oxide synthase (iNOS), and accordingly with this a high nitric oxide (NO) production. Regarding the TLRs expressed in microglia, it is known that *S. suis* induces a high microglial TLR2 upregulation, and in agreement with the cytokines results, the upregulation is higher with the CPS mutant. Once in contact with microglia, virulent encapsulated *S. suis* can modulate intracellular signaling events, such as avoiding the phagocytosis and delaying the activation of the inflammatory response. These findings denote the relevance of CPS in the regulation of pro-inflammatory events (216-219).

1.4.4.2.2 *In vivo*

It has been shown that after 6 days post-injection (intraperitoneal injection), the most important changes in the brain of mice with clinical nervous symptoms comprised hemorrhagic foci and malacia mainly at the somatosensory cortex, striatum, hippocampus, thalamus, and hypothalamus, together with gliosis and the presence of inflammatory foci composed primarily of neutrophils (216). In several mice, the meninges were thickened and severely infiltrated by a mixture of neutrophils, macrophages, and lymphocytes. The expression levels of immune mediators were up-

regulated to different extents in the brain of infected mice. IL-1β was detected in the choroid plexus and cortex as early as 24 h p.i. and gradually increased by day 5 p.i. in mice that have exhibited clinical signs. At this time point, IL-1β signal was clear in the choroid plexus, cortex, corpus callosum, and meninges. In contrast, IL-6 gene expression was not considered significant. For TNF-α, at day 5 p.i., the signal increased and was confined to the entire corpus callosum and the choroid plexus. The microglia/macrophage subset, with a modest participation of astrocytes, express CCL2, which levels peaked by day 5 p.i. Moreover, IL-12, IFN-γ, CXCL1 and CCL5 were observed *in vivo* within 24 h p.i. and might be responsible in part for the sudden death (216). Myelin proteolipid protein (PLP), synthesized by oligodendrocytes, is the most abundant protein of CNS myelin (248). As the infection progressed, the inflammatory response to *S. suis* correlated with a decrease in the expression of PLP mRNA in specific areas of the brain, indicating demyelination. At day 5 p.i., this loss in myelin content was observed in the corpus callosum (216).

1.4.5 Receptors involved in inflammation

1.4.5.1 Pattern recognition receptors (PRRs)

Inflammation is a protective response of the body to ensure removal of detrimental stimuli, as well as a healing process for repairing damaged tissue (239). Germline-encoded pattern recognition receptors are responsible for sensing the presence of microorganisms. They do this by recognizing structures conserved among microbial species, which are called pathogen-associated molecular patterns (PAMPs). Four different classes of PRR families have been identified: transmembrane proteins such as the TLRs and C-type lectin receptors (CLRs), as well as cytoplasmic proteins such as the Retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), NOD-like receptors (NLRs) and cytosolic DNA sensors. These PRRs are expressed not only in macrophages, neutrophils and dendritic cells but also in various nonprofessional immune cells. The sensing of PAMPs by PRRs upregulates the transcription of genes involved in inflammatory responses. These genes encode pro-inflammatory cytokines, type I IFNs, chemokines and antimicrobial proteins, proteins involved in the modulation of PRR signaling, and many uncharacterized proteins (240).

The TLR family is one of the best-characterized PRR families and is responsible for sensing invading pathogens outside of the cell and in intracellular endosomes and lysosomes (241). TLRs are characterized by N-terminal leucine-rich repeats (LRRs) and a transmembrane region followed by a cytoplasmic Toll/IL-1R homology (TIR) domain. Ten TLRs have been identified in humans and twelve in mice (240). Different TLRs recognize different PAMPs and self-components.

The RLR family is composed of RIG-I, melanoma differentiation associated gene 5 (MDA5), and LGP2 (242). They are expressed in cell types such as myeloid cells, epithelial cells, fibroblasts, and cells of the CNS. However, their function is not necessary for IFN production by plasmacytoid dendritic cells (pDCs), despite their expression in this cell type. RLRs are composed of two N-terminal caspase activation recruitment domains (CARDs), a central DEAD box helicase/ATPase domain, and a C-terminal regulatory domain. They recognize the viral genomic RNA of double-stranded RNA (dsRNA) viruses and, viral dsRNA generated as the replication intermediate of single-stranded RNA (ssRNA) viruses. The expression of RLRs is greatly enhanced in response to IFN-I stimulation or virus infection (240).

The NLR family consists of cytoplasmic pathogen sensors composed of a central nucleotide-binding domain and C-terminal leucine-rich repeats (243). The N-terminal portions of most NLRs harbor protein-binding motifs, such as CARDs, a pyrin domain, and a baculovirus inhibitor of apoptosis protein repeat (BIR) domain. NOD1 and NOD2 induce transcriptional upregulation of pro-inflammatory cytokine genes (240).

CLRs comprise a transmembrane receptor family characterized by the presence of a carbohydrate-binding domain. CLRs recognize carbohydrates on microorganisms such as viruses, bacteria, and fungi. CLRs either stimulate the production of pro-inflammatory cytokines or inhibit TLR-mediated immune complexes (240).

DNA sensors recognize microbial or self-DNA present in the cytoplasm as a sign of infection or cell damage and induce the production of type I IFNs, type III IFNs, or IL-1β. They include DNA-dependent activator of Interferon Regulatory Factors (IRFs) (DAI), RNA Polymerase III (Pol III), IFN-γ inducible protein 16 (IFI16), leucine-rich repeat flightless-interacting protein 1 (LRRFP1), extrachromosomal histone H2B, DNA-PK and MRE11. All of them recognize dsDNA to induce IFN-I production. Others DNA sensors were identified such as members of the DExD/H-box helicase family, DHX9, DHX36, and DDX41 but they present different specificities for DNA recognition (254).

PRRs	Localization	PAMP recognized	Key adaptors	Effector response
TLRs				
TLR1	Cell surface	Triacylated lipopeptides	MyD88	IL-6, TNF-α
TLR2	Cell surface	Di/triacylated lipopeptides	MyD88, TIRAP	IL-6, TNF-α, IL-8, MCP-1, RANTES
TLR3	Endosomes	dsRNA	TRIF	IFN-β
TLR4	Cell surface	LPS	MyD88, TRIF, TIRAP, TRAM	IL-6, TNF-α, IFNβ, IP-10
TLR5	Cell surface	Flagellin	MyD88	TNF-α
TLR6	Cell surface	Diacylated lipopeptides	MyD88, TIRAP	TNF-α, IL-6, IL-8, MCP-1, RANTES
TLR7	Endosomes	ssRNA	MyD88	IFN-α
TLR8	Endosomes	ssRNA	MyD88	IFN-α
TLR9	Endosomes	CpG DNA	MyD88	IFN-α
TLR11	Endosomes	Profilin, flagellin	MyD88	IL-12, TNF-α
TLR12	Endosomes	Profilin	MyD88	IL-12p40, IFN-α
TLR13	Endosomes	23s rRNA	MyD88	IL-6, IL-12p40
RLRs				
RIG-I	Cytoplasm	Short dsRNA, ssRNA	IPS-1, STING	IFN-β, IL-6
MDA5	Cytoplasm	Long dsRNA	IPS-1	IFN-β
LGP2	Cytoplasm	dsRNA	IPS-1	IFN-β
DDX3	Cytoplasm	Viral RNA	IPS-1	IFN-β
Cytosolic DNA sensors				
DAI	Cytoplasm	dsDNA	STING	IFN-β
RNA Pol III		AT rich dsDNA	IPS1	IFN-β
IFI16	Nucleus and cytoplasm	dsDNA	STING	IFN-β, IP-10, IL-6, IL-1β
AIM2	Cytoplasm	dsDNA	ASC	IL-1β, IL-18
Ku70	Cytoplasm	dsDNA	?	IFN-γ
MRE11		dsDNA, ISD	STING	IFN-β, IL-6, IP-10
cGAS	Cytoplasm	dsDNA	STING	IFN-β
LRRFIP1	Cytoplasm	dsDNA, dsRNA	β-catenin	IFN-β
DHX36	Cytoplasm	dsDNA	MyD88	TNF-α
DHX9	Cytoplasm	dsDNA	D88	TNF-α
DDX41	Cytoplasm	c-di-GMP, c-di-AI	NG	IFN-α, IFN-β
STING	Cytoplasm	c-di-GMP		IFN-β
HMGB	Cytoplasm	dsDNA, ssDNA	?	IFN-β, IL-6, RANTES
Histone H2B	Nucleus and cytoplasm	Poly (dA:dT), genomic DNA	IPS1	IFN-β

Table 1. PRRs that recognize conserved microbial structures of pathogens. Reproduced from (254).

1.4.5.2 PRRs implicated in the recognition of *S. suis*

PRRs implicated in the recognition of *S. suis* are principally the TLRs and NLRs (244). Inside the TLR family, TLR2 (it recognizes ligands by forming a heterodimer with either TLR1 or TLR6), TLR3, TLR4, and TLR9 could be mentioned (245). Referring to the NLRs, NOD2 becomes activated once its ligand is attached after the

digestion of bacterial cell wall and the muramyl dipeptides (MDP), the largest motif common to Gram-negative and Gram-positive bacteria, is released (246).

Lecours *et al*. used BMDCs in an *in vitro* assay, to evaluate the importance of specific immune receptors in the recognition of *S .suis* serotype 2. They confirmed the participation of myeloid differentiation primary response protein 88 (MyD88) signaling pathways in DC activation and maturation after *S. suis* infection. In fact, the production of the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α, the TH1-driving cytokines IL-12p70 and IL-23, the regulatory cytokine IL-10, and the chemokines CXCL1 and CXCL10, was dramatically impaired in MyD88 -/- DCs. They, also investigated the participation of TLR2 in DC cytokine production following stimulation with *S. suis*, using TLR2 -/- DCs, and they have noticed that levels of IL-1β, IL-6, IL-10, IL-23, TNF-α and CXCL1 were significantly reduced under that condition. In that study, inhibition of TLR9 did not affect DC maturation and activation, but deficiency in TLR2 and blocking of TLR9 together significantly affected the surface expression as well as the production of cytokines. Moreover, Zheng *et al*. have demonstrated that DNA from *S. suis* is a strong stimulus for TLR9 activation and may play a role during the induction of the overpowering inflammatory response (258, 259). TLR4 does not seem to play an important role in DCs maturation and activation (258).

It is known that MyD88-dependent pathway is used by all TLRs except TLR3. However, a partial role for the latter might be suggested, being that transcription of TLR3 mRNA in brains of *S. suis* infected mice has been described (216). Another study with swine DCs showed an upregulation of relative expression of TLR2 and TLR6 mRNA after stimulation with *S. suis* (233).

Regarding the NLRs, the involvement of NOD2 in the release of CXCL1 and IL-23 by DCs after stimulation with *S. suis* has also been shown (258). Finally, it could be said that recognition of *S. suis* by DCs seems to require a multimodal recognition system (258).

2. Type I Interferon (IFN-I)

2.1 General characteristics

Interferons, discovered by Isaacs in 1957, are a family of cytokines which act early in the innate immune response and are famous for inducing an antiviral state in virus

infected cells. In addition to this antiviral activity, they have a role in the regulation of the immune response (12). There are three distinct interferon families. The type I IFN family is a multi-gene cytokine family that encodes 13 partially homologous IFNα subtypes in humans (14 in mice), a single IFNβ and several poorly defined single gene products (IFNε, IFNτ, IFNκ, IFNω, IFNδ and IFNζ) (13). The type II IFN family consists of single gene product, IFNγ, which is predominantly produced by T cells and natural killer cells, and can act on a broad range of cell types that express the IFNγ receptor (IFNγR) (14). The type III IFN family comprises IFNλ1, IFNλ2 and IFNλ3 (also known as IL-29, IL-28A and IL-28B, respectively) and the recently identified IFNλ4 (15), which have similar functions to cytokines of the type I IFN family but restricted activity, as the expressions of their receptor is largely restricted to epithelial cell surfaces (16). Indeed, immune cells are largely unresponsive to IFNλ (16,17).

IFNα and IFNβ (IFNα/β) are the best-defined and most broadly expressed type I IFNs. These are best known for their ability to induce an antiviral state in both virus-infected cells and uninfected, bystander cells, by inducing a program of gene transcription that interferes with multiple stages of the viral replication cycle through various mechanisms (18). However, IFN-I have numerous additional functions that influence the innate and adaptive immune responses not only to viruses but also to bacterial and other pathogens. The outcome of the IFN-I response during infectious disease is highly context dependent. Different conditions are induced during specific infections and affect when and where IFN-I signals are delivered, as well as the signalling pathways that are triggered downstream of the type I IFN receptor (IFNAR). This, in turn, influences which IFN-stimulated genes (ISGs) are activated or repressed, and lead to beneficial or detrimental outcomes for the host (19).

IFNAR is composed of two chains (IFNAR1 and IFNAR2) that coordinately activate the Janus Kinase 1 (Jak1) and Tyrosine kinase 2 (Tyk2) upon IFN-1 binding. Jak1 and Tyk2 phosphorylate STAT1 and STAT2 that, together with IRF9, form the interferon-stimulated gene factor 3 (ISGF3) complex (20). ISGF3 binds to interferon-stimulated response elements (ISRE) to cause upregulation of over 300 genes (21).

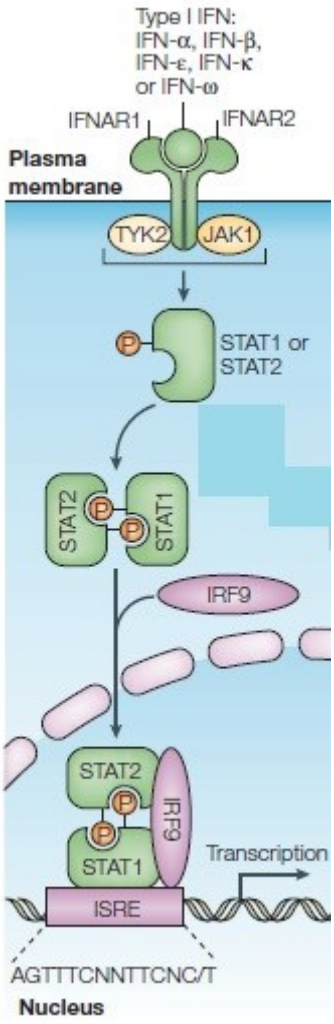

Figure A. Interferon receptors and activation of classical JAK-STAT pathways by type I interferon. Reproduced by permission from Macmillan Publishers Ltd: *Nature Reviews Immunology* (20), 2005.

2.2 Role in immune response

2.2.1 Role in viral infections

The consensus in the field is that IFNAR signaling is protective against most types of viral infection. In most cases, the absence of systemic IFNAR signaling in mice led to an increase in viral titer, lethality, or both compared with mice that keep that signaling (21). IFN-I is important in handling all major genetic classes of viruses including ssRNA, dsRNA and double-stranded DNA (dsDNA) viruses, and acute retroviruses (ssRNA-RT). The two exceptions to the strict requirement for IFNAR are influenza and

dengue virus infections (22). In the case of influenza virus and potentially other respiratory viruses, the type III interferon system (which comprises IFN-λ subtypes and signals using IL-10R2–IFNLR1) plays a dominant role in restricting acute epithelial cell infection, thereby limiting the requirement of IFN-I signaling (22). In dengue, IFN-γ-mediated protection is dominant over IFN-I, although the combined IFNAR–/– × IFNGR–/– mice are more susceptible than the IFNGR–/– mice (23). The effects of IFN-I that limit viral infection are extensive, but several aspects are important to consider: IFN-I signaling enhances the susceptibility of virally infected cells to undergo programmed cell death, thereby limiting viral replication (24, 25), DCs exposed to IFN-I become activated and secrete pro-inflammatory cytokines that lead to activation of the adaptive immune response (26), Natural killer cells become potent killers of virally infected cells (27, 28). In addition, IFN-I has direct effects on adaptive αβ T- cells and sensitizes them to activation via the TCR (29, 30). IFN-I production by pDCs promotes B-cell activation and production of antiviral antibodies (31, 32). In general, these effects are beneficial to the host, as they lead to control of viral replication and spread, but viruses have evolved extensive immune-evasion strategies many of which center around inhibition of the host IFN-I response (33, 34). However, the biological responses to IFN-I do not always lead to beneficial outcomes for the host. In the case of viral infections, the best studied examples of the negative role of IFN-I are chronic viral infections, in particular infection with Lymphocytic Choriomeningitis Virus (LCMV) (35). Mice become chronically infected with LCMV because of T-cell 'exhaustion' that prevents normal clearance (36). Several factors have been implicated. Most salient among them are IL-10 and programmed cell death 1 (PD-1). IL-10 is known to antagonize inflammatory activation on multiple immune cell types and its neutralization prevents chronic infection with LCMV (37). PD-1, a member of the CD28/CTLA4 family of T-cell regulators, is upregulated on exhausted T cells found in chronically infected mice. Its ligands, PD-1L and PD-2L, are broadly expressed and inducible by interferons (38). The interaction of PD-1 with PD-L1 acts to limit T-cell activity during chronic infection. Blockade of the PD-1–PD-L1 interaction using mAbs derepresses CD8 T-cell activity and leads to enhanced adaptive immune responses to LCMV infection (39). In two recent publications, the effects of IL-10 and PD-1 in limiting the response to LCMV infection have been causally linked to IFN-I signaling (40, 41). During the initial stages of infection with a chronically infecting strain (CL-13), the absence of IFN-I signaling allows for an increased viral titer and delayed clearance

during the acute phase of the response (42, 43). Wild-type mice control the primary infection well but become chronic carriers. IFNAR–/– mice also become chronic carriers albeit at higher viral loads. The LCMV - CL13 strain induces higher levels of IFN-α and IFN-β than the acutely infective strain. The major early producers of IFN-I are pDCs when infected with the virus (40). The presence of IFN-I is associated with a prolonged signature of interferon-inducible genes in spleen cells (41). Despite having higher early titers of LCMV, IFNAR–/– mice show reduced IL-10 in serum and reduced PD-L1 expression in myeloid cells. In wild-type mice, blockade of IFNAR signaling with neutralizing mAbs replicates this effect. Furthermore, neutralization of IFNAR after the establishment of chronic infection leads to reduced viral burden (40). Therefore, IFNAR plays a major role in the establishment of chronic infection with LCMV and neutralization of IFNAR has therapeutic potential for mice harboring chronic viral infections.

2.2.2 New described role in bacterial infections

The response of IFNAR–/– mice to bacterial infections varies depending on the species and route of infection. In the majority of the infections produced by *Streptococcus*, *Escherichia coli*, and *Helicobacter*, IFNAR–/– mice have higher titers and/or lethality than wild-type mice controls. Aversely, during the majority of the infections produced by *Brucella*, *Francisella*, *Salmonella, Chlamydia, Mycobacterium*, or *Yersinia*, IFNAR–/– mice control infection better than wild-type mice. Also, the IFNAR–/– mice are more resistant to *L. monocytogenes,* given systemically. Based on these initial studies, the simplest conclusion is that IFNAR signaling is beneficial during extracellular bacterial infection (replication occurs outside host cell) and detrimental during intracellular bacterial infection (ability to survive and multiply inside the host cell) (21).

2.2.2.1 Extracellular bacteria

A protective role for IFN-I has also been reported in mouse models of Group B *Streptococcus* (GBS), *Streptococcus pneumoniae, E. coli, Helicobacter pylori* and *Streptococcus pyogenes* infections (44-47). In all these models, IFNAR1–/– mice had a shorter survival and/or more bacterial growth than wild-type mice controls. In the case

of the immune response against GBS, *E. coli* and *S. pneumoniae*, IFN-I mediated signaling contributed to the optimal activation of macrophages, because of their ability to produce TNF and NO, although plasmatic levels of TNF and IL-6 during *in vivo* infection were much higher in IFNAR1–/– mice than in wild-type mice controls. This may reflect greater inflammation as a result of the higher bacterial burden in the knockout mice or may reflect multiple effects of IFN-I at the systemic level versus the local level (45). In addition, the importance of the correct recruitment of host-protective phagocytic cells by IFN-I dependent chemokine production has been highlighted by results from a caecal ligation and puncture model of infection. In this model, IFNAR1–/– mice presented a shorter survival and elevated bacteremia compared to wild-type mice. These differences were associated with decreased levels of the chemokine CXCL10 and with reduced neutrophil numbers and function. Treatment of IFNAR1–/– mice with recombinant CXCL10 rescued them from fatal infection and restored neutrophil function. Conversely, during subcutaneous *S. pyogenes* infection, IFNAR1–/– mice had increased tissue damage and presented a shorter survival after infection than wild-type mice, and these were associated with uncontrolled neutrophilia, although whether neutrophils had a detrimental role in this case was not confirmed (48). However, a recent study has demonstrated that the development of primary invasive pneumococcal disease caused by serotype 1 pneumococcus is driven by early increased IFN-I response, in the lung (49). In this study, also, they have reported a strain de pendent variation in invasive potential, even within the same serotype. Moreover, while the highly virulent strains could survive in the lungs and invade the blood of mice following intranasal challenge, the noninvasive strains were cleared from the lungs and were not detected in the blood. These results suggest that the difference between both groups of strains was the ability of the invasive ones to survive in the lungs and translocate to the blood, and the pleural cavity acts as the duct through which pneumococci pass from the lungs to the blood (50, 51). In addition, following treatment with anti-IFNAR1 antibodies, a significant decrease in bacterial invasion of the pleural cavity was observed, compared to treatment with the isotype control. So, it reflects a strong induction of an IFN-I response by the invasive strain that leads to rapid invasion of the pleural cavity with subsequent development of severe bacteremia that is absent from mice challenged with the noninvasive one. Suppression of this response in animals challenged with the invasive strain delays the initial invasion of the pleural cavity. These results, contrast with the information proposed above, which attributed a

protective role to IFN-I in infections by *S.pneumoniae*. Interestingly, the effect of IFN-I induction in response to infection is dependent on the strength of the response induced, which varies in a strain-dependent manner (52). Interestingly, an experiment that consisted in the intranasal administration of Poly I:C (a synthetic compound that has been shown to activate TLR3, RIG-I and MDA5) in a murine model of pulmonary infection, followed by intratracheal challenge with *S.pneumoniae*, has shown that Poly I:C, like influenza infection, impairs clearance of pneumococcus (255). It is though that IFN-I appear to mediate, at least in part, the deleterious effect, since this cytokine represents the immune response to influenza. Thus, IFN-1 contributes to the clearance of the virus by the host (255).

2.2.2.2 Intracellular bacteria

The first example of the detrimental effect of IFN-I was discovered following *Listeria monocytogenes* infection (21). Macrophages infected with these bacteria induce expression of IFN-I that is dependent on bacterial expression of the pore-forming toxin listeriolysin O (LLO) (53, 54). LLO is important for the bacterial egress from the nascent phagosome to the cytosol (55), but alone it does not induce strong levels of IFN-I by the infected macrophage, suggesting that the presence of cytosolic bacteria is the driver of IFN-I production (54). The apoptotic cell death of macrophages is enhanced by IFNAR signaling and is also dependent on LLO bacterial expression (56). Since LLO is essential for virulence, it is unclear if it has a direct role in killing the infected macrophage or if it is only important for allowing egress of the bacteria to the cytosol. IFNAR-dependent macrophage death do not occur in IFNAR–/– mice, being that the latest represent a longer survival, and lower bacterial loads in the liver and spleen after infection than wild-type mice, demonstrating a role for IFN-I in sensitization of those cells. It is not known at this time if the macrophages that die *in vivo* are infected by the bacteria. However, the most profound IFNAR-dependent effect seen in mice infected with *L. monocytogenes* is the extensive depletion of white-pulp lymphocytes via apoptotic cell death. This was also associated with lower levels of expression of IFN-inducible apoptosis-associated genes, such as *trail*, *p53* and death domain-associated protein 6 (DAP6; also known as DAXX) in infected IFNAR1–/– mice (57-59). Subsequent induction of immunosuppressive cytokines, particularly IL-10, after this large-scale apoptosis of lymphoid cells was suggested as the

mechanism by which lymphocyte apoptosis led to the IFN-I-dependent increase in susceptibility to infection (60).

Another important mechanism of host immunosuppression by IFN-I was elucidated by MacMicking. During infection with *L. monocytogenes*, the activation of macrophages by T cell-derived and/or NK cell-derived IFNγ is crucial for inducing antimicrobial pathways and for the subsequent eradication of the intracellular bacteria (65). Although IFN-I can induce some of these antimicrobial pathways in particular circumstances, it has now been shown that during infection with *L. monocytogenes*, IFN-I potently inhibit these pathways by blocking the responsiveness of macrophages to IFNγ. This block in responsiveness results from downregulation of *ifnγr* expression by macrophages (66), owing to silencing of new transcription from the gene encoding IFNγR by repressive transcriptional regulators (67).

Something similar occurs in the case of infection with *Mycobacterium tuberculosis*. The importance of IFN-I as a potentially detrimental factor during tuberculosis was suggested by studies of patient cohorts from the United Kingdom and South Africa. Patients with active tuberculosis had a prominent whole blood IFN-I-inducible transcriptional profile that correlated with the extent of radiographic disease and diminished with successful treatment (61). Several other studies have since confirmed these findings in additional patient cohorts from Africa (62, 63) and Indonesia (64), indicating that this IFN-I-inducible signature is broadly applicable to humans and may be detrimental.

The mechanisms that mediate the IFN-I-driven exacerbation of disease are not fully understood but seem to be multifactorial. *M. tuberculosis* strains initially suggested that the suppression of pro-inflammatory cytokines and TH1-type immunity are important (68-70), and there is good evidence both in human cells and in mouse models that IFN-I suppress the production of host-protective cytokines following this type of infection. Moreover, the production of IL-1α and IL-1β, which are crucial for host defence against *M. tuberculosis* (71), is inhibited by IFN-I, both *in vitro* infected human and mouse cells and *in vivo* in mouse models (72-75).

In conclusion, IFN-I may contribute to host protection against bacterial infection by upregulating antimicrobial effectors and pro-inflammatory cytokines. Conversely, IFN-I may impair the host response against bacteria by eliciting the production of IL-10 and IL-1 receptor antagonist, suppressing pro-inflammatory cytokine production, inducing immune cell death (including apoptosis) and restricting host responses to IFNγ (19).

2.2.3 Alternative induction pathways

The ability of multicellular organisms to preserve their integrity and to control growth of smaller organisms colonizing their surfaces depends on their ability to detect the entire universe of "microbial not self" (76). With the possible exception of erythrocytes, all cells of the body are equipped with a wide variety of receptors, encoded in the germline, which are able to sense the presence of evolutionary-conserved microbial substances. Such receptors are particularly abundant in cells of the innate immunity system, which patrol body sites and provoke protective responses, including inflammation and adaptive immunity responses (77).

PRRs mostly associated with the IFN-I induction are RIG-I, MDA5 as well as DNA sensors like DAI, DEAD box, DEAH box and cGAS, and NOD1 and NOD2. Inside the TLR family, TLR3, TLR4, TLR7, TLR8 and TLR9 can lead to IFN-I production. Pathways downstream of these receptors converge on the IRF family of transcription factors that activate the transcription of genes encoding IFN-I. IRF3 and IRF7 are the fundamental IRFs, being that *infβ* and *infα4* genes are induced in an initial wave that relies on IRF3. This IFN burst triggers the transcription of *irf7,* which mediates a positive feedback loop, leading to the induction of a second wave of gene transcription. This "strategy" seems to occur in all cells, with the exception of IFN producing cells (IPCs). Upstream of the IRFs, the kinases IκB kinase-ε (IKKε) and TANK-binding kinase 1 (TBK1) are in charge of the phosphorylation of IRF3 and IRF7. RNA sensors RIG-I and MDA5 depend on the adaptor mitochondrial antiviral signaling protein (MAVS) to activate TBK1, while stimulator of IFN genes (STING) is a fundamental mediator in the recognition of cytosolic DNA. TLR3 and TLR4 use the adaptor TRIF which activates TBK1, leading to the activation of IRF3. In the case of TLR7 and TLR9, which are mostly expressed in pDCs, they transduce signal for IFN-I production through MyD88, and this production by pDCs is a consequence of constitutive expression of IRF7. Nuclear factor-κB (NF-κB) is also required as a cofactor, as it binds to the promoters of pro-inflammatory cytokines (Figure B).

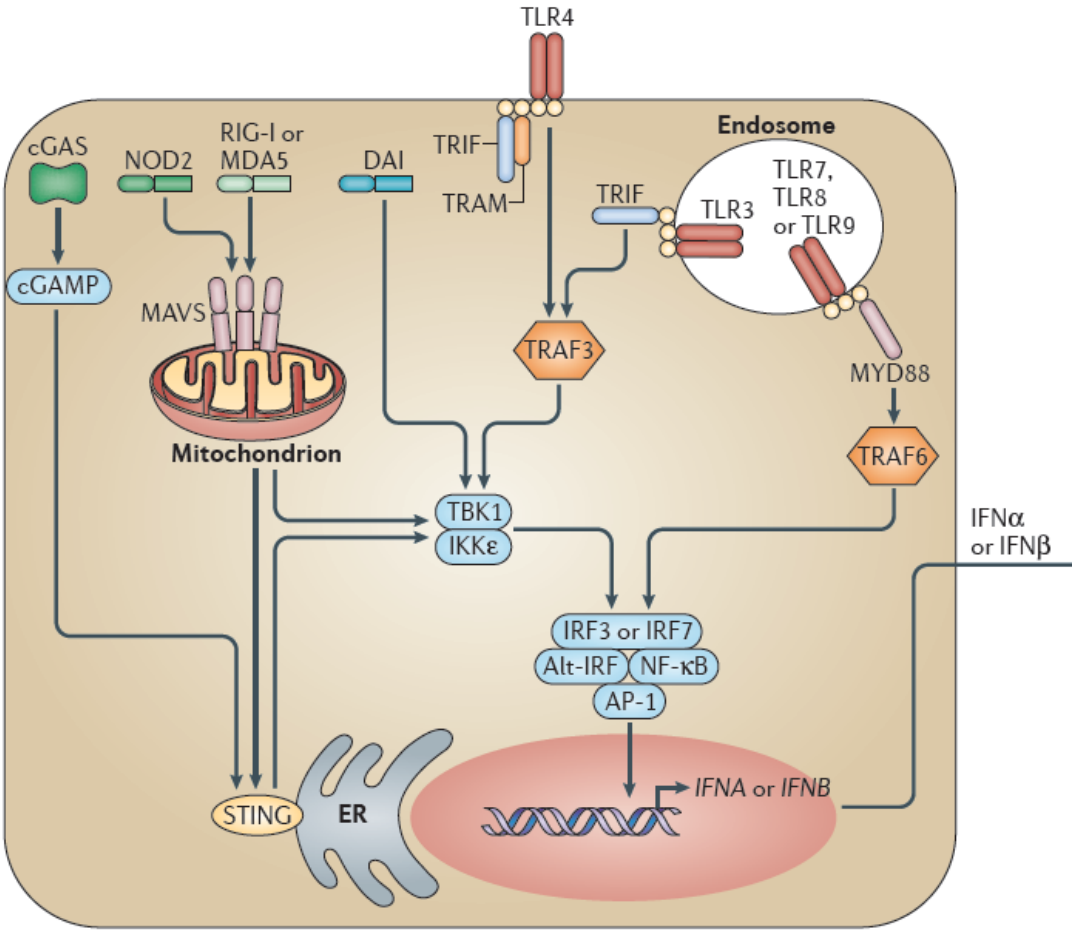

Figure B. Pathways of IFN-I induction. Reproduced by permission from Macmillan Publishers Ltd: *Nature Reviews Immunology* (19), 2015.

Studies with MyD88-deficient mice indicated that there is an additional adaptor allowing TLR4 and TLR3 signalling, called TIR-containing adaptor protein (TIRAP) (78, 79). However, when both the MyD88 and TIRAP adaptors are disrupted, signalling via TLR3 and TLR4 is not completely abrogated, IFN-β is still expressed (80). This lead to the discovery of a third adaptor protein called Toll/IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF), which was mentioned above (81), also known as Toll-interleukin 1 receptor domain (TIR)-containing adaptor molecule (TICAM)-1 (80).

The ability of intracellular TLRs to activate downstream signal transduction pathways, but not their ability to bind ligands, is acquired only in endolysosomes on cleavage of a major portion of their ectodomain by cathepsins (82). Conventional DCs (cDCs) can produce high quantities of IFN-β following bacterial degradation in

phagolysosomes, and such responses required a novel recognition pathway involving TLR7, the adaptor MyD88 and the transcription factor IRF1. It was also found that TLR9, in addition to TLR7, participates in activation of the novel IRF1-dependent bacterial recognition pathway, as suggested by significant reduction in IFN- β production in the absence of TLR9. Collectively these data indicate that TLR7 and TLR9 cooperate in bacterial recognition by recognizing bacterial RNA and DNA, respectively. Morover, it has been proved that blocking *in vitro,* with cytochalasin D, the internalization of GBS by DCs, the expression of IFN-β was totally abrogated, which suggests an internalization-dependent IFN-β production (83).

cDCs produced more IFN-β than did macrophages after bacterial stimulation. In macrophages, IFN-I responses occurred independently of the prevalent intracellular location of the stimulating pathogen and uniformly required IRF3 but not IRF1 or MyD88. In contrast, cDCs were able to use at least two distinct pathways to mount robust IFN responses to bacteria residing in cytosolic and phagosomal compartments. Responses to the former group of pathogens required IRF3, whereas responses to the latter group totally depended on IRF1 and MyD88 and partially depended on IRF7. The activation of distinct sets of transcription factors by cytosolic and phagosomal pathogens may provide further insight into the mechanisms with which the innate immune system responds to organisms residing in different locations. It is likely that by avoiding progression along the phagosomal pathway, some pathogens prevent the activation of IRF1, which promotes pro-inflammatory and often host-protective Th1 responses. Indeed, IRF1 but not IRF3 was required *in vivo* for defense against extracellular bacteria that are normally sequestered in the phagosomal pathway and are unable to escape into the cytosol (83).

The mechanisms underlying the unique ability of cDC to mount robust anti-bacterial IFN responses are presently unclear. It is possible that endophagosomal trafficking in these cells, which entails efficient phagocytosis and rapid translocation of ingested material to late, mature compartments, may facilitate the processing of prokaryotic organisms and subsequent presentation of their nucleic acids to endosomal TLRs (83). It is important to remark, that both pro-inflammatory cytokine and IFN-I responses to bacteria require phagocytosis, phagosomal acidification and receptor translocation to the endosomal compartment (84).

Studies with different extracellular bacteria confirm the distinct ability and “modus operandi” of the phagocytic cells. Particularly, there is not too much data about *S. suis*.

The study performed by Lachance *et al*., has demonstrated that expression levels of the type I IFN, IFN-β, but not IFN-α, are up-regulated *in vivo* following infection with *S. suis* serotype 2 (247). Moreover, this up-regulation of IFN-β was significantly higher in mice infected with an intermediate virulent ST25 strain than in those infected with either a virulent ST1 strain or the highly virulent ST7 strain responsible for a human outbreak (247). These results change the view about IFN-I, being that historically IFN-I responses were considered to be unique to viral infections.

Extracellular pathogen	Experimental conditions	Adaptor used	TLRs implicated	IRFs implicated	Reference
Streptococcus pyogenes	*In vitro* cDCs Mθ	100% MyD88 a) 50% MyD88 b) 50% TRIF	TLR7, TLR9 a)TLR7, TLR9 b)TLR3, TLR4	IRF5, IRF1 a)IRF5, IRF1 b)IRF3	(85)
GBS	*In vitro* cDCs Mθ	100% MyD88 a) 50% MyD88 b) 50% TRIF	TLR7, TLR9 a)TLR7, TLR9 b)TLR3, TLR4	IRF1, IRF7 a) IRF1, IRF7 b) IRF3	(83)
Salmonella typhimurium	*In vitro* Perithoneal Mθ	TRIF	TLR4	NT	(257)
Streptococcus suis	*In vivo* Total RNA was extracted from homogenates of spleen samples	NT	NT	IRF7, IRF1	(247)
Staphylococcus aureus	*In vitro* DCs	MyD88	TLR9	IRF1, IRF7	(256)

Table 2. Summary of IFN-1 induction by different extracellular bacteria, under distinct experimental conditions. cDCs: Bone Marrow conventional Dendritic Cells. Mθ: Bone Marrow Derived Macrophages. NT: Not Tested

3. Problematic, Hypothesis and Objectives of Master's project

As was mention above, the problematic that has motivated this work was the lack of data on the ability of different strains of *S. suis* serotype 2 to induce IFN-β, and the source of that cytokine. Moreover, the cellular receptors and pathways implicated in the IFN-β production during an infection by *S. suis* have barely been studied. In addition, the role of this cytokine in infections caused by *S. suis*, is not known.

The hypothesis was that IFN-β plays a protective role in infections caused by *S. suis* serotype 2. Moreover, based on the aforementioned effect of North American strains, it is further hypothesized that the increased production of this type I interferon by them would be responsible, at least in part, of their lower virulence.

The first specific objective of this Master research project was to study the *in vitro* induction of IFN-β by murine bone marrow-derived dendritic cells and macrophages activated by strains of *S. suis* serotype 2 from different backgrounds and virulence. Secondly, the assessment of the role of different cell receptors in the induction of IFN-β by *S. suis* was studied.

The last objective of this research consisted in studying the role of IFN-β during an *in vivo* infection with *S. suis* serotype 2. To do that, we use a murine model of infection, including IFNAR knockout mice and wild-type mice.

III - MATERIALS, METHODS AND RESULTS

Article: Type I Interferon Induced by *Streptococcus suis* Serotype 2 is Strain-Dependent and May Be Beneficial for Host Survival

Submitted to Frontiers in Immunology Journal

Type I Interferon Induced by *Streptococcus suis* Serotype 2 is Strain-Dependent and May Be Beneficial for Host Survival

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Running title: *Streptococcus suis*-induced type I interferon

Keywords: *Streptococcus suis* serotype 2; type I interferon; dendritic cells; inflammation; virulence

Abstract

Streptococcus suis serotype 2 is an important porcine bacterial pathogen and emerging zoonotic agent mainly responsible for sudden death, septic shock, and meningitis, with exacerbated inflammation being a hallmark of the infection. However, serotype 2 strains are genotypically and phenotypically heterogeneous, being composed of a multitude of sequence types (ST) whose virulence greatly varies: the virulent ST1 (Eurasia), highly virulent ST7 (responsible for the human outbreaks in China), and intermediate virulent ST25 (North America) are the most important worldwide. Even though type I interferons (IFNs) are traditionally associated with important anti-viral functions, recent studies have demonstrated that they may also play an important role during infections with extracellular bacteria. Up-regulation of IFN-β levels was previously observed in mice following infection with this pathogen. Consequently, the implication of IFN-β in the *S. suis* serotype 2 pathogenesis, which has always been considered a strict extracellular bacterium, was evaluated using strains of varying virulence. This study demonstrates that intermediate virulence strains are significantly more susceptible to phagocytosis than virulent strains. Hence, subsequent localization of these strains within the phagosome results in recognition of bacterial nucleic acids by Toll-like receptors 7 and 9, leading to activation of the interferon regulatory factors 1, 3, and 7 and production of IFN-β. Type I IFN, whose implication depends on the virulence level of the *S. suis* strain, is involved in host defense by participating in the modulation of systemic inflammation, which is responsible for the clearance of blood bacterial burden. As such, when induced by intermediate, and to a lesser extent, virulent *S. suis* strains, type I IFN plays a beneficial role in host survival. The highly virulent ST7 strain, however, hastily induces a septic shock that cannot be controlled by type I IFN, leading to rapid death of the host. A better understanding of the underlying mechanisms involved in the control of inflammation and subsequent bacterial burden could help to develop control measures for this important porcine and zoonotic agent.

1. Introduction

Streptococcus suis is an important porcine bacterial pathogen and emerging zoonotic agent mainly responsible for sudden death (pigs), septic shock (humans), and meningitis (both species) (1). Of the different described serotypes based on the presence of the capsular polysaccharide (CPS) or its respective genes, serotype 2 is regarded as not only the most widespread worldwide, but also the most virulent serotype, responsible for the majority of porcine and human cases of infection (2). However, serotype 2 strains are genotypically and phenotypically heterogeneous, being composed of a multitude of sequence types (STs), as determined by multilocus sequence typing, whose distribution greatly varies worldwide (2). Virulence of the most important STs (ST1, ST7, and ST25) has been evaluated using mouse models of infection (3, 4). Indeed, the ST7 strain responsible for the human outbreaks of 1998 and 2005 in China is highly virulent whereas European ST1 strains are virulent; on the other hand, ST25 strains, typically recovered in North America, are of intermediate virulence (3, 5).

Of the various virulence factors described for *S. suis*, the CPS, suilysin (SLY), and cell wall modifications have been demonstrated to play important roles (6, 7). Indeed, the CPS, which is antigenically identical for all serotype 2 strains, is a critical factor implicated in a multitude of functions, most importantly in resistance to phagocytosis by innate immune cells (8-12); its presence also masks bacterial surface proteins responsible for host cell activation (12, 13). Meanwhile, the SLY, a cholesterol-dependent cytolysin similar to the pneumolysin of *Streptococcus pneumoniae*, is responsible for causing cell toxicity and inducing pro-inflammatory cytokines (12, 14). This toxin is present in serotype 2 ST1 and ST7 strains, but not in ST25 strains (14, 15). Cell wall modifications, such as the D-alanylation of the lipoteichoic acid and N-deacetylation of the peptidoglycan, of a ST1 strain were shown to interfere with host defense and to be partially responsible for cell activation (12, 16, 17). Finally, several cell-wall associated proteins, mainly reported for ST1 and ST7 strains, have also been described as critical virulence factors, though many of these remain controversial in the literature (7).

Recognition of *S. suis* by innate immune cells involves a multitude of membrane-associated and cytoplasmic receptors (6, 18). Of these, the Toll-like receptor (TLR)

pathway is implicated in recognition of *S. suis* by phagocytic cells, including dendritic cells (DCs) and macrophages (19). Abrogation of MyD88, the adaptor protein central to the TLR pathway, results in near complete lack of pro-inflammatory cytokine production *in vitro* following infection with *S. suis* (13, 20). Furthermore, being a classical extracellular pathogen, recognition of *S. suis* has been mostly associated with surface TLRs, where TLR2, in cooperation with TLR6, plays a predominant role (13, 20, 21). Pathogen recognition by TLRs classically results in the production of pro-inflammatory cytokines via the NF-κB or interferon (IFN) pathways (19). Pathways involved in activation of NF-κB by *S. suis* have been somewhat described in recent years (20, 22), while those regarding the IFN pathways are less known, having mainly focused on type II IFN (3, 23). Nonetheless, it was recently demonstrated that expression levels of the type I IFN, IFN-β, but not those of IFN-α, are up-regulated *in vivo* following infection with *S. suis* serotype 2 (3). Moreover, this up-regulation of IFN-β was significantly higher in mice infected with an intermediate virulent ST25 strain than in those infected with either a virulent ST1 strain or the highly virulent ST7 strain responsible for the human outbreaks (3). However, no other study has addressed the production of type I IFN or its role during the *S. suis* serotype 2 infection.

Type I IFN regroups various members of the IFN family of which IFN-α, composed of sixteen different subtypes, and IFN-β, its most potent member, are the best characterized (24). Classical production of these cytokines is the result of endosomal TLR (TLR3, TLR7, and TLR9 in mice) activation, which leads to phosphorylation and translocation of interferon regulatory factors (IRFs) to the nucleus (25). Nonetheless IRF1 and IRF3/IRF7 are usually associated with type II and type I IFN, respectively, all three can result in transcription of type I IFNs (24, 25). Following their production, both IFN-α and IFN-β bind a common heterodimeric receptor, the IFN-αβ receptor (IFNAR) (25). Binding to this receptor activates the JAK/STAT pathway, transcription of various genes associated with host defense, and modulation of the inflammatory response (25).

Even though type I IFNs are traditionally associated with important anti-viral functions, recent studies have demonstrated that they may also play an important role, particularly for IFN-β, during bacterial infections, including pathogenic streptococci (25, 26). However, their role, whether beneficial or detrimental, may depend on the bacterial species and/or infection models (27-31). As aforementioned, little information is

available regarding type I IFN, and more specifically IFN-β, during the *S. suis* infection, which is considered a strict extracellular pathogen. Consequently, its implication in the *S. suis* serotype 2 pathogenesis was evaluated using strains of varying virulence. Herein, we demonstrated that following phagocytosis by DCs, to which intermediate virulence strains are more susceptible, *S. suis* is located within the phagosome where bacterial nucleic acids are recognized by TLR7 and TLR9, leading to activation of IRF1, IRF3, and IRF7 and production of IFN-β. When induced by intermediate, and to a lesser extent, virulent *S. suis* strains, but not by a highly virulent strain, type I IFN plays a beneficial role, being involved in the control of blood bacterial burden via modulation of systemic inflammation.

2. Materials and Methods

2.1. Ethics statement

This study was carried out in accordance with the recommendations of the guidelines and policies of the Canadian Council on Animal Care (CCAC) and the principles set forth in the Guide for the Care and Use of Laboratory Animals. The protocols and procedures were approved by the Animal Welfare Committee of the University of Montreal (protocol number 15- Rech-1570).

2.2. Endotoxin-free conditions

Endotoxin (lipopolysaccharide)-free material and solutions were used for bacterial and cell cultures work throughout this study.

2.3. *Streptococcus suis* serotype 2 strains and growth conditions

The different well-encapsulated *S. suis* serotype 2 strains, belonging to the most important STs (ST1, ST7, and ST25), and isogenic mutants used in this study are listed in **Table 1**. A highly virulent ST7 strain, isolated during the 2005 human outbreak in China (SC84) (32), a virulent archetypal European ST1 strain (P1/7), and an intermediate virulent North American ST25 strain (89-1591) (3), were used throughout

this study. Isogenic mutants were derived from P1/7 (ST1) or a genotypically and phenotypically similar strain, 31533 (ST1), also included in this study. For comparison purposes, two additional intermediate virulent ST25 strains (91-1804 and LPH4) were used in selected experiments. Virulence of the wild-type strains was previously reported (3, 4). *S. suis* strains were grown in Todd Hewitt broth (THB; Becton Dickinson, Mississauga, ON, Canada) as previously described (10), diluted in culture medium before experiments, and the number of colony-forming units (CFU)/mL in the final suspension was determined by plating on THB agar.

2.4. Mice

MyD88$^{-/-}$ (B6.129P2(SJL)-*MyD88*$^{tm1.Defr}$/J), TLR2$^{-/-}$ (B6.129-*Tlr2*^{tmKir}/J), TLR3$^{-/-}$ (B6;129S1-*Tlr3*^{tm1Flv}/J), TLR4$^{-/-}$ (B6.B10ScN-*Tlr4*$^{lps-del}$/JthJ), TLR7$^{-/-}$ (B6.129S1-*Tlr7*^{tm1Flv}/J), TLR9$^{-/-}$ (C57BL/6J-*Tlr9*^{M7Btlr}/Mmjax), IRF1$^{-/-}$ (B6.129S2-*Irf1*^{tm1Mak}/J), IRF3$^{-/-}$ (33), IRF7$^{-/-}$ (34), and IFNAR1$^{-/-}$ (B6.129S2-*Ifnar1*^{tm1Agt}/Mmjax) mice on C57BL/6 background were housed under specific pathogen-free conditions alongside their wild-type counterparts (C57BL/6J). Mice were purchased from Jackson Research Laboratories (Bar Harbor, ME, USA), with the exception of IRF3$^{-/-}$ and IRF7$^{-/-}$ mice, which were provided by Dr. K. Mossman (Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada).

2.5. Generation of bone marrow-derived dendritic cells and macrophages

The femur and tibia of wild-type and knock-out mice were used to generate bone marrow-derived dendritic cells (DCs) and macrophages, as described previously (12, 35). Briefly, hematopoietic bone marrow cells were cultured in RPMI-1640 medium supplemented with 5% (DCs) or 10% (macrophages) heat-inactivated fetal bovine serum, 10 mM HEPES, 2 mM L-glutamine, and 50 µM 2-mercaptoethanol (Gibco, Burlington, ON, Canada). Complete medium was complemented with 20% granulocyte macrophage-colony stimulating factor from mouse-transfected Ag8653 cells (35) for DCs or L929 cell-derived macrophage-colony stimulating factor for macrophages (36). Cell purity was at least 85% CD11c^{+} and F4/80^{+} cells for DCs and macrophages, respectively (12).

2.6. *S. suis* infection of dendritic cells and macrophages

Cells were resuspended at $1x10^6$ cells/mL in complete medium and stimulated with the different strains of *S. suis* serotype 2 listed in **Table 1** (10^6 CFU/mL; initial multiplicity of infection = 1). The conditions used were based on those previously published (12, 20). Cells were harvested in TRIzol (Invitrogen, Burlington, ON, Canada) for mRNA expression 3 h, 6 h or 12 h following infection, and supernatants collected for cytokine measurement 16 h (tumor necrosis factor (TNF), interleukin (IL)-6, IL-12p70, C-C motif chemokine ligand (CCL) 2, CCL3, and C-X-C motif chemokine ligand (CXCL) 1 or 24 h (IFN-β) post-infection (p.i.). Non-infected cells served as negative controls. For neutralization of TLR9, DCs were pretreated with 5 μM ODN2088 (TLR9 inhibitor; InvivoGen, Burlington, ON, Canada) or 5 μM ODN2088-control (Ctrl) for 1 h prior to infection with *S. suis*. Different TLR ligands were used to stimulate cells as positive controls: 1 μg/mL PAM3CSK4 (TLR1/2; InvivoGen), 1 μg/mL FSL-1 (TLR2/6; InvivoGen), 10 μg/mL poly(I:C) (TLR3; Novus Biologicals, Littleton, CO, USA), 100 ng/mL ultrapure *Escherichia coli* lipopolysaccharide (TLR4; InvivoGen), 5 μg/mL imiquimod (TLR7; Novus Biologicals), and 1 μM CpG ODN1826 (TLR9; InvivoGen).

2.7. Determination of cell mRNA expression by quantitative RT-PCR (RT-qPCR)

Cell mRNA was extracted according to the manufacturer's instructions (TRIzol) and cDNA generated using the Quantitect cDNA Synthesis Kit (Qiagen, Mississauga, ON, Canada). Real-time qPCR was performed on the CFX-96 Rapid Thermal Cycler System (Bio-Rad, Hercules, CA, USA) using 250 nM of primers (Integrated DNA technologies, Coralville, IA, USA) and SsoFast Evagreen Supermix Kit (Bio-Rad). The cycling conditions were 3 min of polymerase activation at 98°C, followed by 40 cycles at 98°C for 2 sec and 57°C for 5 sec. Melting curves were generated after each run to confirm the presence of a single PCR product. The sequences of primers used in this study are shown in **Supplementary Table 1 (in Supplementary Material)** and were verified to have reaction efficiencies between 90 % and 110 %. The housekeeping genes *Atp5b* and *Gapdh* were determined to be the most stably expressed and used as reference genes to normalize the data. Fold changes in gene expression were calculated using the

quantification cycle threshold (Cq) method using the CFX software manager v.3.0 (Bio-Rad). Samples from mock-infected cells served as calibrators.

2.8. Cytokine quantification in cell culture supernatants

Levels of IFN-β, TNF, IL-6, IL-12p70, CCL2 (MCP-1), CCL3 (MIP-1α), and CXCL1 (KC) in cell culture supernatants were measured by sandwich ELISA using pair-matched antibodies from BioLegend (Burlington, ON, Canada) for IFN-β and from R&D Systems (Minneapolis, MN, USA) for the other cytokines, according to the manufacturer's recommendations.

2.9. Phagocytosis assays

Cells were infected with the different *S. suis* strains and phagocytosis was left to proceed for different times (0.5 to 4 h) at 37°C with 5% CO_2. Multiplicity of infection and assay conditions were chosen based on previous studies regarding the kinetics of *S. suis* phagocytosis by DCs (12). After incubation, penicillin G (5 mg/mL; Sigma-Aldrich, Oakville, ON, Canada) and gentamicin (100 mg/mL; Gibco) were directly added to the wells for 1 h to kill extracellular bacteria. Supernatant controls were taken in every test to confirm that extracellular bacteria were efficiently killed by the antibiotics. After antibiotic treatment, cells were washed three times and sterile water added to lyse the cells. Where required, cells were pretreated with either 5 μM cytochalasin D (Santa Cruz Biotech, Dallas, TX, USA), 8 μM dynasore (Sigma-Aldrich), 1 μM bafilomycin A1 (Santa Cruz Biotech) or their vehicle, DMSO (Sigma-Aldrich), for 45 min prior to infection with bacteria, and phagocytosis was allowed to proceed for 2 h. The number of CFU recovered per well was determined by plating viable intracellular bacteria on THB agar.

2.10. *S. suis* DNA and RNA preparation and transfection of cells

For bacterial RNA and DNA isolation, bacterial cultures were grown to mid-log phase. Total RNA was extracted using the Aurum Total RNA Mini Kit (Bio-Rad) according to the manufacturer's instructions, including treatment with DNase I. For DNA preparation, bacteria were harvested in 10 mM Tris, 1 mM EDTA, pH 8.0, and treated

with 10% SDS and 20 mg/mL proteinase K (Sigma-Aldrich) for 1 h at 37°C. DNA was isolated using phenol/chloroform/isoamyl alcohol (Sigma-Aldrich) (27). After isolation, bacterial DNA was treated with 10 mg/mL RNase A (Roche, Mississauga, ON, Canada) for 30 min at 37°C. DCs were transfected with 1 μg of RNA or DNA using DOTAP liposomal transfection agent (Sigma-Aldrich) as described for transfection of bacterial extracts (27, 37).

2.11. *S. suis* serotype 2 mouse model of infection

Six-week-old wild-type C57BL/6 and IFNAR$^{-/-}$ mice were used. Mice were acclimatized to standard laboratory conditions with unlimited access to water and rodent chow (38). These studies were carried out in strict accordance with the recommendations of and approved by the University of Montreal Animal Welfare Committee guidelines and policies, including euthanasia to minimize animal suffering, applied throughout this study when animals were seriously affected since mortality was not an endpoint measurement. The different *S. suis* serotype 2 strains, or the vehicle solution (sterile THB), were administered at a dose of 1x10^7 CFU by intraperitoneal inoculation to groups of 10 to 15 mice for survival and blood bacterial burden. Mice were monitored at least three times daily until 72 h p.i. and twice thereafter until 14 days p.i. Blood bacterial burden was assessed 12 h and 48 h p.i. by collecting 5 μL of blood from the caudal vein, appropriately diluting and plating on THB agar as described above. Blood bacterial burden was also measured prior to euthanasia.

2.12. Measurement of plasma (systemic) pro-inflammatory cytokine levels

Eight wild-type and IFNAR$^{-/-}$ mice per group were infected with each strain as described above and the blood collected 12 h p.i. by intracardiac puncture following euthanasia and stabilization with EDTA (Sigma-Aldrich) as previously described (3, 4). Plasma supernatants were collected following centrifugation at 10 000 x *g* for 10 min at 4ºC, and stored at -80ºC. Plasmatic concentrations of TNF, IL-6, IL-12p70, CCL2, CCL3, and CXCL1 were measured using a custom-made cytokine Bio-Plex Pro™ assay (Bio-Rad) according to the manufacturer's instructions. Acquisition was performed on

the MAGPIX platform (Luminex®) and data analyzed using the Bio-Plex Manager 6.1 software (Bio-Rad).

2.13. Transmission electron microscopy

Unless otherwise indicated, chemicals were purchased from Sigma-Aldrich. Bacteria were grown to mid-logarithmic phase and washed in 0.1 M cacodylate buffer, pH 7.3 (Canemco & Marivac, Canton de Gore, QC, Canada). The CPS was stabilized using specific antibodies as previously described (39). Anti- *S. suis* serotype 2 rabbit serum, produced as previously described (40), was used to gently resuspend bacteria. Next, cells were immobilized in 4% (w/v) agar in 0.1 M cacodylate buffer, pH 7.3. Pre-fixation was performed by adding 0.1 M cacodylate buffer, pH 7.3, containing 0.5% (v/v) glutaraldehyde and 0.15% (w/v) ruthenium red for 30 min. Fixation was performed for 2 h at room temperature with 0.1 M cacodylate buffer, pH 7.3, containing 5% (v/v) glutaraldehyde and 0.05% (w/v) ruthenium red. Post-fixation was carried out with 2% (v/v) osmium tetroxide in water overnight at 4˚C. Samples were washed with water every 20 min for 2 h to remove osmium tetroxide and dehydrated in an increasing graded series of acetone. Specimens were then washed twice in propylene oxide and embedded in Spurr low-viscosity resin (Electron Microscopy Sciences, Hatfield, PA, USA). Thin sections were post-stained with uranyl acetate and lead citrate and examined using a transmission electron microscope at 80 kV (Hitachi model HT7770, Chiyoda, Tokyo, Japan).

2.14. Statistical analyses

The software used was Graphpad Prism 6.01. Normality of data was verified using the Shapiro-Wilk test. Accordingly, parametric (unpaired t-test and one-way ANOVA) or non-parametric tests (Mann-Whitney rank sum test and one-way ANOVA on ranks), where appropriate, were performed to evaluate statistical differences between groups. Log-rank (Mantel-Cox) tests were used to compare survival between wild-type and $IFNAR^{-/-}$ mice. Each test was repeated in at least three independent experiments. $P < 0.05$ and $p < 0.01$ values were considered as statistically significant and highly significant, respectively.

3. Results

3.1 Strain- and cell type-dependent induction of IFN-β by *S. suis* serotype 2

It is already known that DCs and macrophages are important for IFN-β production during bacterial infections. Moreover, it was also shown that these cells produce high levels of other pro-inflammatory cytokines following infection with *S. suis* (12,13,20,41). Consequently, the capacity of these cells to produce IFN-β following infection with three different *S. suis* serotype 2 strains (highly virulent ST7 strain SC84, virulent ST1 strain P1/7, and intermediate virulent ST25 strain 89-1591) was evaluated. This cytokine was chosen as a representative of type I IFN since *S. suis* was previously demonstrated to up-regulate levels of IFN-β expression *in vivo*, but not those of IFN-α (3). Moreover, DCs and macrophages have been demonstrated to mainly produce IFN-β, but only low levels of IFN-α, following infection with pathogenic streptococci (27, 29, 37). As shown in **Fig. 1A**, DCs expressed low levels of IFN-β 3 h after infection with *S. suis* (**Fig. 1A**). However, expression levels quickly and significantly increased, peaking at 6 h ($p < 0.05$). Importantly, levels induced by the intermediate virulent ST25 strain 89-1591 were significantly higher ($p < 0.001$) than those induced by the two more virulent strains. These levels were not specific to 89-1591, since similar high levels were also obtained using two other intermediate virulent ST25 strains (**Figure S1 in Supplementary Material**). In contrast, IFN-β expression levels of *S. suis*-infected macrophages remained relatively low and unchanged, regardless of the incubation time and strain used (**Fig. 1B**). Indeed, IFN-β expression levels by macrophages were significantly lower than those by DCs and this, for all three strains tested ($p < 0.01$). A clear induction of IFN-β expression was detected with the positive control, poly(I:C), indicating that the low response observed with *S. suis* was a consequence of the stimulus rather than the cells (**Fig. 1B**).

In order to evaluate if these differences in expression between cell types were also observed at the protein level, IFN-β was measured in the supernatant of cells 24 h after infection by ELISA (**Fig. 1C**). Indeed, IFN-β mRNA expression and protein production correlated. Results demonstrated that only DCs produce important protein levels of IFN-β following infection with *S. suis* serotype 2, which were significantly higher than control cells ($p < 0.001$) (**Fig. 1C**). However, as with mRNA expression, the

intermediate virulent strain 89-1591 induced significantly higher protein levels of IFN-β by DCs than the other two *S. suis* strains ($p < 0.001$). Meanwhile, macrophages produced significant levels of IFN-β when stimulated with poly(I:C) ($p < 0.001$), but not following *S. suis* infection, confirming results observed at the transcriptional (mRNA) level (**Fig. 1C**). Based on these results, all subsequent experiments in this study were performed using DCs.

3.2. The presence of capsular polysaccharide interferes with *S. suis*-induced IFN-β expression by dendritic cells, while the suilysin (when present) is partially responsible for activation

Of the different described virulence factors for *S. suis* serotype 2, the presence of the CPS, SLY, and cell wall modifications have been reported to modulate and/or participate in cytokine production by DCs (12). Consequently, their role in the induction of IFN-β expression by DCs was evaluated using isogenic mutants (**Fig. 2**). Absence of the CPS in a ST1 strain resulted in a significant increase of IFN-β expression by DCs ($p < 0.001$), suggesting that its presence interferes with cell activation. On the other hand, absence of the SLY, which is a pore-forming toxin present in ST1 and ST7 strains, resulted in a significant decrease of IFN-β expression ($p < 0.01$). Meanwhile, cell wall modifications (D-alanylation of the lipoteichoic acid and the N-acetylation of the peptidoglycan) did not modulate *S. suis*-induced IFN-β by DCs. Interestingly, levels of IFN-β induced by the ST1 strain 31533 were very similar to those observed with the prototype strain P1/7.

3.3. Recognition of *S. suis* by the Toll-like receptor pathway is required for IFN-β induction in dendritic cells

The TLR pathway has been traditionally associated with IFN-β production following pathogen recognition by the endosomal TLRs (TLR3, TLR7, and TLR9 in mice) (25). However, being considered a classical extracellular pathogen, recognition of *S. suis* has been mostly associated with surface TLRs (TLR1, TLR2, and TLR6) (13, 20). Consequently, the role of the TLR pathway in *S. suis*-induced IFN-β by DCs was evaluated. In the absence of the adaptor protein MyD88, used by the majority of TLRs, a significant decrease of IFN-β expression by DCs ($p < 0.001$) was observed with the

three *S. suis* strains, corresponding to a near complete abrogation (**Fig. 3A**). This result suggests that *S. suis*-induced IFN-β expression by DCs is overwhelmingly MyD88-dependent since only 5 to 10% of expression remained independent of MyD88 (**Fig. 3A**).

Given the near complete MyD88-dependence of *S. suis*-induced IFN-β by DCs and the fact that the surface TLR2 (13, 20) and, possibly TLR4 via the SLY (42), may be implicated in recognition of this pathogen, their role in *S. suis*-induced IFN-β by DCs was evaluated. TLR2 was not implicated in IFN-β expression by DCs following *S. suis* infection, regardless of the strain used (**Fig. 3B**). Moreover, it was not possible to induce IFN-β expression following stimulation of wild-type DCs with PAM3CSK4 (for TLR1/2) and FSL-1 (for TLR2/6) (43), which are synthetic bacterial TLR2 ligands frequently used for most cell types (**Figure S2 in Supplementary Material**). However, TLR2 was involved in expression of the pro-inflammatory cytokines IL-6 and CXCL1 by DCs following infection with *S. suis* and stimulation by both TLR2 ligands (**Figure S3 in Supplementary Material**). These results demonstrate that though not capable of producing IFN-β following stimulation with control ligands (44), the cells remained responsive to TLR2-dependent *S. suis* stimulation. Surprisingly, TLR4 was partially implicated in IFN-β expression by DCs following infection with both the ST7 strain SC84 and the ST1 strain P1/7 ($p < 0.05$), which corresponded to a reduction of approximately 15%. TLR4 involvement was not observed with the ST25 strain 89-1591 (**Fig. 3B**). A notable difference between the ST1/ST7 and ST25 strains is the absence of SLY in the latter strain. As such, IFN-β expression by wild-type and TLR4$^{-/-}$ DCs was evaluated following infection with the SLY-deficient mutant (**Fig. 3C**). Indeed, the wild-type and SLY-deficient strains induced similar levels of IFN-β by TLR4$^{-/-}$ DCs, indicating that recognition of the SLY by TLR4 contributes to the induction of this cytokine.

Despite the fact that *S. suis* has been described as remaining extracellularly, the largely surface TLR-independence of *S. suis*-induced IFN-β expression by DCs suggested that endosomal TLRs might participate in its induction. In accordance with *S. suis*-induced IFN-β production by DCs being mostly MyD88-dependent, the TLR3, which is MyD88-independent and recruits TRIF, was only minimally implicated, and only following infection with the SLY-negative ST25 strain 89-1591 ($p < 0.05$) (**Fig. 3D**). Meanwhile, both TLR7 and TLR9 were responsible for IFN-β expression by DCs

following *S. suis* recognition, regardless of the strain (**Fig. 3D**). Indeed, their absence resulted in a 40 to 60% reduction of IFN-β expression, which was significantly lower when compared with expression by wild-type DCs ($p < 0.01$). In order to evaluate if recognition of *S. suis* by TLR7 and TLR9 was the result of a cooperative effect, DCs dually deficient for TLR7 and TLR9 were created by pretreating cells from either wild-type or TLR7$^{-/-}$ mice with the TLR9 antagonist ODN2088 or its control, ODN2088-Ctrl (**Fig. 3E**). Antagonizing wild-type DCs with ODN2088 resulted in a phenotype similar to that obtained using TLR9$^{-/-}$ DCs. Use of ODN2088-Ctrl on wild-type and TLR7$^{-/-}$ DCs confirmed the specificity of the treatment. When TLR7$^{-/-}$ DCs were antagonized with ODN2088, creating a dual TLR7$^{-/-}$/TLR9$^{-/-}$ phenotype, a greater decrease, resulting in nearly 80% abrogation of IFN-β expression by wild-type DCs ($p < 0.001$), was observed regardless of the strain. Results of IFN-β by DCs following stimulation with the different purified or synthetic TLR ligands are presented in **Figure S2 in Supplementary Material**.

3.4. The interferon regulatory factors 1, 3, and 7 play important though partially redundant roles in IFN-β expression by dendritic cells following *S. suis* infection

Activation of the TLR pathway via the endosomal TLRs usually leads to phosphorylation of IRF3/IRF7 and subsequent production of IFN-β (25). Moreover, though IRF1 is typically associated with induction of type II IFN, crosstalk within the cell and/or simultaneous activation of various cellular pathways may result in IRF1 phosphorylation leading to IFN-β induction (45). Consequently, given that TLR7 and TLR9 are the main TLRs responsible for *S. suis*-induced IFN-β expression by DCs, the expression levels of IRF1, IRF3, and IRF7 in DCs was determined (**Fig. 4A**). All three strains of *S. suis* induced up-regulation of both IRF1 and IRF7, but not IRF3, with no significant differences amongst strains. However, IRF7 expression was significantly more up-regulated than that of IRF1 ($p < 0.05$). Subsequently, in order to evaluate the role of IRF1, IRF3, and IRF7 in *S. suis*-induced IFN-β expression by DCs, cells isolated from wild-type and knock-out mice infected with the three strains of *S. suis* were evaluated. All three IRFs were significantly implicated in *S. suis*-induced IFN-β expression by DCs ($p < 0.01$), with reductions ranging between 40 and 70% (**Fig. 4B**).

3.5. *S. suis*-induced IFN-β by dendritic cells requires internalization and phagosome maturation

Previous studies with group A *Streptococcus* (GAS) and group B *Streptococcus* (GBS) have demonstrated that internalization of the pathogen and maturation of the phagosome are required for IFN-β production by DCs (27, 37). However, and differently from *S. suis*, these two pathogens are well-known to be internalized by phagocytes (46). Given that the endosomal TLR7 and TLR9 are implicated in *S. suis*-induced IFN-β expression by DCs, it was hypothesized that internalization could be a critical step, even for this classical extracellular pathogen whose CPS protects against phagocytosis (10-12, 47). However, no study has evaluated the capacity of DCs to internalize strains of *S. suis* other than ST1. Consequently, the kinetics of internalization of the three *S. suis* strains by DCs was evaluated. In accordance with previous studies (12), the well-encapsulated ST1 strain P1/7 was poorly internalized, even after 4 h of infection (**Fig. 5A**). Similar results were obtained with the ST7 strain SC84, for which information was previously unavailable. On the other hand, the intermediate virulent ST25 strain 89-1591, which induces the highest levels of IFN-β, was surprisingly and significantly more internalized by DCs than the ST1 and ST7 strains ($p < 0.01$). Since the CPS is the most important anti-phagocytic factor possessed by *S. suis* serotype 2, these results could have suggested that the strain 89-1591 was less encapsulated. However, it was possible to observe, using transmission electron microscopy, that the ST1 strain P1/7 (**Figure S4A in Supplementary Material**) and the ST25 strain 89-1591 (**Figure S4B in Supplementary Material**) are similarly well-encapsulated, suggesting that the greater internalization of strain 89-1591 by DCs is not the result of a thinner CPS.

In order to determine if *S. suis* internalization is indeed required for IFN-β expression by DCs, cells were pretreated with cytochalasin D, an inhibitor of actin polymerization, or its vehicle, DMSO. In the absence of actin polymerization, IFN-β expression was completely abrogated ($p < 0.001$) following infection with all three strains of *S. suis* (**Fig. 5B**). In contrast to IFN-β expression, actin polymerization was only partially implicated in DC expression of IL-6 and CXCL1 following infection with the *S. suis* strain P1/7 (**Figure S5 in Supplementary Material**).

Once internalized by phagocytes, the pathogen will find itself within the phagosome, which must undergo maturation (48). Amongst the different proteins involved in internalization is the GTPase dynamin, which is required in the case of coated endosomal vesicles (49). As such, dynamin frequently contributes to endosomal signaling of IFN-β (27, 50). Indeed, when inhibiting dynamin activity using the inhibitor dynasore, a near complete abrogation of IFN-β expression ($p < 0.001$) was observed (**Fig. 5C**). However, though dynamin was essential for *S. suis*-induced IFN-β expression by DCs, its role was not internalization-dependent since internalization levels of all three *S. suis* strains did not differ when inhibiting dynamin (**Fig. 5D**).

Following phagosome formation, destruction of the pathogen requires acidification of the compartment, which occurs following fusion with the lysosome (51). This fusion, resulting in the creation of the phagolysosome, leads to acidification of the vesicle in which vacuolar-type H^+ ATPases are implicated (52). Inhibition of these vacuolar-type H^+ ATPases using bafilomycin A1 resulted in a significant ($p < 0.001$) and near complete abrogation of *S. suis*-induced IFN-β expression by DCs (**Fig. 5C**). It should be noted that bafilomycin A1-treatment did not affect internalization of *S. suis* (**Fig. 5D**).

3.6. *S. suis* nucleic acids are responsible for inducing IFN-β expression by dendritic cells following endosomal delivery

The requirement of internalization and phagosome maturation in *S. suis*-induced IFN-β expression by DCs suggests that destruction of the pathogen within the phagolysosome is necessary. TLR7 and TLR9 recognize single-stranded RNA and unmethylated CpG motifs of DNA, respectively (53, 54). To evaluate this hypothesis, bacterial RNA and DNA, isolated from all three *S. suis* strains, were complexed with DOTAP liposomal transfection agent which allows for delivery within the phagosome. As shown in **Fig. 6**, both *S. suis* RNA (**Fig. 6A**) and DNA (**Fig. 6B**) induced significant levels of IFN-β in DCs ($p < 0.001$). Moreover, levels of IFN-β expression were similar between the different *S. suis* strains and between RNA and DNA.

3.7. *S. suis*-induced type I interferon by dendritic cells modulates autocrine cytokine production

Once produced, type I IFN, including IFN-β, will bind to its receptor, IFNAR, located on the surface of most cell types, including DCs (25). Consequently, type I IFN can modulate autocrine production of other inflammatory mediators. As such, the production of certain inflammatory cytokines known to be induced by *S. suis* (20), by DCs derived from wild-type and IFNAR$^{-/-}$ mice, was compared (**Fig. 7**). A significant role of type I IFN ($p < 0.01$) was observed in TNF, IL-6, IL-12p70, and CCL2 (**Fig. 7A-D**) production induced by the ST1 strain P1/7 and the ST25 strain 89-1591, but not by the ST7 strain SC84. On the other hand, no type I IFN-downstream modulation of CCL3 and CXCL1 production was observed for any of the strains tested.

3.8. Type I interferon is beneficial for host survival during the *S. suis* serotype 2 systemic infection: Implication in the modulation of systemic inflammation which controls blood bacterial burden

The *in vitro* production of IFN-β by DCs observed in this study, coupled with the up-regulation of this cytokine in mice infected with *S. suis* (3), suggests that IFN-β may be implicated in the balance and/or exacerbation of the systemic inflammation induced by this pathogen, and subsequently, host survival. Consequently, the role of type I IFN during the *S. suis* systemic infection caused by the three strains was evaluated using a well-standardized intraperitoneal C57BL/6 mouse model of infection comparing wild-type and IFNAR$^{-/-}$ mice (38). No role of type I IFN was observed in mouse survival during the systemic infection with the highly virulent ST7 strain SC84, with wild-type or IFNAR$^{-/-}$ mice, with both equally succumbing to the infection (**Fig. 8A**). Meanwhile, type I IFN played a significant role in the survival of mice infected with the virulent ST1 strain P1/7 ($p < 0.05$), with IFNAR$^{-/-}$ mice succumbing at a greater rate than their wild-type counterparts (45% of wild-type mice survived the systemic infection vs. only 10% of the IFNAR$^{-/-}$ mice) (**Fig. 8B**). Interestingly, no role of type I IFN was observed during early (first 72 h p.i.) systemic infection with the intermediate virulent ST25 strain 89-1591 (**Fig. 8C**), which induced high levels of IFN-β *in vitro*. However, given the lower virulence of this strain, which caused only 10 to 20% of mortality at 72 h p.i., this result is not entirely surprising. Less virulent *S. suis* strains are known to cause a delayed mortality, usually by meningitis, as a result of persistent bacteremia (4). As such, survival of mice was evaluated until 14 days p.i. (**Fig. 8D**). Four days p.i., mortality in the wild-type group increased but then remained stable until the end of the

experiment. On the other hand, IFNAR$^{-/-}$ mice were significantly more susceptible to the infection than their wild-type counterparts ($p < 0.01$) (**Fig. 8D**). Taken together, these results suggest that type I IFN plays a beneficial role during the *S. suis* infection, at least for the ST25 and, to a certain extent, the ST1 strains, but not with the ST7 strain.

Host death during the *S. suis* systemic infection is usually the result of uncontrolled blood bacterial burden resulting from excessive bacterial growth, concomitant with an exacerbated systemic inflammatory response (4, 55). As such, the role of type I IFN on aggravated inflammation was evaluated by measuring plasma (systemic) cytokines of both wild-type and IFNAR$^{-/-}$ mice 12 h p.i., as previously described (**Fig. 9**) (4, 38). For TNF, IL-6 , IL-12p70, CCL2, CCL3, and CXCL1 (**Fig. 9A-F**), a significant decrease in the levels produced by IFNAR$^{-/-}$ mice, in comparison to their wild-type counterparts, was only observed for the strains P1/7 and 89-1591 ($p < 0.05$), with the difference being more pronounced in mice infected with the strain 89-1591.

The second factor responsible for host death during the *S. suis* systemic infection is uncontrolled blood bacterial burden (4). No differences were observed between the acute blood bacterial burden of wild-type and IFNAR$^{-/-}$ mice early after infection (12 h), regardless of the strain (**Fig. 10A**). However, since differences in mortality only became important at later time points, the effect of type I IFN during the *S. suis* infection was possibly not immediate, but rather delayed. Indeed, blood bacterial burden of mice infected with the ST1 strain P1/7 and the ST25 strain 89-1591, but not of those infected with the ST7 strain SC84, was significantly higher in IFNAR$^{-/-}$ mice 48 h p.i. than in their wild-type counterparts ($p < 0.05$) (**Fig. 10B**). Consequently, type I IFN is implicated in the modulation of systemic inflammatory mediators required for control of blood bacterial burden.

4. Discussion

S. suis serotype 2, an important porcine and emerging human pathogen, has always been considered a prototypical extracellular bacterium whose CPS confers important anti-phagocytic properties (6, 18). Consequently, recognition of *S. suis* by the innate immune response was long thought to occur following interaction of bacterial

lipoproteins (or other cell wall components) with surface-associated receptors, in particular TLR2 (13, 20, 21, 56). More recently, however, partial implication of the endosomal TLR9 and cytoplasmic nucleotide-binding oligomerization domain-containing protein (NOD) 2 in recognition of *S. suis* by DCs was reported (20), suggesting that *S. suis* or its products might also activate cellular intracellular pathways (in-out signals). Nonetheless, the implications of such intracellular receptors in the pathogenesis of the infection caused by this bacterial pathogen were, so far, unknown.
Since *S. suis* was previously reported to up-regulate levels of IFN-β *in vivo* (3), but not IFN-α, the ability of DCs and macrophages, which are usually important sources of pro-inflammatory mediators, to produce IFN-β following *S. suis* infection was evaluated. In response to *S. suis*, DCs produced higher levels of IFN-β than macrophages, suggesting different activation levels and/or intrinsic differences in cytokine production by both cell types, resulting in part from the activation of varying signaling pathways and cascades (37). Since results obtained with positive controls revealed that DCs and macrophages are both able to induce high IFN-β levels, the differential activation of these two cell types by *S. suis* reflects an intrinsic feature of this pathogen, a characteristic than can be extended to other pathogenic streptococci (27, 37).

Lachance *et al*. suggested that *in vivo* levels of IFN-β were inversely associated with virulence of the strain used (3), a fact that was confirmed in this study with DCs, where the intermediate virulent ST25 strain 89-1591 induced higher levels of IFN-β than the virulent ST1 and ST7 strains. This inverse association is not a trait unique to the strain used, since two other intermediate virulent ST25 strains, as well as an additional ST1 strain (31533), presented results similar to their respective prototypical ST strains. To our knowledge, the inversed association of virulence with IFN-β induction has not been previously described for other bacterial pathogens. However, this association does not apply to strains of highly virulent to virulent phenotypes, as ST7 and ST1 strains induce similar levels of IFN-β.

The TLR pathway has been traditionally associated with IFN-β induction following recognition by the endosomal TLRs (25), yet, as aforementioned, recognition of *S. suis* has been mostly demonstrated to occur via surface TLRs (13, 20). *S. suis*-induced IFN-β by DCs was MyD88-dependent, indicating that the TLR pathway is almost exclusively implicated in its production. However, even though production of many *S. suis*-induced

pro-inflammatory cytokines by DCs has been reported to be mainly TLR2-dependent (20), we were unable to induce TLR2-dependent IFN-β expression by DCs. Though TLR2 activation can result in IFN-β production by macrophages, it was previously suggested that this is not the case for DCs (57, 58). On the other hand, a partial role of TLR4 was observed in IFN-β expression induced by ST1/ST7 strains, but not by ST25 strains: this activation was related to SLY production. This toxin was previously reported to be recognized by TLR4 expressed on peritoneal macrophages (42).

The limited contribution of surface TLRs to *S. suis*-induced IFN-β expression by DCs suggested that endosomal TLRs, of which TLR7 and TLR9 are MyD88-dependent (54), might participate in its induction. The involvement of endosomal TLRs in recognition of *S. suis* has been little evaluated since it has been considered an extracellular pathogen. Consequently, it was unexpected that the TLR7 and TLR9 were equally and primarily responsible for *S. suis*-induced IFN-β. Though TLR7, and to a lesser extent TLR9, were responsible for IFN-β production following recognition of GBS (37), no individual TLR could be identified for GAS (27). Even though *S. suis* and GBS share a similarity in this regard, the pathogenesis of these two encapsulated bacteria greatly differs: the most important difference between these two pathogens is that while *S. suis* is a classical extracellular bacterium protected from phagocytosis by its CPS, well-encapsulated GBS is highly internalized (46). In the case of GBS, IFN-β production by DCs was shown to be completely dependent on IRF1 and only partially IRF7-dependent (37). Interestingly, IRF1, IRF3, and IRF7 were all implicated in *S. suis*-induced IFN-β by DCs, suggesting a partial redundancy in signaling pathways, not observed for GBS. Indeed, localization of pathogens within the phagosome usually triggers IRF1 and IRF7 (37). Furthermore, IFN-β induced by TLR9 agonists results in IRF1 activation via a phagosome-dependent pathway (59). Participation of IRF3 may be the result of TLR3 or TLR4 activation by SLY-negative and SLY-positive strains, respectively, via a MyD88-independent, TRIF-dependent pathway (45). Feedback loops resulting from crosstalk between pathways could also be responsible for implication of IRF3 (34). In agreement with activation of these intracellular pathways, *S. suis*-induced IFN-β levels inversely correlate with strain-dependent capacity to resist phagocytosis. This hypothesis is supported by the significantly higher levels of IFN-β observed in this study when using the non-encapsulated ST1 mutant strain, which was previously demonstrated to be highly internalized by DCs (20), and by the complete abrogation of IFN-β induction following blockage of actin-dependent internalization. Interestingly,

non-encapsulated *S. suis* strains have traditionally been shown to increase cytokine induction by hindering recognition of surface cell wall components, responsible for cell activation by surface-associated receptors (12, 13, 20), mechanism that would not be involved in IFN-β modulation.

Previous studies with GAS and GBS have demonstrated that internalization of the pathogen and maturation of the phagosome are required for IFN-β production by DCs (27, 37). Results obtained in this study demonstrate that though dynamin is required for IFN-β production, this protein is implicated in early pre-acidification steps of phagosome maturation rather than in phagosome formation as evidenced by lack of effect on *S. suis* internalization. Subsequent acidification of the phagosome is required for IFN-β expression by DCs following infection with *S. suis*, suggesting that bacterial processing via hydrolytic degradation is essential for the liberation of TLR7 and TLR9 ligands (27, 37). These results indicate that bacterial nucleic acids were the ligands of TLR7 and TLR9. Indeed, both bacterial RNA, and to a lesser extent, DNA, from GAS and GBS are also responsible for IFN-β production by DCs following stimulation of TLR7 and TLR9 (27, 37). In contrast to GAS and GBS, however, the *S. suis* RNA and DNA induced similar levels of IFN-β, suggesting that both nucleic acids have comparable stimulatory effects. This is supported by the dual implication of TLR7 and TLR9 in *S. suis*-induced IFN-β production by DCs. Nucleic acids isolated from the three *S. suis* strains induced similar levels of IFN-β by DCs, indicating that regardless of virulence, the different *S. suis* strains possess similar stimulatory properties and differences observed would be mainly attributed to intracellular bacterial levels.

Once produced, type I IFN will bind to its receptor, IFNAR, located on the surface of most cell types, including DCs (25). This autocrine effect modulates *S. suis*-induced pro-inflammatory cytokines by DCs, as well as distal production of cytokines and chemokines by other cells as observed *in vivo* during *S. suis* infection. The IFNAR down-stream effect is complex as evidenced by a lack of effect on DC chemokine production at the single-cell level (60). Yet, when analyzing the global systemic response, the release of the chemokines CCL3 and CXCL1 is indeed modulated by the type I IFN pathway. This pathway was previously demonstrated to amplify TNF signaling following infection with GBS, *S. pneumoniae*, and *E. coli* (28). Pro-inflammatory cytokine signaling is the result of a cascade triggered by TNF, among

other mediators, leading to production of IL-6 and IL-12p70 (61). An amplification of these downstream cytokines by type I IFN is thus expected. Similarly, type I IFN was shown to modulate the recruitment of myeloid cells by influencing CCL2 signaling during infection with the intracellular pathogens *Listeria monocytogenes* and *Mycobacterium tuberculosis* (62, 63). Consequently, these results indicate a mechanism complementary to surface-associated receptor activation whereby higher internalization of *S. suis* leads to increased IFN-β induction and subsequent regulation of the pro-inflammatory loop. Our data also suggest that this IFN-β-modulated inflammatory response contributes to control bacterial burden during *S. suis* infection and improves the clinical outcome of infected animals.

It has been previously reported with other pathogens that the induction of IFN-β may be either beneficial or detrimental for the host, as shown using experimental infections. For example, a similar beneficial role was described for GAS (27), GBS (28, 37), and *S. pneumoniae* (28-30). On the other hand, the induction of a strong type I IFN response may also be considered a key factor in early progression of invasive *S. pneumoniae* beyond the lung during development of invasive pneumococcal disease (31). Moreover, type I IFN is associated with suppression of the innate response to certain bacterial infections, such as *L. monocytogenes* and *Francisella tularensis*, resulting in hindered bacterial clearance and deleterious host effects (45).

Taken together, type I IFN is produced by the host following *S. suis* infection and contributes to a regulated inflammatory response. In the case of the intermediate virulent ST25 strain, the elevated IFN-β production modulates systemic pro-inflammatory mediators and appears responsible for the decreased blood bacterial burdens, which ultimately results in a reduction of meningitis and increased host survival. Indeed, it was previously reported that persistent blood bacterial burden is a prerequisite for the development of *S. suis* meningitis (4). Albeit lower levels of IFN-β production, a beneficial effect is also noticed after infection with the virulent ST1 strain. In contrast, type I IFN is unable to counteract the exacerbated inflammatory response and/or bacterial burden induced by the highly virulent ST7 strain. This observation might be related to its genetic particularities, including the presence of a pathogenicity island, and its capacity to induce exaggerated inflammation unparalleled by other *S. suis*

strains, resulting in streptococcal toxic shock-like syndrome characterized by a cytokine storm (64-66).

In conclusion, this study demonstrates that, depending on the virulence level of the strain, type I IFN is involved in host defense during the *S. suis* infection by participating in clearance of blood bacterial burden and/or modulation of systemic inflammation. Results also showed that the lower virulence of the North American serotype 2 ST25 strains might be related to a lower resistance to phagocytosis that would lead to increased intracellular receptor activation with consequent IFN-β induction. Underlying mechanisms involved in the control of inflammation and subsequent bacterial burden could help to develop control measures for this important zoonotic infection.

5. Conflict of interest

The authors declare they have no conflict of interest.

6. Author contribution

Conceived and designed the experiments: JPA, MS, and MG. Performed the experiments: JPA, AS, and DR. Analyzed the data: JPA, MS, and MG. Provided research tools: KM and JX. Contributed to the writing of the manuscript: JPA, MS, and MG. All authors have read and approved the manuscript.

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8. Abbreviations

CCL, C-C motif chemokine ligand; CFU, colony-forming unit; CPS, capsular polysaccharide; CXCL, C-X-C motif chemokine ligand; DC, dendritic cell; GAS, group

A *Streptococcus*; GBS, group B *Streptococcus*; IFN, interferon; IFNAR, interferon-α/β receptor; IL, interleukin; IRF, interferon regulatory factor; p.i., post-infection; SLY, suilysin; ST, sequence type; THB, Todd Hewitt broth; TLR, Toll-like receptor; TNF, tumor necrosis factor.

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10. References

1. Gottschalk M, Xu J, Calzas C, Segura M. *Streptococcus suis*: a new emerging or an old neglected zoonotic pathogen? *Future Microbiol* (2010) **5**:371-91.

2. Goyette-Desjardins G, Auger JP, Xu J, Segura M, Gottschalk M. *Streptococcus suis*, an important pig pathogen and emerging zoonotic agent-an update on the worldwide distribution based on serotyping and sequence typing. *Emerg Microbes Infect* (2014) **3**:e45.

3. Lachance C, Gottschalk M, Gerber PP, Lemire P, Xu J, Segura M. Exacerbated type II interferon response drives hypervirulence and toxic shock by an emergent epidemic strain of *Streptococcus suis*. *Infect Immun* (2013) **81**:1928-39.

4. Auger JP, Fittipaldi N, Benoit-Biancamano MO, Segura M, Gottschalk M. Virulence studies of different sequence types and geographical origins of *Streptococcus suis* serotype 2 in a mouse model of infection. *Pathogens* (2016) **5**:48.

5. Ye C, Bai X, Zhang J, Jing H, Zheng H, Du H, et al. Spread of *Streptococcus suis* sequence type 7, China. *Emerg Infect Dis* (2008) **14**:787-91.

6. Fittipaldi N, Segura M, Grenier D, Gottschalk M. Virulence factors involved in the pathogenesis of the infection caused by the swine pathogen and zoonotic agent *Streptococcus suis*. *Future Microbiol* (2012) **7**:259-79.

7. Segura M, Fittipaldi N, Calzas C, Gottschalk M. Critical *Streptococcus suis* virulence factors: are they all really critical? *Trends Microbiol* (2017):In Press.

8. Chabot-Roy G, Willson P, Segura M, Lacouture S, Gottschalk M. Phagocytosis and killing of *Streptococcus suis* by porcine neutrophils. *Microb Pathog* (2006) **41**:21-32.

9. Benga L, Fulde M, Neis C, Goethe R, Valentin-Weigand P. Polysaccharide capsule and suilysin contribute to extracellular survival of *Streptococcus suis* co-cultivated with primary porcine phagocytes. *Vet Microbiol* (2008) **132**:211-9.

10. Segura M, Gottschalk M, Olivier M. Encapsulated *Streptococcus suis* inhibits activation of signaling pathways involved in phagocytosis. *Infect Immun* (2004) **72**:5322-30.

11. Segura MA, Cléroux P, Gottschalk M. *Streptococcus suis* and group B *Streptococcus* differ in their interactions with murine macrophages. *FEMS Immunol Med Microbiol* (1998) **21**:189-95.

12. Lecours MP, Gottschalk M, Houde M, Lemire P, Fittipaldi N, Segura M. Critical role for *Streptococcus suis* cell wall modifications and suilysin in resistance to complement-dependent killing by dendritic cells. *J Infect Dis* (2011) **204**:919-29.

13. Graveline R, Segura M, Radzioch D, Gottschalk M. TLR2-dependent recognition of *Streptococcus suis* is modulated by the presence of capsular polysaccharide which modifies macrophage responsiveness. *Int Immunol* (2007) **19**:375-89.

14. Tenenbaum T, Seitz M, Schroten H, Schwerk C. Biological activities of suilysin: role in *Streptococcus suis* pathogenesis. *Future Microbiol* (2016) **11**:941-54.

15. Fittipaldi N, Xu J, Lacouture S, Tharavichitkul P, Osaki M, Sekizaki T, et al. Lineage and virulence of *Streptococcus suis* serotype 2 isolates from North America. *Emerg Infect Dis* (2011) **17**:2239-44.

16. Fittipaldi N, Sekizaki T, Takamatsu D, de la Cruz Dominguez-Punaro M, Harel J, Bui NK, et al. Significant contribution of the *pgdA* gene to the virulence of *Streptococcus suis*. *Mol Microbiol* (2008) **70**:1120-35.

17. Fittipaldi N, Sekizaki T, Takamatsu D, Harel J, Dominguez-Punaro Mde L, Von Aulock S, et al. D-alanylation of lipoteichoic acid contributes to the virulence of *Streptococcus suis*. *Infect Immun* (2008) **76**:3587-94.

18. Segura M, Calzas C, Grenier D, Gottschalk M. Initial steps of the pathogenesis of the infection caused by *Streptococcus suis*: fighting against nonspecific defenses. *FEBS Lett* (2016) **590**:3772-99.

19. Kawasaki T, Kawai T. Toll-like receptor signaling pathways. *Front Immunol* (2014) **5**:461.

20. Lecours MP, Segura M, Fittipaldi N, Rivest S, Gottschalk M. Immune receptors involved in *Streptococcus suis* recognition by dendritic cells. *PLoS One* (2012) **7**:e44746.

21. Wichgers Schreur PJ, Rebel JM, Smits MA, van Putten JP, Smith HE. Differential activation of the Toll-like receptor 2/6 complex by lipoproteins of *Streptococcus suis* serotypes 2 and 9. *Vet Microbiol* (2010) **143**:363-70.

22. de Greeff A, Benga L, Wichgers Schreur PJ, Valentin-Weigand P, Rebel JM, Smith HE. Involvement of NF-kappaB and MAP-kinases in the transcriptional response of alveolar macrophages to *Streptococcus suis*. *Vet Microbiol* (2010) **141**:59-67.

23. Lecours MP, Letendre C, Clarke D, Lemire P, Galbas T, Benoit-Biancamano MO, et al. Immune-responsiveness of CD4+ T cells during *Streptococcus suis* serotype 2 infection. *Sci Rep* (2016) **6**:38061.

24. Monroe KM, McWhirter SM, Vance RE. Induction of type I interferons by bacteria. *Cell Microbiol* (2010) **12**:881-90.

25. McNab F, Mayer-Barber K, Sher A, Wack A, O'Garra A. Type I interferons in infectious disease. *Nat Rev Immunol* (2015) **15**:87-103.

26. Stetson DB, Medzhitov R. Type I interferons in host defense. *Immunity* (2006) **25**:373-81.

27. Gratz N, Hartweger H, Matt U, Kratochvill F, Janos M, Sigel S, et al. Type I interferon production induced by *Streptococcus pyogenes*-derived nucleic acids is required for host protection. *PLoS Pathog* (2011) **7**:e1001345.

28. Mancuso G, Midiri A, Biondo C, Beninati C, Zummo S, Galbo R, et al. Type I IFN signaling is crucial for host resistance against different species of pathogenic bacteria. *J Immunol* (2007) **178**:3126-33.

29. Parker D, Martin FJ, Soong G, Harfenist BS, Aguilar JL, Ratner AJ, et al. *Streptococcus pneumoniae* DNA initiates type I interferon signaling in the respiratory tract. *mBio* (2011) **2**:e00016-11.

30. LeMessurier KS, Hacker H, Chi L, Tuomanen E, Redecke V. Type I interferon protects against pneumococcal invasive disease by inhibiting bacterial transmigration across the lung. *PLoS Pathog* (2013) **9**:e1003727.

31. Hughes CE, Harvey RM, Plumptre CD, Paton JC. Development of primary invasive pneumococcal disease caused by serotype 1 pneumococci is driven by early increased type I interferon response in the lung. *Infect Immun* (2014) **82**:3919-26.

32. Ye C, Zhu X, Jing H, Du H, Segura M, Zheng H, et al. *Streptococcus suis* sequence type 7 outbreak, Sichuan, China. *Emerg Infect Dis* (2006) **12**:1203-8.

33. Sato M, Suemori H, Hata N, Asagiri M, Ogasawara K, Nakao K, et al. Distinct and essential roles of transcription factors IRF-3 and IRF-7 in response to viruses for IFN-alpha/beta gene induction. *Immunity* (2000) **13**:539-48.

34. Honda K, Taniguchi T. IRFs: master regulators of signalling by Toll-like receptors and cytosolic pattern-recognition receptors. *Nat Rev Immunol* (2006) **6**:644-58.

35. Segura M, Su Z, Piccirillo C, Stevenson MM. Impairment of dendritic cell function by excretory-secretory products: a potential mechanism for nematode-induced immunosuppression. *Eur J Immunol* (2007) **37**:1887-904.

36. Weischenfeldt J, Porse B. Bone marrow-derived macrophages (BMM): isolation and applications. *CSH protocols* (2008) **2008**:5080.

37. Mancuso G, Gambuzza M, Midiri A, Biondo C, Papasergi S, Akira S, et al. Bacterial recognition by TLR7 in the lysosomes of conventional dendritic cells. *Nat Immunol* (2009) **10**:587-94.

38. Dominguez-Punaro Mde L, Segura M, Radzioch D, Rivest S, Gottschalk M. Comparison of the susceptibilities of C57BL/6 and A/J mouse strains to *Streptococcus suis* serotype 2 infection. *Infect Immun* (2008) **76**:3901-10.

39. Jacques M, Gottschalk M, Foiry B, Higgins R. Ultrastructural study of surface components of *Streptococcus suis*. *J Bacteriol* (1990) **172**:2833-8.

40. Higgins R, Gottschalk M. An update on *Streptococcus suis* identification. *J Vet Diagn Invest* (1990) **2**:249-52.

41. Segura M, Vadeboncoeur N, Gottschalk M. CD14-dependent and -independent cytokine and chemokine production by human THP-1 monocytes stimulated by *Streptococcus suis* capsular type 2. *Clin Exp Immunol* (2002) **127**:243-54.

42. Bi L, Pian Y, Chen S, Ren Z, Liu P, Lv Q, et al. Toll-like receptor 4 confers inflammatory response to suilysin. *Frontiers in microbiology* (2015) **6**:644.

43. Kaisho T, Akira S. Regulation of dendritic cell function through Toll-like receptors. *Curr Mol Med* (2003) **3**:373-85.

44. Barbalat R, Lau L, Locksley RM, Barton GM. Toll-like receptor 2 on inflammatory monocytes induces type I interferon in response to viral but not bacterial ligands. *Nat Immunol* (2009) **10**:1200-7.

45. Trinchieri G. Type I interferon: friend or foe? *J Exp Med* (2010) **207**:2053-63.

46. Segura M. Fisher scientific award lecture - the capsular polysaccharides of group B *Streptococcus* and *Streptococcus suis* differently modulate bacterial interactions with dendritic cells. *Can J Microbiol* (2012) **58**:249-60.

47. Houde M, Gottschalk M, Gagnon F, Van Calsteren MR, Segura M. *Streptococcus suis* capsular polysaccharide inhibits phagocytosis through destabilization of lipid microdomains and prevents lactosylceramide-dependent recognition. *Infect Immun* (2012) **80**:506-17.

48. Gruenberg J, van der Goot FG. Mechanisms of pathogen entry through the endosomal compartments. *Nat Rev Mol Cell Biol* (2006) **7**:495-504.

49. Macia E, Ehrlich M, Massol R, Boucrot E, Brunner C, Kirchhausen T. Dynasore, a cell-permeable inhibitor of dynamin. *Dev Cell* (2006) **10**:839-50.

50. Stetson DB, Medzhitov R. Recognition of cytosolic DNA activates an IRF3-dependent innate immune response. *Immunity* (2006) **24**:93-103.

51. Kinchen JM, Ravichandran KS. Phagosome maturation: going through the acid test. *Nat Rev Mol Cell Biol* (2008) **9**:781-95.

52. Maxson ME, Grinstein S. The vacuolar-type H(+)-ATPase at a glance - more than a proton pump. *J Cell Sci* (2014) **127**:4987-93.

53. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell* (2006) **124**:783-801.

54. Akira S, Takeda K. Toll-like receptor signalling. *Nat Rev Immunol* (2004) **4**:499-511.

55. Dominguez-Punaro MC, Segura M, Plante MM, Lacouture S, Rivest S, Gottschalk M. *Streptococcus suis* serotype 2, an important swine and human pathogen, induces strong systemic and cerebral inflammatory responses in a mouse model of infection. *J Immunol* (2007) **179**:1842-54.

56. Draing C, Sigel S, Deininger S, Traub S, Munke R, Mayer C, et al. Cytokine induction by Gram-positive bacteria. *Immunobiology* (2008) **213**:285-96.

57. Stack J, Doyle SL, Connolly DJ, Reinert LS, O'Keeffe KM, McLoughlin RM, et al. TRAM is required for TLR2 endosomal signaling to type I IFN induction. *J Immunol* (2014) **193**:6090-102.

58. Hoshino K, Kaisho T, Iwabe T, Takeuchi O, Akira S. Differential involvement of IFN-beta in Toll-like receptor-stimulated dendritic cell activation. *Int Immunol* (2002) **14**:1225-31.

59. Schmitz F, Heit A, Guggemoos S, Krug A, Mages J, Schiemann M, et al. Interferon-regulatory-factor 1 controls Toll-like receptor 9-mediated IFN-beta production in myeloid dendritic cells. *Eur J Immunol* (2007) **37**:315-27.

60. Dorhoi A, Yeremeev V, Nouailles G, Weiner J, 3rd, Jorg S, Heinemann E, et al. Type I IFN signaling triggers immunopathology in tuberculosis-susceptible mice by modulating lung phagocyte dynamics. *Eur J Immunol* (2014) **44**:2380-93.

61. Cavaillon J-M, Adib-Conquy M. "The pro-inflammatory cytokine cascade". In: Marshall JC, Cohen J, editors. *Immune Response in the Critically Ill*. Berlin, Germany: Springer Berlin Heidelberg (2002). p. 37-66.

62. Antonelli LR, Gigliotti Rothfuchs A, Goncalves R, Roffe E, Cheever AW, Bafica A, et al. Intranasal poly(I:C) treatment exacerbates tuberculosis in mice through the pulmonary recruitment of a pathogen-permissive monocyte/macrophage population. *J Clin Invest* (2010) **120**:1674-82.

63. Jia T, Leiner I, Dorothee G, Brandl K, Pamer EG. MyD88 and type I interferon receptor-mediated chemokine induction and monocyte recruitment during *Listeria monocytogenes* infection. *J Immunol* (2009) **183**:1271-8.

64. Ye C, Zheng H, Zhang J, Jing H, Wang L, Xiong Y, et al. Clinical, experimental, and genomic differences between intermediately pathogenic, highly pathogenic, and epidemic *Streptococcus suis*. *J Infect Dis* (2009) **199**:97-107.

65. Yu H, Jing H, Chen Z, Zheng H, Zhu X, Wang H, et al. Human *Streptococcus suis* outbreak, Sichuan, China. *Emerg Infect Dis* (2006) **12**:914-20.

66. Holden MT, Hauser H, Sanders M, Ngo TH, Cherevach I, Cronin A, et al. Rapid evolution of virulence and drug resistance in the emerging zoonotic pathogen *Streptococcus suis*. *PLoS One* (2009) **4**:e6072.

67. Slater JD, Allen AG, May JP, Bolitho S, Lindsay H, Maskell DJ. Mutagenesis of *Streptococcus equi* and *Streptococcus suis* by transposon Tn917. *Vet Microbiol* (2003) **93**:197-206.

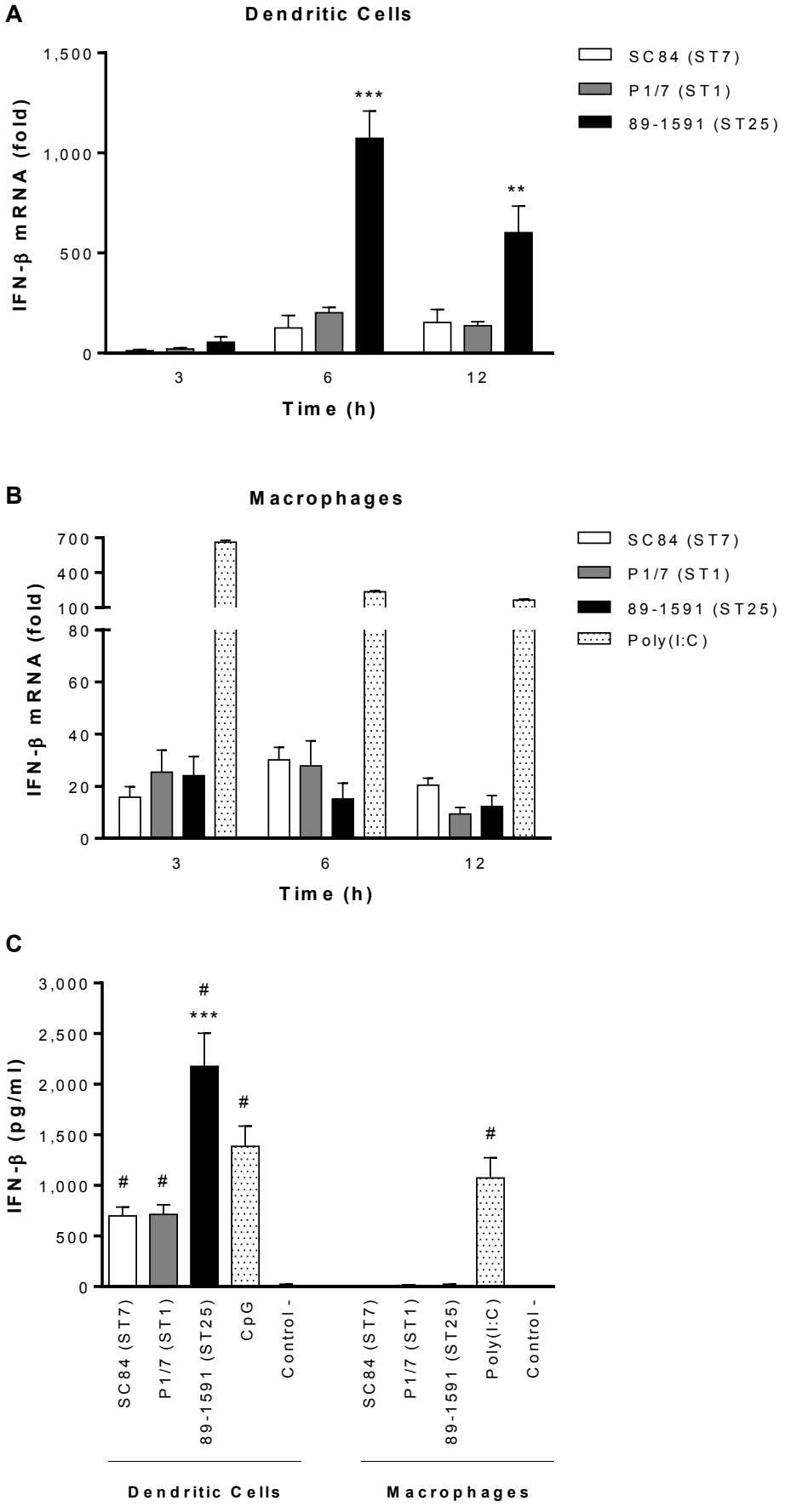
68. Kobisch M, Gottschalk M, Morvan M, Cariolet G, Bénévent G, Joly JP. Experimental infection of SPF piglets with *Streptococcus suis* serotype 2. *J Rech Porcine Fr* (1995) **27**:97-102.

69. Lun S, Perez-Casal J, Connor W, Willson PJ. Role of suilysin in pathogenesis of *Streptococcus suis* capsular serotype 2. *Microb Pathog* (2003) **34**:27-37.

70. Gottschalk M, Higgins R, Jacques M, Dubreuil D. Production and characterization of two *Streptococcus suis* capsular type 2 mutants. *Vet Microbiol* (1992) **30**:59-71.

71. Trottier S, Higgins R, Brochu G, Gottschalk M. A case of human endocarditis due to *Streptococcus suis* in North America. *Rev Infect Dis* (1991) **13**:1251-2.

72. Takamatsu D, Wongsawan K, Osaki M, Nishino H, Ishiji T, Tharavichitkul P, et al. *Streptococcus suis* in humans, Thailand. *Emerg Infect Dis* (2008) **14**:181-3.


A
Dendritic Cells
IFN-β mRNA (fold)
1,500
1,000
500
0
***
**
3
6
12
Time (h)
SC84 (ST7)
P1/7 (ST1)
89-1591 (ST25)
B
Macrophages
IFN-β mRNA (fold)
700
400
100
80
60
40
20
0
3
6
12
Time (h)
SC84 (ST7)
P1/7 (ST1)
89-1591 (ST25)
Poly(I:C)
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IFN-β (pg/ml)
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SC84 (ST7)
P1/7 (ST1)
89-1591 (ST25)
CpG
Control -
SC84 (ST7)
P1/7 (ST1)
89-1591 (ST25)
Poly(I:C)
Control -
Dendritic Cells
Macrophages

Figure 1. Dendritic cells (DCs) produce higher levels of IFN-β than macrophages following infection with *S. suis* serotype 2. IFN-β mRNA expression kinetics, measured by RT-qPCR, following infection of DCs **(A)** and macrophages **(B)**, with the highly virulent ST7 strain SC84, virulent ST1 strain P1/7, and intermediate virulent ST25 strain 89-1591. IFN-β protein production by DCs and macrophages was measured by ELISA 24 h following infection with the different *S. suis* strains **(C)**. Data represent the mean ± SEM from four independent experiments. ** ($p < 0.01$) and *** ($p < 0.001$) indicate a significant difference between 89-1591 and P1/7 or SC84; # ($p < 0.001$) between *S. suis* or CpG and the negative control (control -) for DCs or between poly(I:C) and negative control (control -) for macrophages.

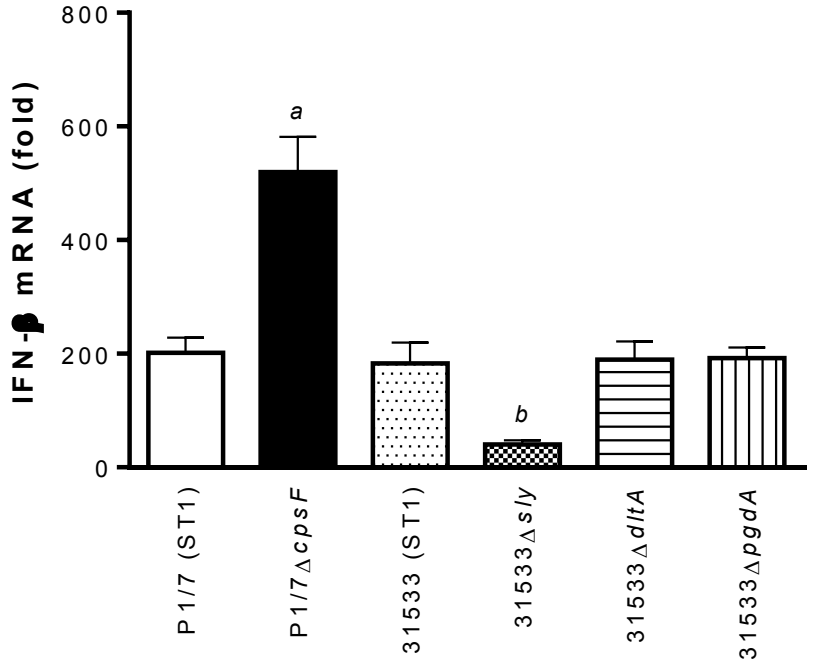

Figure 2. The presence of the capsular polysaccharide interferes with *S. suis*-induced IFN-β expression by dendritic cells, while the suilysin is partially responsible for its activation. Role of the capsular polysaccharide (CPS), suilysin (SLY), and cell wall modifications (D-alanylation of the lipoteichoic acid, Δ*dlta* or N-deacetylation of the peptidoglycan, Δ*pgdA*) in IFN-β mRNA expression by dendritic cells 6 h following infection with the wild-type or mutant *S. suis* strains. Data represent the mean ± SEM from four independent experiments. *a* ($p < 0.001$) indicates a significant difference between P1/7 and P1/7Δ*cpsF*; *b* ($p < 0.01$) between 31533 and 31533Δ*sly*.

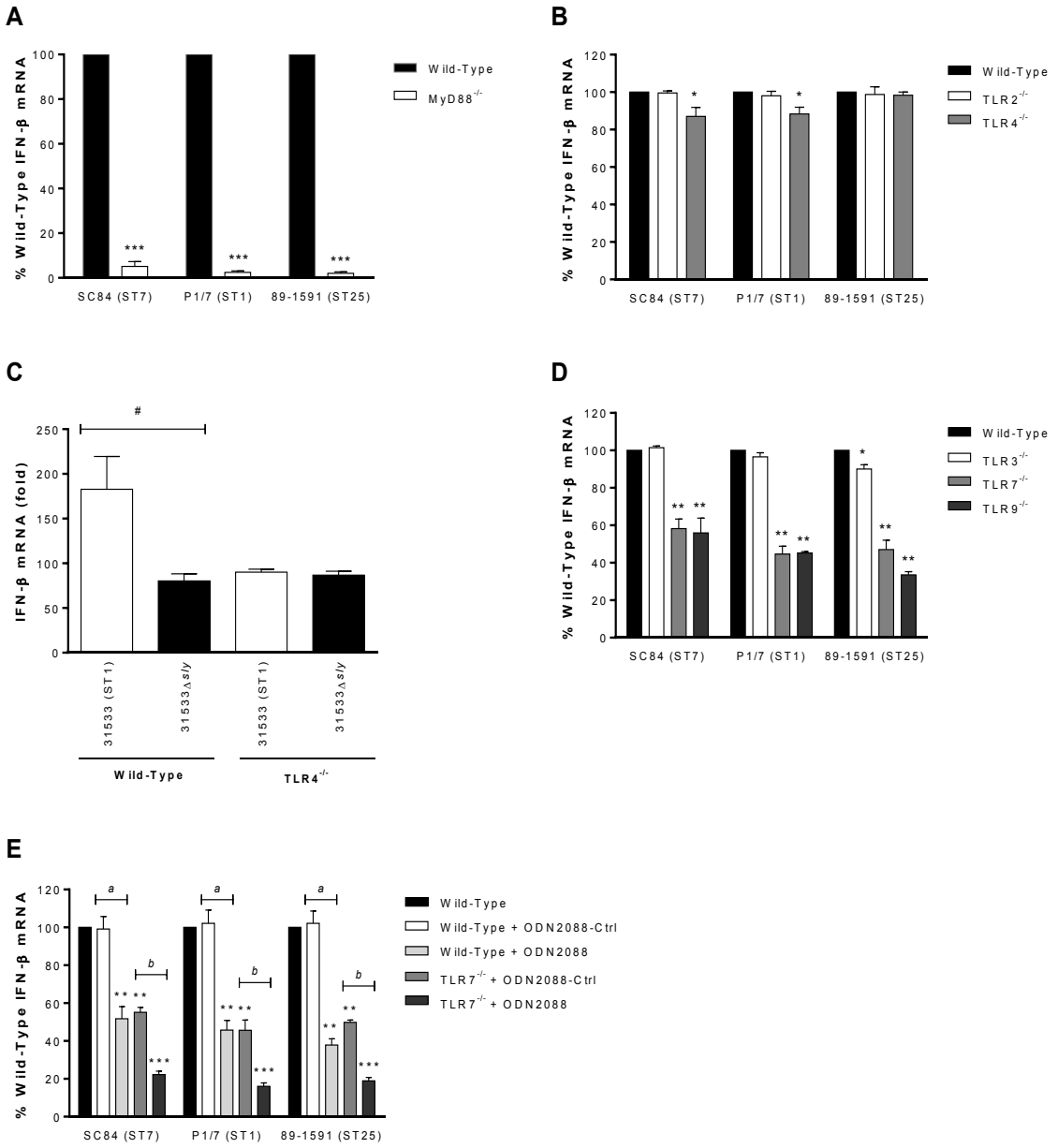

Figure 3. Recognition of *S. suis* by the Toll-like receptor (TLR) pathway is required for induction of IFN-β expression by dendritic cells (DCs). IFN-β mRNA expression induced by the different *S. suis* strains 6 h following infection of DCs deficient for MyD88 **(A)**, TLR2 or TLR4 **(B)**, or for the endosomal TLR3, TLR7, or TLR9 **(D)**. The suilysin (SLY) is responsible for TLR4-dependent IFN-β expression by DCs **(C)**. The cooperative role of TLR7 and TLR9 was evaluated using wild-type or

TLR7$^{-/-}$ cells pretreated with the TLR9 antagonist ODN2088 or its control, ODN2088-Ctrl, resulting in TLR7$^{-/-}$ cells non-responsive for TLR9 (dual deficiency) **(E)**.

Data represent the mean ± SEM from three or four independent experiments. * ($p < 0.05$), ** ($p < 0.01$), or *** ($p < 0.001$) indicates a significant difference between expression by wild-type and deficient DCs; # ($p < 0.01$) between 31533 and 31533Δ*sly*; *a* ($p < 0.01$) between expression by wild-type DCs pretreated with ODN2088-Ctrl or ODN2088; *b* ($p < 0.01$) between expression by TLR7$^{-/-}$ DCs pretreated with ODN2088-Ctrl or ODN2088.

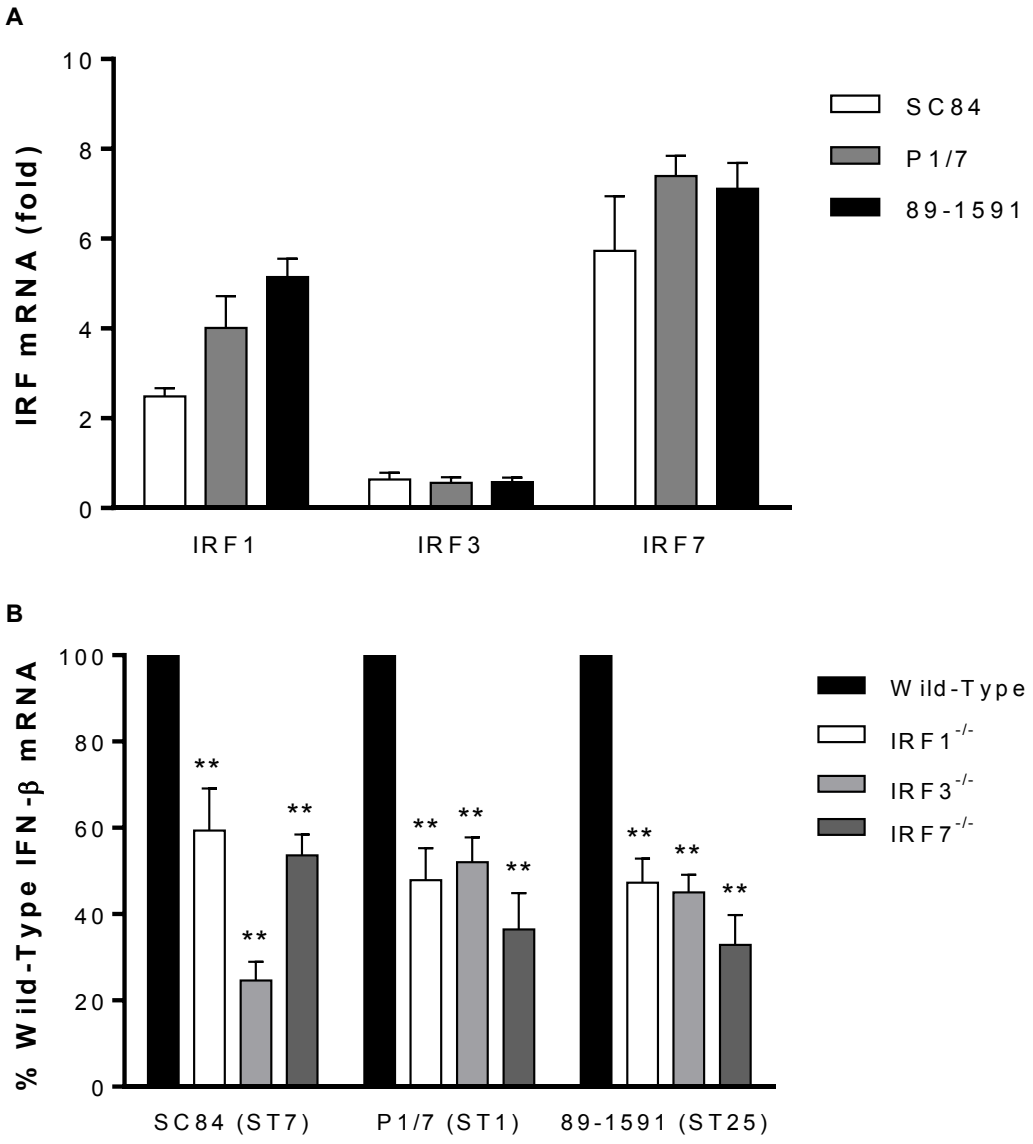

Figure 4. The interferon regulatory factors (IRFs) 1, 3, and 7 are involved in IFN-β expression by dendritic cells (DCs) following infection with *S. suis*. *S. suis*-induced IRF1, IRF3, and IRF7 mRNA expression by DCs 6 h following infection with the different strains **(A)**. IFN-β mRNA expression induced by the different *S. suis* strains following infection of IRF1$^{-/-}$, IRF3$^{-/-}$, or IRF7$^{-/-}$ DCs in comparison with cells from wild-type mice **(B)**. Data represent the mean ± SEM from three or four independent experiments. ** ($p < 0.01$) indicates a significant difference between expression by wild-type and deficient DCs.

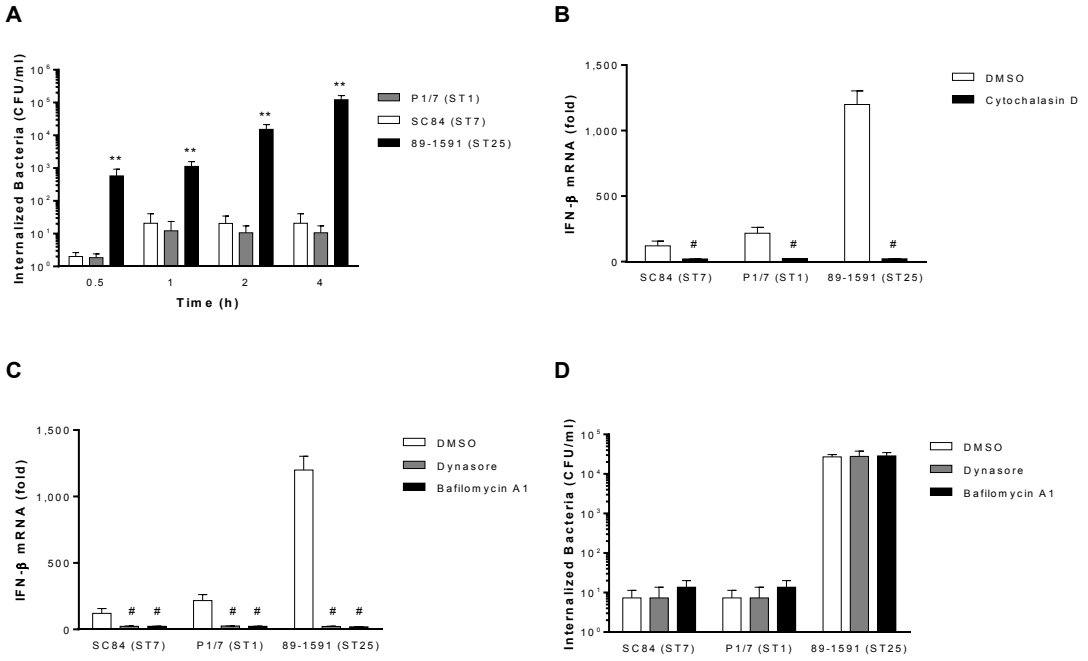

Figure 5. *S. suis*-induced IFN-β expression by dendritic cells requires internalization and phagosome maturation. Internalization kinetics of different *S. suis* strains by dendritic cells (DCs) **(A)**. Implication of actin polymerization (5 μM cytochalasin D) **(B)**, dynamin (8 μM dynasore), and vacuolar-type H^+ ATPase-dependent phagosome acidification (1 μM bafilmoycin A1) **(C)** on IFN-β mRNA expression by DCs 6 h following infection with *S. suis*. Effect of dynamin remodeling and phagosome acidification on internalization of *S. suis* by DCs 2 h following infection **(D)**. Data represent the mean ± SEM from three independent experiments. ** ($p < 0.05$) indicates a significant difference between 89-1591 and P1/7 or SC84; # ($p < 0.001$) between DCs treated with DMSO (vehicle) and DCs treated with the inhibitors (cytochalasin D, dynasore or bafilomycin A1).

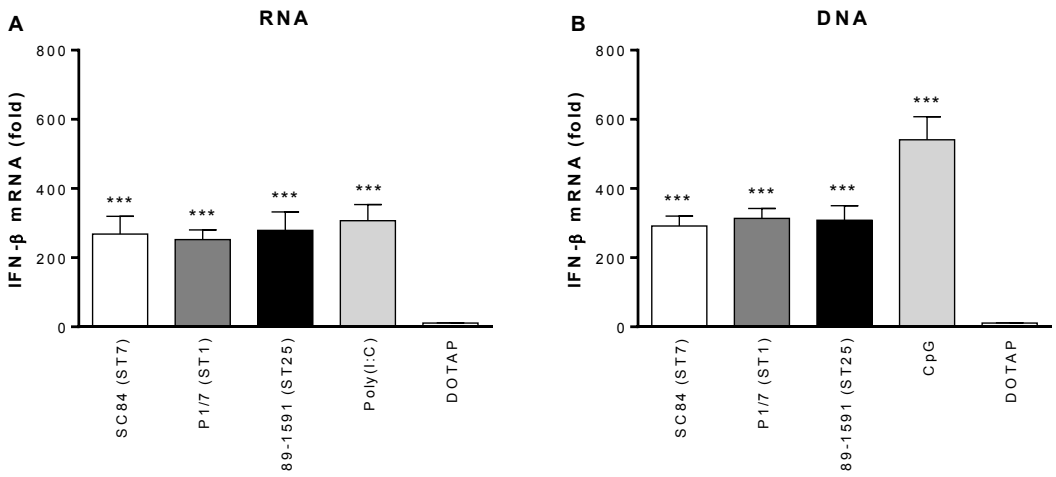

Figure 6. The *S. suis* nucleic acids are responsible for inducing IFN-β expression by dendritic cells following phagosomal delivery. IFN-β mRNA expression by dendritic cells 6 h following transfection with RNA **(A)** or DNA **(B)** isolated from the different *S. suis* strains, poly(I:C) or CpG. Nucleic acids were complexed with DOTAP liposomal transfection agent prior to transfection of dendritic cells. Data represent the mean ± SEM from three independent experiments. *** ($p < 0.001$) indicates a significant difference with DOTAP alone (vehicle, negative control).

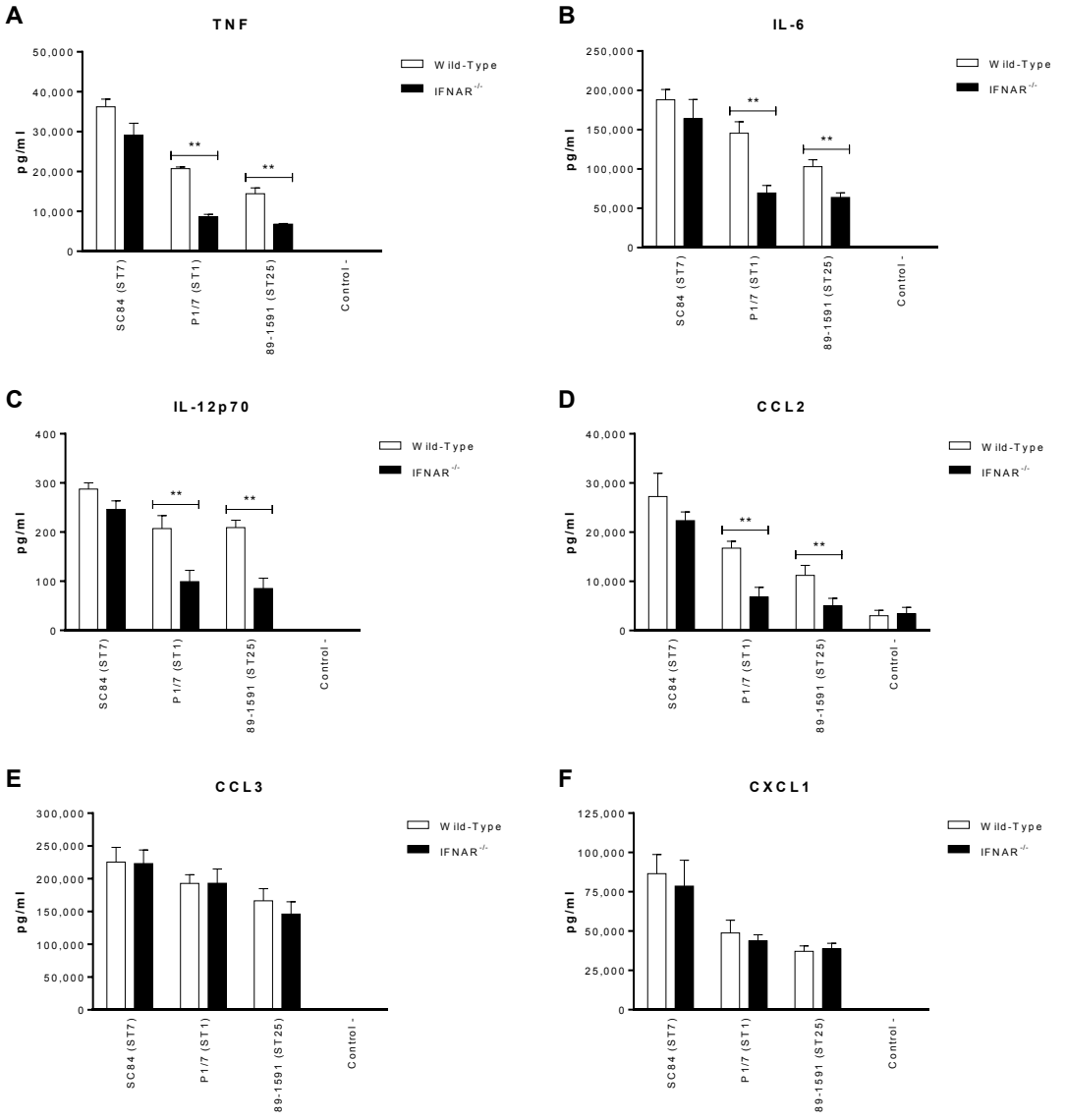

Figure 7. *S. suis*-induced type I interferon produced by dendritic cells modulates autocrine cytokine production. Production of TNF **(A)**, IL-6 **(B)**, IL-12p70 **(C)**, CCL2 **(D)**, CCL3 **(E)**, and CXCL1 **(F)** by dendritic cells 16 h following infection with the different *S. suis* strains. Data represent the mean ± SEM from four independent experiments. ** ($p < 0.01$) indicates a significant difference in cytokine production between wild-type and IFNAR$^{-/-}$ DCs.

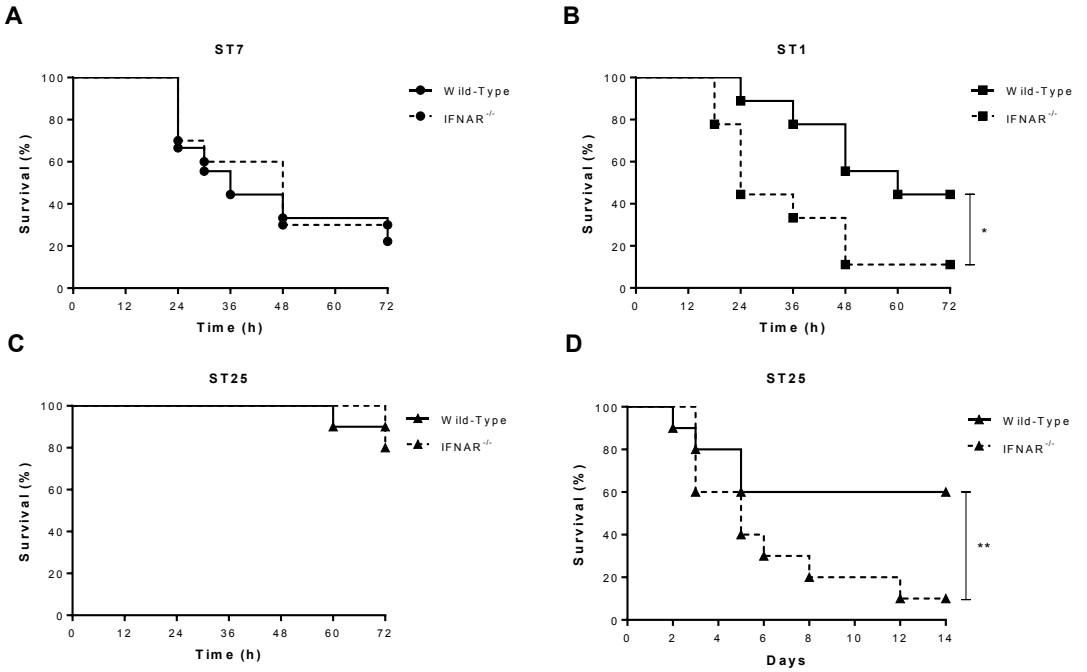

Figure 8. Type I interferon is beneficial for host survival following infection with intermediate virulent and virulent *S. suis* serotype 2 strains. Survival of wild-type and IFNAR$^{-/-}$ mice infected with the different *S. suis* strains: the highly virulent ST7 strain SC84 **(A)**, the virulent ST1 strain P1/7 **(B)**, and the intermediate virulent ST25 strain 89-1591 **(C)** during the systemic infection (until 72 h post-infection). Survival of wild-type and IFNAR$^{-/-}$ mice infected with strain 89-1591 following both the systemic and central nervous system infections (14 days post-infection) **(D)**. Data represent the survival curves of 15 mice/group. * ($p < 0.05$) and ** ($p < 0.01$) indicate a significant difference between survival of wild-type and IFNAR$^{-/-}$ mice.

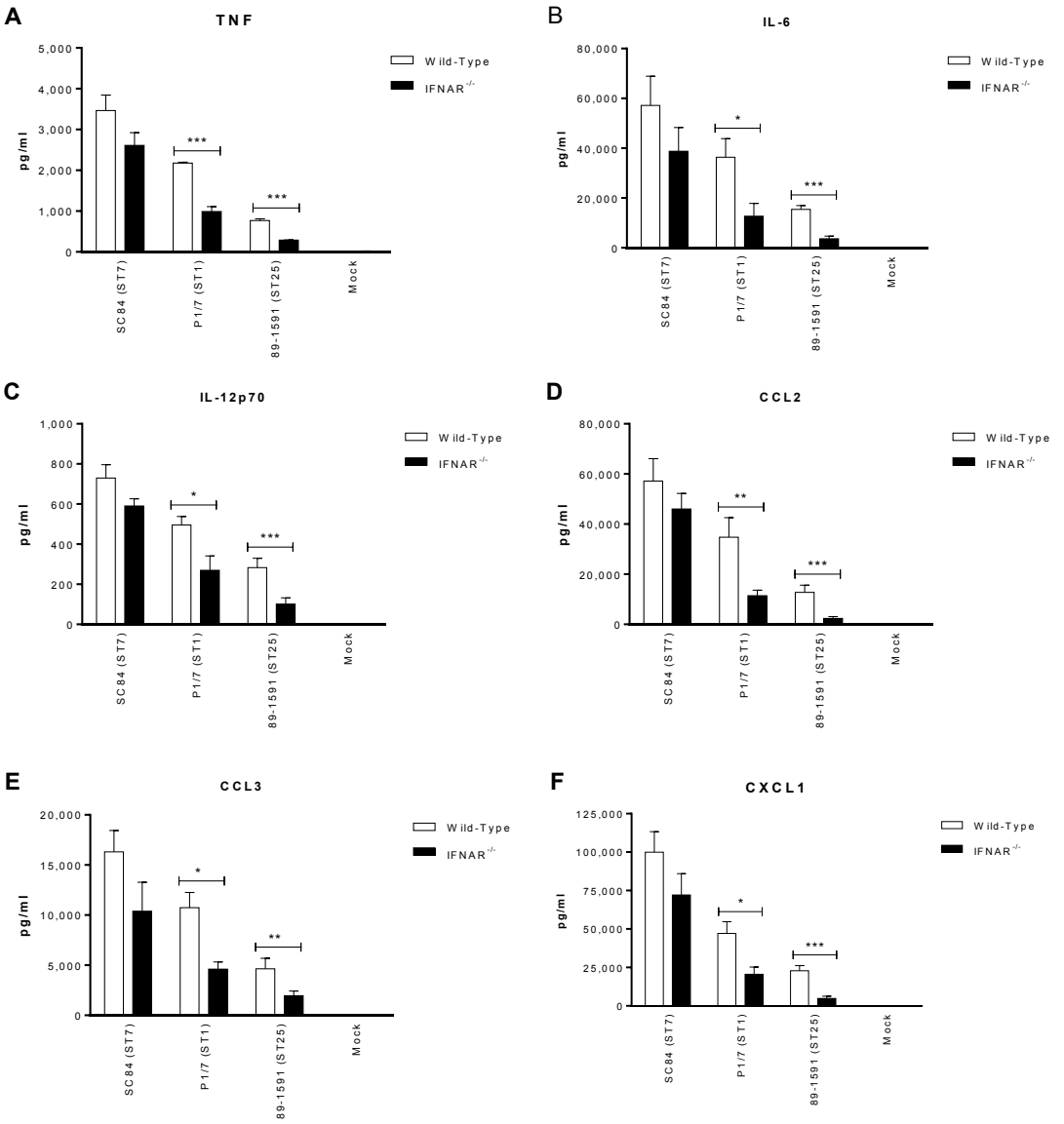

Figure 9. Type I interferon modulates plasma pro-inflammatory cytokines involved in *S. suis*-induced systemic inflammation. Plasma levels of TNF **(A)**, IL-6 **(B)**, IL-12p70 **(C)**, CCL2 **(D)**, CCL3 **(E)**, and CXCL1 **(F)** in wild-type and IFNAR$^{-/-}$ mice 12 h following infection with the different *S. suis* strains. Data represent the mean ± SEM from 8 mice/group. * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$) indicate a significant difference in plasma levels between wild-type and IFNAR$^{-/-}$ mice.

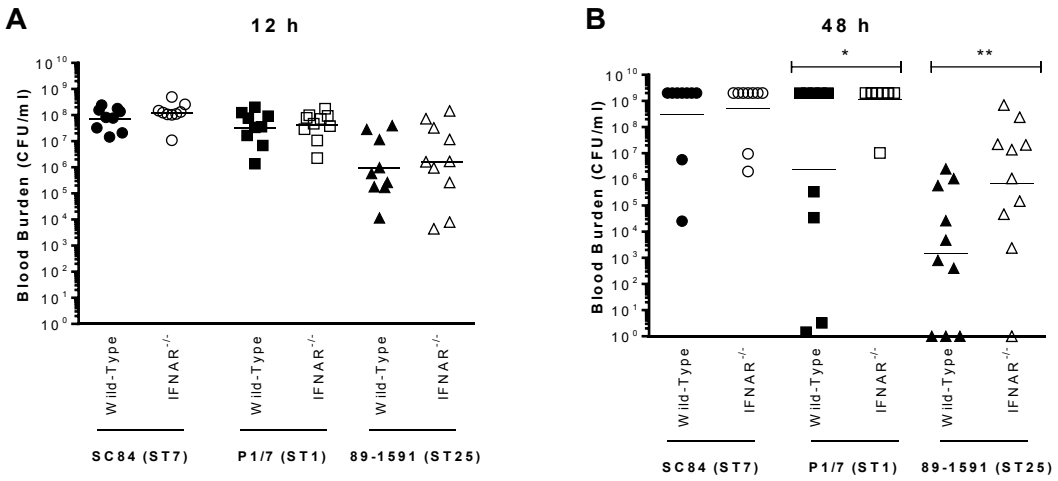

Figure 10. Type I interferon is required for control of blood bacterial burden following infection with intermediate virulent and virulent *S. suis* serotype 2 strains. Blood bacterial burden of wild-type and IFNAR$^{-/-}$ mice infected with the different *S. suis* strains 12 h post-infection **(A)** or 48 h post-infection **(B)**. Data represent the geometric mean of 15 mice/group. A blood bacterial burden of 2 x 10^9 CFU/mL, corresponding to the average burden upon euthanasia, was attributed to euthanized mice. * ($p < 0.05$) and ** ($p < 0.01$) indicate a significant difference between blood bacterial burden of wild-type and IFNAR$^{-/-}$ mice.

Strain	General characteristics	Reference
P1/7	Virulent European ST1 strain isolated from a case of pig meningitis in the United Kingdom	(67)
P1/7Δ*cpsF*	Isogenic non-encapsulated mutant derived from P1/7; in frame deletion of *cpsF* gene	(12)
31533	Virulent European ST1 strain isolated from a case of pig meningitis in France	(68)
31533Δ*sly*	Isogenic suilysin-deficient mutant derived from 31533; in frame deletion of *sly* gene	(69)
31533Δ*dltA*	Isogenic D-alanylation of lipoteichoic acid-deficient mutant derived from 31533; in frame deletion of *dltA* gene	(17)
31533Δ*pgdA*	Isogenic N-deacetylation of peptidoglycan-deficient mutant derived from 31533; in frame deletion of *pgdA* gene	(16)
SC84	Highly virulent ST7 strain isolated from a case of human streptococcal toxic shock-like syndrome during the 2005 outbreak in China	(32)
89-1591	Intermediate virulent North American ST25 strain isolated from a case of pig sepsis in Canada	(70)
91-1804	Intermediate virulent North American ST25 strain isolated from a case of human endocarditis in Canada	(71)
LPH4	Intermediate virulent Asian ST25 strain isolated from a case of human sepsis in Thailand	(72)

Table 1. *Streptococcus suis* serotype 2 strains used in this study.

Supplementary Material

Type I Interferon Induced by *Streptococcus suis* Serotype 2 is Strain-Dependent and May Be Beneficial for Host Survival

Jean-Philippe Auger, Agustina Santinon, David Roy, Karen Mossman, Jianguo Xu, Mariela Segura, and Marcelo Gottschalk*

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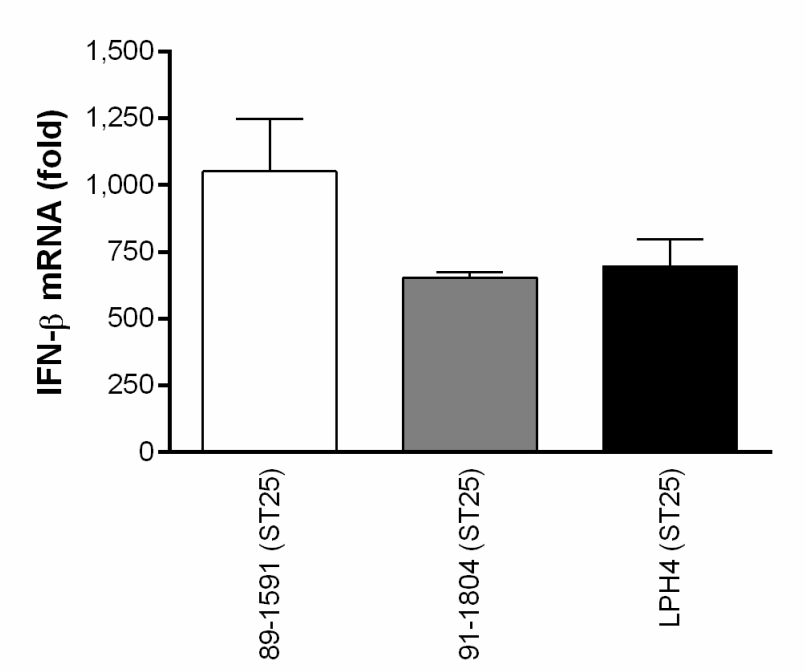
Supplementary Table

Table S1. Primer sequences used for real-time qPCR

Supplementary Figures

Supplementary Table S1. Primer sequences used for real-time qPCR.

Gene	Forward (F) and reverse (R) primers
Atp5b	F: ACC AGC CCA CCC TAG CCA CC
	R: TGC AGG GGC AGG GTC AGT CA
Gapdh	F: CCC GTA GAC AAA ATG GTG AAG
	R: GAC TGT GCC GTT GAA TTT G
Ifnb	F: CCC AGT GCT GGA GCC ATT GT
	R: CCC TAT GGA GAT GAC GGA GA
Irf1	F: AGG CAT CCT TGT TGA TGT CC
	R: AAT TCC AAC CAA ATC CCA GG
Irf3	F:GAT GGC TGA CTT TGG CAT CT
	R: ACC GGA AAT TCC TCT TCC AG
Irf7	F: AGC ATT GCT GAG GCT CAC TT
	R: TGA TCC GCA TAA GGT GTA CG
Il6	F: ATG GTA GCT ACC AAA CTG GAT
	R: TGA AGG ACT CTG GCT TTG TCT
Cxcl1	F: TCT CCG TTA CTT GGG GAC AC
	R: CCA CAC TCA AGA ATG GTC GC



Supplementary Figure S1. Intermediate virulent *S. suis* ST25 strains induce high levels of IFN-β expression by dendritic cells. IFN-β mRNA expression by dendritic cells 6 h following infection with the intermediate virulent ST25 strains 89-1591, 91-1804, and LPH4. Virulence of strains 91-1804 and LPH4 was previously described.

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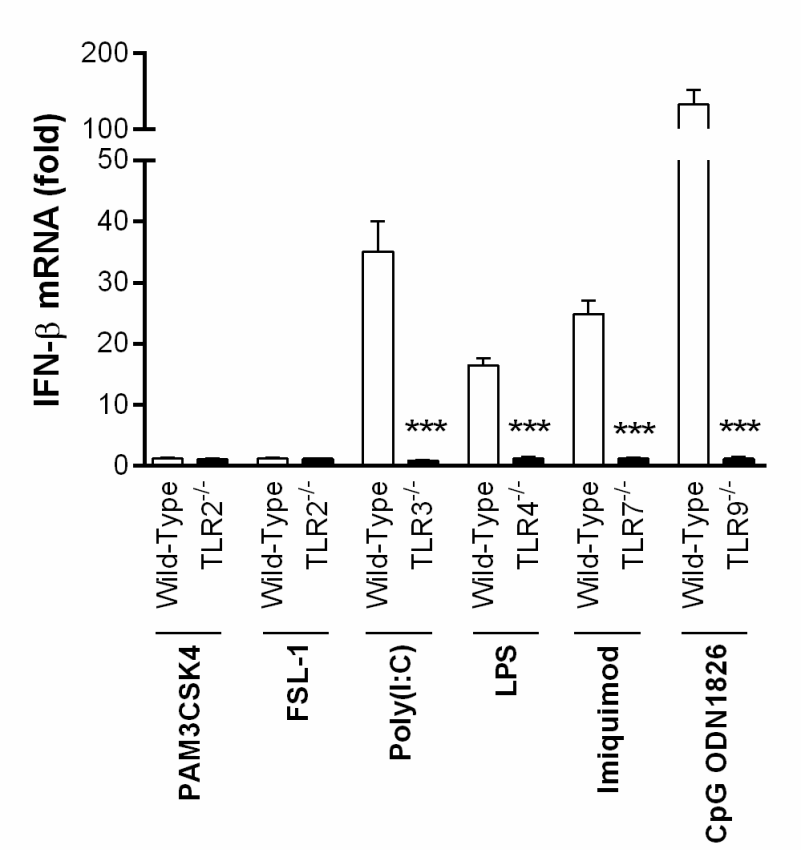

Supplementary Figure S2. Ligands of the different Toll-like receptors (TLRs) evaluated in this study, with the exception of TLR2, induce IFN-β by dendritic cells. IFN-β mRNA expression by wild-type and deficient dendritic cells 6 h following activation with the different TLR ligands: 1 μg/mL PAM3CSK4 (TLR1/2), 1 μg/mL FSL-1 (TLR2/6), 10 μg/mL poly(I:C) (TLR3), 100 ng/mL lipopolysaccharide (LPS) (TLR4), 5 μg/mL imiquimod (TLR7), and 1 μM CpG ODN1826 (TLR9). Data represent the mean ± SEM from three or four independent experiments. *** ($p < 0.001$) indicates a significant difference in IFN-β expression by wild-type and deficient dendritic cells.

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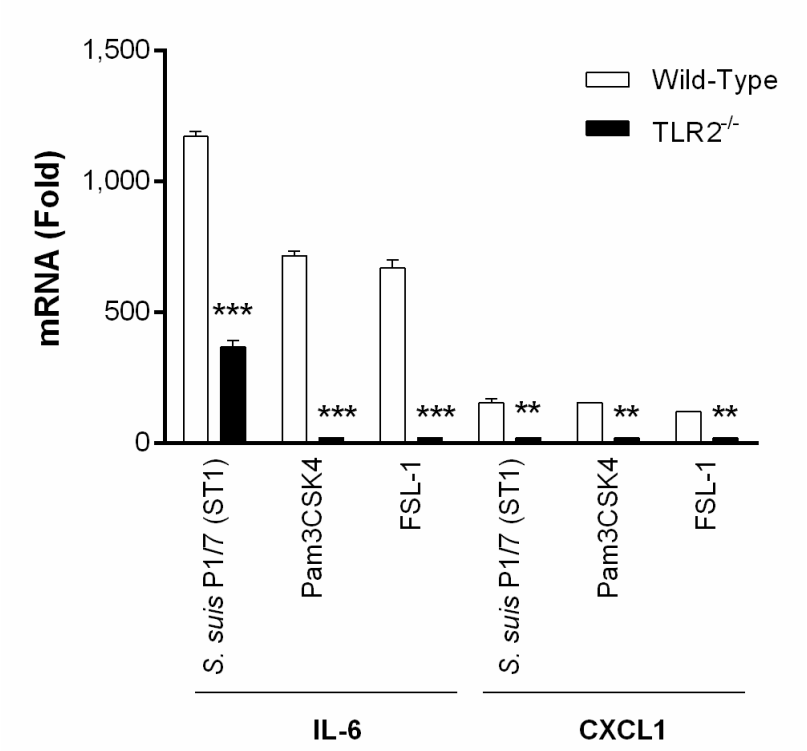

Supplementary Figure S3. IL-6 and CXCL1 induced by *S. suis* and bacterial TLR2 ligands partially requires TLR2 expression by dendritic cells. IL-6 and CXCL1 expression by wild-type or TLR2$^{-/-}$ dendritic cells 6 h following infection with *S. suis* ST1 strain P1/7 or activation with the TLR2 ligands PAM3CSK4 (1 μg/mL) and FSL-1 (1 μg/mL). Data represent the mean ± SEM from three independent experiments. ** ($p < 0.01$) and *** ($p < 0.001$) indicate a significant difference between expression by wild-type and TLR2$^{-/-}$ dendritic cells.

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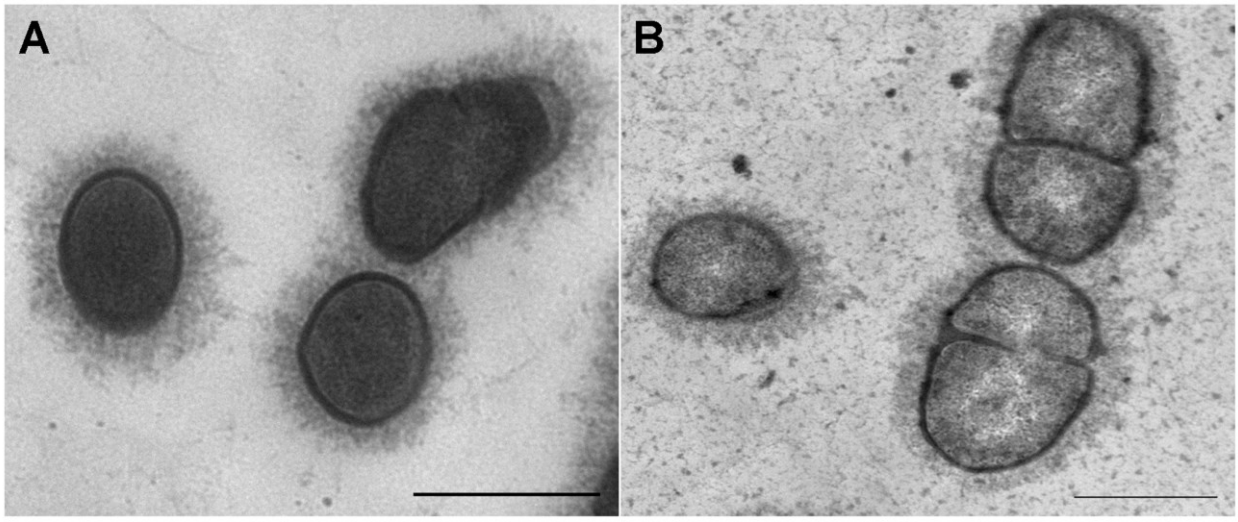

Supplementary Figure S4. The intermediate virulent *S. suis* strain 89-1591 is highly encapsulated. Transmission electron micrographs following antibody stabilization of the capsular polysaccharide, using an anti-*S. suis* serotype 2 rabbit serum of ST1 strain P1/7 (*A*) and ST25 strain 89-1591 (*B*). Black bars = 1 μm.

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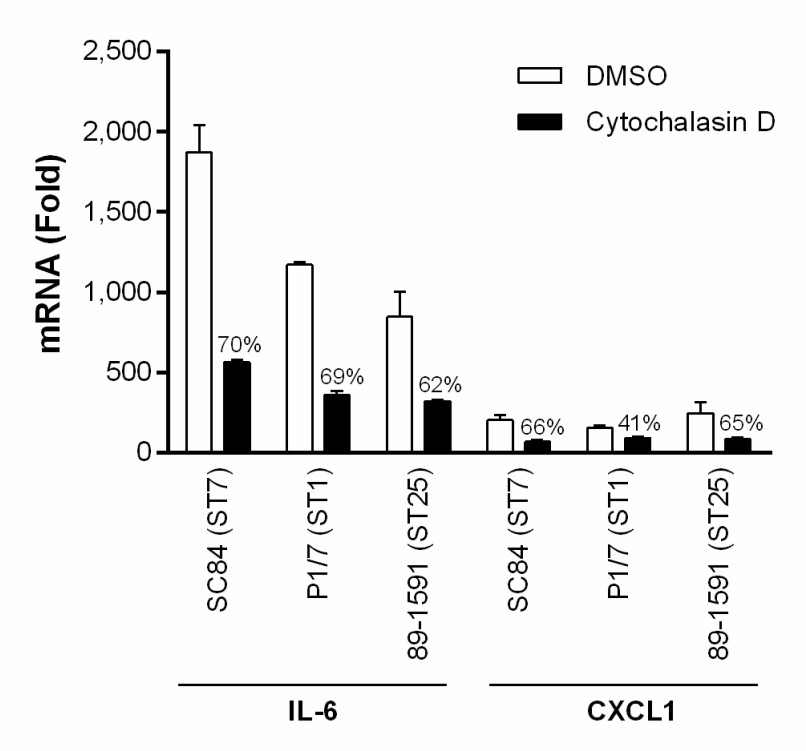

Supplementary Figure S5. *S. suis*-induced IL-6 and CXCL1 expression by dendritic cells is partially internalization-dependent. IL-6 and CXCL1 expression by dendritic cells pretreated with cytochalasin D (5 μM) to inhibit actin polymerization 6 h following infection with *S. suis*. Mock-treated cells (DMSO) were used as controls. Data represent the mean ± SEM from three independent experiments. The percentages of cytochalasin D-mediated inhibition of cytokine expression are indicated.

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IV- DISCUSSION

The mechanisms involved in the innate and adaptive immune responses to *S. suis* remain poorly known, and the increasing number of cases of *S. suis* infections in humans underscores the urgent need for a better understanding of the interactions between the pathogen and the immune system, in order to develop an effective therapeutic and control strategies against *S. suis*.

The interactions between this pathogen and murine DCs have been described earlier (262). Once activated by *S. suis*, DCs start a maturation process implicating the up-regulation of costimulatory molecules and the production of several pro-inflammatory mediators, like IFN-β (233, 262). The confounding role (protective/detrimental)of tis cytokine, following infection with extracellular bacteria, has already been described (45, 46, 52, 85, 283). However, the study of its role during *S. suis* serotype 2 infection, for which no information was previously available, became interesting. The present work went into further details, also testing the capacity of macrophages to produce IFN-β, since they are also known as an important source of pro-inflammatory mediators. Results obtained following infection of DCs and macrophages with *S. suis* serotype 2 have shown that only DCs are able to produce high levels of IFN-β, which is in agreement with previous studies with other streptococci, such as *S. pyogenes*, GBS and *S. pneumoniae* (83, 85). This could suggest different activation levels by *S. suis* and/or intrinsic differences in cytokine production by both cell types, in part resulting from the activation of varying signaling pathways and cascades (83). Moreover, the intermediate virulent ST25 strain, the least virulent ST tested, was identified as the strongest IFN-β inducing strain. However, the ST7 and ST1 strains, induced similar levels of IFN-β mRNA expression (confirmed at protein level). These latter results agree with previous *in vivo* studies in a murine model of infection (247).

The TLR pathway has been traditionally associated with IFN-β induction following recognition by the endosomal TLRs (19), yet recognition of *S. suis* has been mostly demonstrated to occur via surface TLRs (232, 258). For a better understanding of the pathways involved, DCs from wild-type and knock-out mice for several proteins that, according to the literature, could be implicated in the induction of IFN-β, were activated. Results have shown that the production of IFN-β is MyD88 dependent, suggesting that the TLR pathway is almost exclusively implicated in IFN-β production. In addition, TLR2 was confirmed not to be implicated in the production of this cytokine by DCs, following an infection with *S. suis*. Similar results were obtained for TLR3. Inversely, TLR4, TLR7, and TLR9 were implicated in *S. suis*-induced IFN-β by DCs.

Indeed, a partial role of TLR4 was observed in IFN-β induced by the ST1/ST7 strains, but not the ST25: the SLY, which was reported to be recognized by TLR4 (253), is produced by ST1/ST7 but not by ST25 strains. Thus, results obtained in this study show that the SLY is responsible for the TLR4-dependent IFN-β induced by *S. suis*.

The involvement of endosomal TLRs has been poorly evaluated for this pathogen since *S. suis* has been considered an extracellular pathogen. Therefore, it was unexpected that the TLR7 and TLR9 were equally and primarily responsible for *S. suis*-induced IFN-β. Though TLR7, and to a lesser extent TLR9, were responsible for GBS-induced IFN-β (83), neither were implicated in GAS recognition (85). Even though *S. suis* and GBS share a similarity in this regard, the main difference between them relies on the pathogenesis: while *S. suis* is considered a classical extracellular bacterium protected from phagocytosis by its CPS, well-encapsulated GBS is highly internalized (275). Furthermore, activation of IRFs by a classical activation of the TLR pathway via the endosomal TLRs can lead to the production of IFN-β. Our results show that the IRF1, IRF3 and IRF7 are implicated in *S. suis*-induced IFN-β by DCs, suggesting a partial redundancy. Morover, the equal contribution of IRF1 and IRF7 could be explained by the mechanisms similar to those involved in GBS-induced IFN-β: i.e., localization of pathogens within the phagosome usually triggers IRF1 and IRF7 activation (83). In addition, IFN-β induced by TLR9 agonists results in IRF1 activation via a phagosome-dependent pathway (287). On the other hand, the involvement of IRF3 may be the result of TLR3 or TLR4 activation by SLY-negative and -positive strains, respectively, via a MyD88-independent, TRIF-dependent pathway (276). Overall, the implication of IRF3 could be a consequence of feedback loops resulting from crosstalk between pathways (277). This information contributes significantly to our knowledge of the pathway involved in the induction and the production of IFN-β following infection with *S. suis* serotype 2.

As mentioned above, since DCs represent a more important source of IFN-β than macrophages, the formers have been used for subsequent experiments. To test the role of virulence factors such as the CPS and SLY, in IFN-β induction, DCs were challenged with the wild-type and mutant *S. suis* strains. Results showed that production of IFN-β was up-regulated in the absence of the CPS, and down-regulated in the absence of the SLY. These results are in agreement with previous studies, which have described that *S. suis* virulence factors regulate DCs release of several cytokines (233, 262).

The fact that TLR7 and TLR9 are endosomal receptors means that the activating bacterial components are located intracellularly. As such, the capacity of DCs to internalize *S. suis* was tested using an internalization assay. Our results showed that the intermediate virulent ST25 strain was more internalized, despite the presence of its CPS, previously demonstrated to plays an anti-phagocytic role (233), and that all of the strains are well-encapsulated as confirmed by electron microscopy (269-271). The more elevated intracellular localization of this strain could be responsible for the increased activation of the intracellular receptors, resulting in increased IFN-β production. These results suggest that *S. suis*-induced IFN-β could be internalization-dependent. To confirm this, the internalization capacity of DCs was blocked, prior to infection with *S. suis*. This resulted, in almost complete abrogation of IFN-β expression, which resembles the result obtained with GBS (83).

Following internalization by phagocytes, the pathogen is located within the phagosome, which must undergo maturation (278). Amongst the different proteins involved in these steps is the GTPase dynamin (279). This study demonstrates that despite dynamin being required for IFN-β production, this protein is implicated in early pre-acidification steps of phagosome maturation rather than its formation as evidenced by lack of effect on *S. suis* internalization. Subsequent acidification of the phagosome is required for IFN-β production by DCs following infection with *S. suis*, as was previously demonstrated for both GAS and GBS, suggesting that bacterial processing via hydrolytic degradation is essential for the liberation of the TLR7 and TLR9 ligands (83, 85). Thus, results indicated that bacterial nucleic acids were the ligands of TLR7 and TLR9. Indeed, both bacterial RNA, and to a lesser extent, DNA, from GAS and GBS are also responsible for IFN-β production by DCs following stimulation of TLR7 and TLR9 (83, 85). In the case of *S. suis* RNA and DNA induced similar levels of IFN-β, suggesting that both nucleic acids have comparable stimulatory effects. Nucleic acids isolated from the three *S. suis* strains induced similar levels of IFN-β by DCs, indicating that despite the differences of virulence, they all possess similar stimulatory properties and differences observed would be attributed to differences in intracellular bacterial levels.

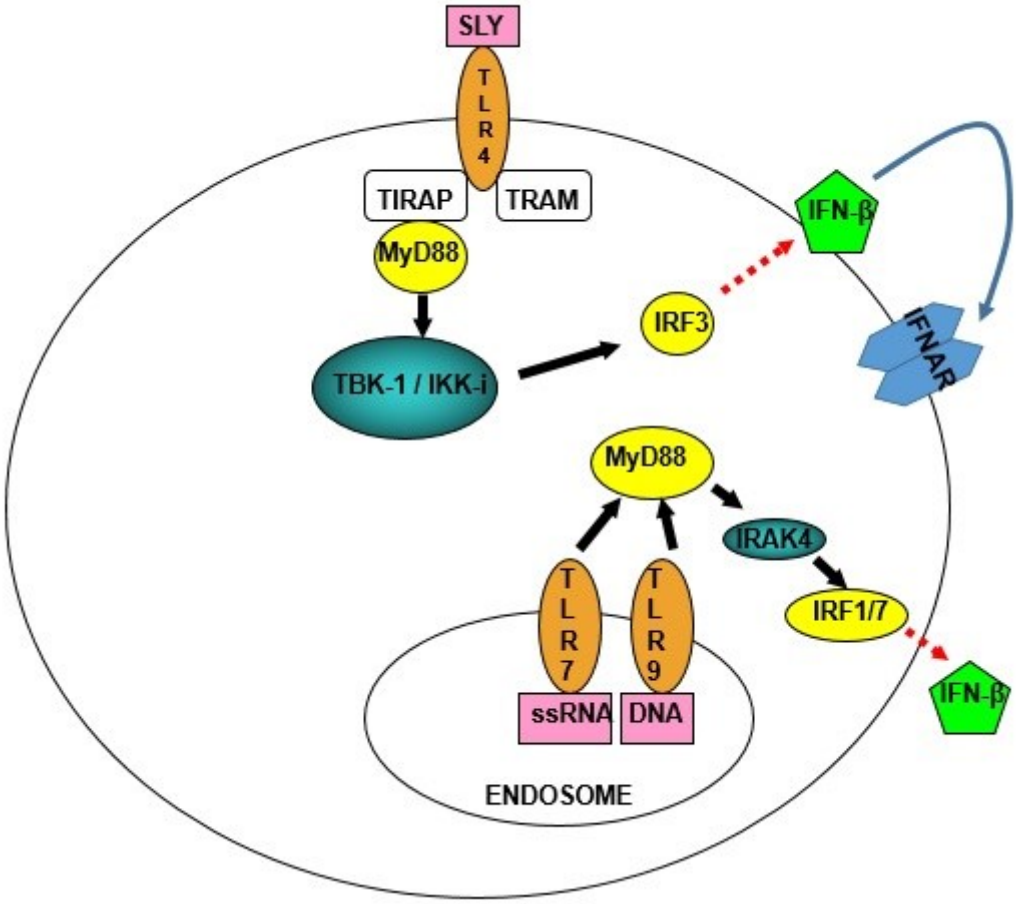
As was mentioned earlier, type I IFN binds its receptor, IFNAR, located on the surface of most cell types, including DCs (19), and following production is able to modulate autocrine production of other inflammatory mediators (45, 280). This autocrine production could play an important role in the modulation of *S. suis*-induced

pro-inflammatory cytokines by DCs, levels of which are elevated following infection (258, 262). *S. suis*-induced type I IFN produced by DCs modulated TNF, IL-6, IL-12p70, and CCL2, but not CCL3 and CXCL1. Indeed, type I IFN was previously demonstrated to amplify TNF signaling following infection with GBS, *S. pneumonaie*, and *E. coli* (45). Being that pro-inflammatory cytokine signaling is the result of a cascade triggered by TNF, leading to production of IL-6 and IL-12p70 (281), an amplification of these downstream cytokines by type I IFN is expected. However, type I IFN had no effect on levels of CCL3 and CXCL1, which are known to be produced by DCs at high levels. This lack of modulation was also observed in a septic peritonitis model, whereby IFNAR-deficiency did not alter levels of CXCL1, and for macrophage-produced CCL3 induced by *M. tuberculosis* (276, 282). As such, these results indicate a mechanism complementary to surface-associated receptor activation, whereby the higher internalization of *S. suis* leads to increased IFN-β induction and subsequent autocrine pro-inflammatory cytokine production.

It was previously reported with other pathogens that the induction of IFN-β may be beneficial or detrimental for the host, as shown using experimental infections. For example, a beneficial role was described for GAS (85), GBS (45), and *S. pneumoniae* (45, 46, 283). Aversely, the type I IFN response may also be considered a key factor in early progression of invasive *S. pneumoniae* beyond the lung during development of invasive pneumococcal disease (52). Moreover, type I IFN is also associated with suppression of the innate immune response to infections with *L. monocytogenes* and *Francisella tularensis*, resulting in hindered bacterial clearance and deleterious host effects. Results obtained in this work have shown that type I IFN plays a beneficial role during the *S. suis* infection since IFNAR-knock-out mice were more susceptible to infection than their wild-type counterparts, especially for the intermediate virulent ST25 strain, the most potent *in vitro* IFN-β inducer and, to a lesser extent, for the ST1 strain. Host death during the *S. suis* systemic infection is generally a consequence of an exacerbated systemic inflammatory response leading to an uncontrolled blood bacterial burden resulting from excessive bacterial growth (216, 284). Modulation of both of these factors by type I IFN might explain its beneficial role during infection with *S. suis*. In fact, IFNAR KO mice infected with the ST1 and ST25 strains induced lower levels of pro-inflammatory cytokines leading to a lower cell activation, and a lower capacity to control bacterial burden. A similar effect was previously reported for GBS and *S. pneumoniae* (83, 283). Following infection with the intermediate virulent ST25

strain, the elevated IFN-β production modulates systemic pro-inflammatory mediators, as observed 12 h p.i., and seems to be responsible for the decreased blood bacterial burden 48 h p.i. This indicates a required, though delayed role of type I IFN in blood bacterial burden clearance. Thus, type I IFN induced by that strain counterbalances systemic inflammation, resulting in a reduction of meningitis and increased host survival. Indeed, it was previously reported that persistent blood bacterial burden is a prerequisite for the development of meningitis by *S. suis* (284). Contrarily, the virulent ST1 and highly virulent ST7 strains induced similar lower IFN-β production *in vitro*. However, in the case of ST1-infected mice, type I IFN played a beneficial role, even though these two virulent strains induce elevated inflammation. Because of its genetic particularities, including the presence of a pathogenicity island, the ST7 strain induces exacerbated inflammation resulting in streptococcal toxic shock-like syndrome characterized by a cytokine storm (285, 286). Consequently, low levels of type I IFN induced by the ST7 strain are probably not sufficient to modulate the deleterious effects of very high pro-inflammatory cytokine levels.

Proposed model of IFN-β production during *S. suis* serotype 2 infection



The endosomal receptors TLR7 and TLR9, are activated by nucleic acids, while the surface TLR4 is activated by the suilysin of the pathogen. The production follows a pathway that use the adaptor molecule MyD88, resulting in activation of IRF1, IRF7, and to a lesser extent, IRF3. These factors then translocate to the cell nucleus, to trigger the production of IFN-β, which then, binds IFNAR. If IFNAR is on the surface of the DC, this results in autocrine regulation of pro-inflammatory cytokines. But, if IFNAR is located on another cell type, the same also occurs but, in this case, the regulation is paracrine. Following infection with the ST25, ST1 or ST7 strains, the pro-inflammatory mediators induced by IFNAR binding, clear, control bacterial burden or not, which results in host survival, some death, or death of all, respectively.

V- GENERAL CONCLUSION AND PERSPECTIVES

In conclusion, this study demonstrated that, depending on the virulence level of the strain, type I IFN is involved in host defense during the *S. suis* infection by participating in clearance of blood bacterial burden following modulation of systemic inflammation. Also, the present work has demonstrated that DCs are an important source of *S. suis*-induced IFN-β in the case of the virulent ST1 and highly virulent ST7 strains, following recognition of bacterial nucleic acids and, to a lesser extent, the SLY. Moreover, this induction was mainly through a MyD88 pathway, requiring TLR7 and TLR9. Our study has evidenced that *S. suis* internalization is required for IFN-β production. In addition, the intermediate virulent ST25 strain, which was the main IFN-β inducing strain, was also the most internalized by DCs. Hence, our results suggest the following important conclusion: less virulence strain, more effective internalization, higher IFN-β induction.

A better understanding of the mechanisms involved in the control of inflammation and subsequent bacterial burden can provide biomedical foundation for the developing of effective control measures for this important zoonotic infection.

VI- REFERENCES

1. Lun ZR, Wang QP, Chen XG, Li AX, and Zhu XQ. *Streptococcus suis*: an emerging zoonotic pathogen. *Lancet Infect Dis*; 7:201–9 (2007).

2. Gottschalk M. Streptococcosis. In : Diseases of Swine. Karriker L, Ramirez A, Schwartz KJ, Stevenson G, Zimmerman J (Eds). *Wiley Publishers*, NJ, USA (2011).

3. Wisselink HJ, Smith HE, Stockhofe-Zurwieden N, Peperkamp K, Vecht U. Distribution of capsular types and production of muramidase-released protein (MRP) and extracellular factor (EF) of *Streptococcus suis* strains isolated from diseased pigs in seven European countries. *Vet. Microbiol.* 74(3), 237-248 (2000).

4. Perch B, Kristjansen P, Skadhauge K. Group R streptococci pathogenic for man. Two cases of meningitis and one fatal case of sepsis. *Acta Pathol. Microbiol. Scand*. 74(1), 69-76 (1968).

5. Gottschalk M, Xu J, Calzas C, Segura M. *Streptococcus suis*: a new emerging ora n old neglected zoonotic pathogen? *Fut. Microbiol* 5(3), 371-391 (2010).

6. Wertheim HF. Nghia HD, Taylor W, Schultsz C. *Streptococcus suis* : an emerging human pathogen. *Clin. Infec. Dis* 48(5), 617-625 (2009).

7. Yu H, Jing H, Chen Z *et al*. Human *Streptococcus suis* outbreak, Sichuan, China. *Emerg. Infec. Dis* 12(6), 914-920 (2006).

8. Hui AC, Ng KC, Tong PY *et al*. Bacterial meningitis in Hong Kong : 10-years experience. *Clin. Neurol. Neurosurg*. 107(5), 366-370 (2005).

9. Ip M, Fung KS, Chi F *et al. Streptococcus suis* in Hong Kong. *Diagn. Microbiol. Infect. Dis.* 57(1), 15-20 (2007).

10. Mai NT, Hoa NT, Nga TV *et al*. *Streptococcus suis* meningitis in adults in Vietnam. *Clin. Infect. Dis*. 46(5), 659-667 (2008).

11. Suankratay C, Intalapaporn P, Nunthapisud P, Arunyingmongkol K, Wilde H. *Streptococcus suis* meningitis in Thailand. Southeast Asian J. Trop. *Med. Pub. Health* 35(4), 868-876 (2004).

12. Peter L. Smith, Giovanna Lombardi, Graham R. Foster. Type I interferons and the innate immune response – more than just antiviral cytokines. *Molecular Immunology* 42 ;869–877 (2005).

13. Pestka, S., Krause, C. D. & Walter, M. R. Interferons, interferon-like cytokines, and their receptors. *Immunol. Rev*. 202, 8–32 (2004).

14. Schoenborn, J. R. & Wilson, C. B. Regulation of interferon-γ during innate and adaptive immune responses. *Adv. Immunol*. 96, 41–101 (2007).

15. O'Brien, T. R., Prokunina-Olsson, L. & Donnelly, R. P. IFN-λ4: the paradoxical new member of the interferon λ family. *J. Interferon Cytokine Res*. 34, 829–838 (2014).

16. Witte, K., Witte, E., Sabat, R. & Wolk, K. IL-28A, IL-28B, and IL-29: promising cytokines with type I interferon-like properties. *Cytokine Growth Factor Rev*. 21, 237–251 (2010).

17. Durbin, R. K., Kotenko, S. V. & Durbin, J. E. Interferon induction and function at the mucosal surface. *Immunol. Rev*. 255, 25–39 (2013).

18. Yan, N. & Chen, Z. J. Intrinsic antiviral immunity. *Nature Immunol*. 13, 214–222 (2012).

19. Finlay McNab, Katrin Mayer-Barber, Alan Sher, Andreas Wack and Anne O'Garra. Type I interferons in infectious disease. *Nature Reviews Immunology*. 15, 87-103 (2015).

20. Platanias, L. C. Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nature Reviews Immunology*. 5:375 (2005).

21. Javier Antonio Carrero. Confounding roles of type I interferons during bacterial and viral pathogenesis. *International Immunology*, Vol. 25, No. 12, pp. 663–669 (2013).

22. Levy, D. E., Marié, I. J. and Durbin, J. E. Induction and function of type I and III interferon in response to viral infection. *Curr. Opin. Virol*. 1:476 (2011).

23. Shresta, S., Kyle, J. L., Snider, H. M., Basavapatna, M., Beatty, P. R. and Harris, E. Interferon-dependent immunity is essential for resistance to primary dengue virus infection in mice, whereas T- and B-cell-dependent immunity are less critical. *J. Virol*. (2004).

24. Stark, G. R., Kerr, I. M., Williams, B. R., Silverman, R. H. and Schreiber, R. D. How cells respond to interferons. *Annu. Rev. Biochem*. 67:227 (1998).

25. Stetson, D. B. and Medzhitov, R. Type I interferons in host defense. *Immunity* 25:373 (2006).

26. Theofilopoulos, A. N., Baccala, R., Beutler, B. and Kono, D. H. Type I interferons (alpha/beta) in immunity and autoimmunity. *Annu. Rev. Immunol*. 23:307 (2005).

27. Garcia-Sastre, A. and Biron, C. A. Type 1 interferons and the virus-host relationship: a lesson in detente. *Science* 312:879 (2006).

28. Nguyen, K. B., Salazar-Mather, T. P., Dalod, M. Y. *et al*. Coordinated and distinct roles for IFN-alpha beta, IL-12, and IL-15 regulation of NK cell responses to viral infection. *J. Immunol.* 169:4279 (2002).

29. Huber, J. P. and Farrar, J. D. Regulation of effector and memory T-cell functions by type I interferon. *Immunology* 132:466 (2011).

30. Sun, S., Zhang, X., Tough, D. F. and Sprent, J. Type I interferon- mediated stimulation of T cells by CpG DNA. *J. Exp. Med.* 188:2335 (1998).

31. Deal, E. M., Lahl, K., Narváez, C. F., Butcher, E. C. and Greenberg, H. B. Plasmacytoid dendritic cells promote rotavirusinduced human and murine B cell responses. *J. Clin. Invest.* 123:2464 (2013).

32. Jego, G., Palucka, A. K., Blanck, J. P., Chalouni, C., Pascual, V. and Banchereau, J. Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6. *Immunity* 19:225 (2003).

33. Diamond, M. S. Mechanisms of evasion of the type I interferon antiviral response by flaviviruses. *J. Interferon Cytokine Res*. 29:521 (2009).

34. Taylor, K. E. and Mossman, K. L. Recent advances in understanding viral evasion of type I interferon. *Immunology* 138:190 (2013).

35. Salvato, M., Borrow, P., Shimomaye, E. and Oldstone, M. B. Molecular basis of viral persistence: a single amino acid change in the glycoprotein of lymphocytic choriomeningitis virus is associated with suppression of the antiviral cytotoxic T-lymphocyte response and establishment of persistence. *J. Virol*. 65:1863 (1991).

36. Moskophidis, D., Lechner, F., Pircher, H. and Zinkernagel, R. M. Virus persistence in acutely infected immunocompetent mice by exhaustion of antiviral cytotoxic effector T cells. *Nature* 362:758 (1993).

37. Moore, K. W., de Waal Malefyt, R., Coffman, R. L. and O'Garra, A. Interleukin-10 and the interleukin-10 receptor. Annu. Rev. *Immunol*. 19:683 (2001).

38. Keir, M. E., Butte, M. J., Freeman, G. J. and Sharpe, A. H. PD-1 and its ligands in tolerance and immunity. Annu. Rev. *Immunol*. 26:677 (2008).

39. Barber, D. L., Wherry, E. J., Masopust, D. *et al*. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* 439:682 (2006).

40. Teijaro, J. R., Ng, C., Lee, A. M. *et al*. Persistent LCMV infection is controlled by blockade of type I interferon signaling. *Science* 340:207 (2013).

41. Wilson, E. B., Yamada, D. H., Elsaesser, H. *et al.* Blockade of chronic type I interferon signaling to control persistent LCMV infection. *Science* 340:202 (2013).

42. Müller, U., Steinhoff, U., Reis, L. F. *et al*. Functional role of type I and type II interferons in antiviral defense. *Science* 264:1918 (1994).

43. Ou, R., Zhou, S., Huang, L. and Moskophidis, D. Critical role for alpha/beta and gamma interferons in persistence of lymphocytic choriomeningitis virus by clonal exhaustion of cytotoxic T cells. *J. Virol*. 75:8407 (2001).

44. Watanabe, T. *et al*. NOD1 contributes to mouse host defense against *Helicobacter pylori* via induction of type I IFN and activation of the ISGF3 signaling pathway. *J. Clin. Invest*. 120, 1645–1662 (2010).

45. Mancuso, G. *et al.* Type I IFN signaling is crucial for host resistance against different species of pathogenic bacteria. *J. Immunol*. 178, 3126–3133 (2007).

46. Parker, D. *et al*. *Streptococcus pneumoniae* DNA initiates type I interferon signaling in the respiratory tract. *MBio 2*, e00016-11 (2011).

47. Weigent, D. A., Huff, T. L., Peterson, J. W., Stanton, G. J. & Baron, S. Role of interferon in streptococcal infection in the mouse. *Microb. Pathog*. 1, 399–407 (1986).

48. Kelly-Scumpia, K. M. *et al*. Type I interferon signaling in hematopoietic cells is required for survival in mouse polymicrobial sepsis by regulating CXCL10. *J. Exp. Med*. 207, 319–326 (2010).

49. Harvey RM, Stroeher UH, Ogunniyi AD, Smith-Vaughan HC, Leach AJ, Paton JC. A variable region within the genome of *Streptococcus pneumoniae* contributes to strain-strain variation in virulence. *PLoS One* 6:e19650 (2011).

50. Mitchell AM, Mitchell TJ. *Streptococcus pneumoniae*: virulence factors and variation. *Clin. Microbiol. Infect*. 16:411-418 (2010).

51. Wilkosz S, Edwards LA, Bielsa S, Hyams C, Taylor A, Davies RJ, Laurent GJ, Chambers RC, Brown JS, Lee YC. Characterization of a new mouse model of empyema and the mechanisms of pleural invasion by *Streptococcus pneumoniae*. Am. *J. Respir. Cell Mol. Biol*. 46:180–187 (2012).

52. Catherine E. Hughes, Richard M. Harvey, Charles D. Plumptre and James C. Paton. Development of Primary Invasive Pneumococcal Disease Caused by Serotype 1 *Pneumococci* Is Driven by Early Increased Type I Interferon Response in the Lung. *Infect. Immun*. 82(9):3919-3926 (2014).

53. Stockinger, S., Materna, T., Stoiber, D. *et al*. Production of type I IFN sensitizes macrophages to cell death induced by *Listeria monocytogenes*. *J. Immunol*. 169:6522 (2002).

54. McCaffrey, R. L., Fawcett, P., O'Riordan, M. *et al*. A specific gene expression program triggered by Gram positive bacteria in the cytosol. *Proc. Natl Acad. Sci*. USA 101:11386 (2004).

55. Schnupf, P. and Portnoy, D. A. Listeriolysin O: a phagosome- specific lysin. *Microbes Infect*. 9:1176 (2007).

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

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

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

59. O'Connell, R. M. *et al*. Type I interferon production enhances susceptibility to *Listeria monocytogenes* infection. *J. Exp. Med*. 200, 437–445 (2004).

60. Carrero, J. A., Calderon, B. & Unanue, E. R. Lymphocytes are detrimental during the early innate immune response against *Listeria monocytogenes. J. Exp. Med*. 203, 933–940 (2006).

61. Berry, M. P. *et al*. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature* 466, 973–977 (2010).

62. Cliff, J. M. *et al.* Distinct phases of blood gene expression pattern through tuberculosis treatment reflect modulation of the humoral immune response. *J. Infect. Dis*. 207, 18–29 (2013).

63. Maertzdorf, J. *et al.* Human gene expression profiles of susceptibility and resistance in tuberculosis. *Genes Immun.* 12, 15–22 (2011).

64. Ottenhoff, T. H. *et al*. Genome-wide expression profiling identifies type 1 interferon response pathways in active tuberculosis. *PLoS ONE* 7, e45839 (2012).

65. MacMicking, J. D. Interferon-inducible effector mechanisms in cell-autonomous immunity. *Nature Rev. Immunol*. 12, 367–382 (2012).

66. Rayamajhi, M., Humann, J., Penheiter, K., Andreasen, K. & Lenz, L. L. Induction of IFN-α/β enables *Listeria monocytogenes* to suppress macrophage activation by IFN-γ. *J. Exp. Med.* 207, 327–337 (2010).

67. Kearney, S. J. *et al.* Type I IFNs downregulate myeloid cell IFN-γ receptor by inducing recruitment of an early growth response 3/NGFI-A binding protein 1 complex that silences ifngr1 transcription. *J. Immunol*. 191, 3384–3392 (2013).

68. Manca, C. *et al.* Hypervirulent M. tuberculosis W/Beijing strains upregulate type I IFNs and increase expression of negative regulators of the Jak-Stat pathway. *J. Interferon Cytokine Res*. 25, 694–701 (2005).

69. Ordway, D. *et al.* The hypervirulent *Mycobacterium tuberculosis* strain HN878 induces a potent TH1 response followed by rapid down-regulation. *J. Immunol*. 179, 522–531 (2007).

70. Manca, C. *et al*. Virulence of a *Mycobacterium tuberculosis* clinical isolate in mice is determined by failure to induce Th1 type immunity and is associated with induction of IFN-α/β. *Proc. Natl Acad. Sci*. USA 98, 5752–5757 (2001).

71. Mayer-Barber, K. D. *et al*. Caspase-1 independent IL-1β production is critical for host resistance to *Mycobacterium tuberculosis* and does not require TLR signaling *in vivo. J. Immunol.* 184, 3326–3330 (2010).

72. Mayer-Barber, K. D. *et al.* Innate and adaptive interferons suppress IL-1α and IL-1β production by distinct pulmonary myeloid subsets during *Mycobacterium tuberculosis* infection. *Immunity* 35, 1023–1034 (2011).

73. de Paus, R. A. *et al.* Inhibition of the type I immune responses of human monocytes by IFN-α and IFN-β. *Cytokine* 61, 645–655 (2013).

74. Novikov, A. *et al. Mycobacterium tuberculosis* triggers host type I IFN signaling to regulate IL-1β production in human macrophages. *J. Immunol*. 187, 2540–2547 (2011).

75. McNab, F. W. *et al.* Type I IFN induces IL-10 production in an IL-27-independent manner and blocks responsiveness to IFN-γ for production of IL-12 and bacterial killing in *Mycobacterium tuberculosis*-infected macrophages. *J. Immunol*. 193, 3600–3612 (2014).

76. Uematsu S, Akira S. Toll-like receptors and innate immunity. *J Mol Med.* 84: 712-725 (2006).

77. C. Biondo, G. Mancuso, C. Beninati, C. Iaria, O. Romeo, A. Cascio, G. Teti. The role of endosomal toll-like receptors in bacterial recognition. *European Review for Medical and Pharmacological Sciences*. 16: 1506-1512 (2012).

78. Kawai, T., Adachi, O., Ogawi, T., Takeda, K., Akira, S. Unresponsiveness MyD88-deficient mice to endotoxin. *Immunity* 11, 115– 122 (1999).

79. Yamamoto, M., Sato, S., Hemmi, H., Sanjo, H., Uematsu, S., Kaisho, T., Hoshino, K., Takeuchi, O., Kobayashi, M., Fujita, T., Takeda, K., Akira, S. Essential role for TIRAP in activation of the signalling cascade shared by TLR2 and TLR4. *Nature* 420, 324–329 (2002).

80. Oshiumi, H., Matsumoto, M., Funami, K., Akazawa, T., Seya, T. TICAM-1, an adaptor molecule that participates in Toll-like receptor 3-mediated interferon-β induction. *Nature Immunol*. 4 (2), 161– 167 (2003).

81. Yammamoto, M., Sato, S., Mori, K., Hoshino, K., Takeuchi, O., Takeda, K., Sato, A. Cutting edge: a novel Toll/IL-1 receptor domaincontaining adaptor that preferentially activates the IFN-β promoter in the Toll-like receptor signalling. *J. Immunol*. 169, 6668–6672 (2002).

82. Ewald SE, Lee BL, Lau L, Wickliffe KE, Shi Gp, Chapman HA, Barton GM. The ectodomain of Toll-like receptor 9 is cleaved to generate a functional receptor. *Nature*; 456: 658-662 (2008).

83. Mancuso G, Gambuzza M, Midiri A, Biondo C, Papasergi S, Akira S, Teti G, Beninati C. Bacterial recognition by TLR7 in the lysosomes of conventional dendritic cells. *Nat Immunol*; 10: 587- 594 (2009).

84. Costa A, Gupta R, Signorino G, Malara A, Cardile F, Biondo C, Midiri A, Galbo R, Trieu-Cuot P, Papasergi S, Teti G, Henneke P, Mancuso G, Golenbock DT, Beninati C. Activation of the NLRP3 inflammasome by group B streptococci. *J Immunol*; 188: 1953-1960 (2012).

85. Nina Gratz, Harald Hartweger, Ulrich Matt, Franz Kratochvill, Marton Janos, Stefanie Sigel, Barbara Drobits, Xiao-Dong Li, Sylvia Knapp, Pavel Kovarik. Type I Interferon Production Induced by *Streptococcus pyogenes*-Derived Nucleic Acids Is Required for Host Protection. *PLoS Pathogens*; Volume 7; Issue 5; 1-16 (2011).

86. Wangsomboonsiri W, Luksananun T, Saksornchai S, Ketwong K, Sungkanuparph S. *Streptococcus suis* infection and risk factors for mortality. *J Infect*; 57: 392–396 (2008).

87. Ho Dang Trung N, Le Thi Phuong T, Wolbers M *et al*. Aetiologies of central nervous system infection in Viet Nam: a prospective provincial hospital-based descriptive surveillance study. *PLoS ONE*; 7: e37825 (2012).

88. Taylor WR, Nguyen K, Nguyen D *et al*. The spectrum of central nervous system infections in an adult referral hospital in Hanoi Vietnam. *PLoS ONE*; 7: e42099 (2012).

89. Staats JJ, Feder I, Okwumabua O, Chengappa MM. *Streptococcus suis*: past and present. *Vet Res Commun*; 21:381–407 (1997).

90. Marois C, Le Devendec L, Gottschalk M, Kobisch M. Detection and molecular typing of *Streptococcus suis* in tonsils from live pigs in France. *Can J Vet Res*; 71:14–22 (2007).

91. Huang YT, Teng LJ, Ho SW, Hsueh PR. *Streptococcus suis* infection. *J Microbiol Immunol Infect*; 38:306–13 (2005).

92. Higgins R, Gottschalk M. *Streptococcal diseases*. In: Straw BE, D'Allaire S, Mengeling WL, Taylor DJ (eds.) *Diseases of swine*. Ames, IA: *Iowa State University Press*: 769–783 (2006).

93. Sanford SE,Tilker ME. *Streptococcus suis* type II-associated diseases in swine: observations of a one-year study. *J Am Vet Med Assoc*; 181: 673–676 (1982).

94. Rosenkranz M, Elsner HA, Sturenburg HJ, Weiller C, Rother J, Sobottka I. *Streptococcus suis* meningitis and septicemia contracted from a wild boar in Germany. *J Neurol*; 250: 869–70 (2003).

95. Mazokopakis EE, Kofteridis DP, Papadakis JA, Gikas AH, Samonis GJ. First case report of *Streptococcus suis* septicaemia and meningitis from Greece. *Eur J Neurol*; 12: 487–89 (2005).

96. Matsuo H, Sakamoto S. Purulent meningitis caused by *Streptococcus suis* in a pig breeder. *Kansenshogaku Zasshi*; 77: 340–42 (2003).

97. Fongcom A, Pruksakorn S, Mongkol R, Tharavichitkul P, Yoonim N. *Streptococcus suis* infection in northern Thailand. *J Med Assoc Thai*; 84: 1502–08 (2001).

98. Zhang XY, Ding JQ, Qin HP. Clinical analysis of 22 cases of the disease contracted both by man and pigs with swine streptococcus infections. *J Trop Med*; 2: 361–63 (2002).

99. Tambyah PA, Kumarasinghe G, Chan HL, Lee KO. *Streptococcus suis* infection complicated by purpura fulminans and rhabdomyolysis: case report and review. *Clin Infect Dis*; 24: 710–12 (1997).

100. Watkins EJ, Brooksby P, Schweiger MS, Enright SM. Septicaemia in a pig-farm worker. *Lancet*; 357: 38 (2001).

101. Gottschalk M, Higgins R, Jacques M, Mittal KR, Henrichsen J. Description of 14 new capsular types of *Streptococcus suis*. *J Clin Microbiol*; 27: 2633–36 (1989).

102. Hill JE, Gottschalk M, Brousseau R, Harel J, Hemmingsen SM, Goh SH. Biochemical analysis, cpn60 and 16S rDNA sequence data indicate that *Streptococcus suis* serotypes 32 and 34, isolated from pigs, are *Streptococcus orisratti. Vet Microbiol*; 107: 63–69 (2005).

103. Costa AT, Lobato FC, Abreu VL, Assis RA, Reis R, Uzal FA. Serotyping and evaluation of the virulence in mice of *Streptococcus suis* strains isolated from diseased pigs. *Rev Inst Med Trop Sao Paulo*; 47: 113–15 (2005).

104. Higgins R, Gottschalk M, Mittal KR, Beaudoin M. *Streptococcus suis* infection in swine. A sixteen month study. *Can J Vet Res*; 54: 170–73 (1990).

105. Robertson ID, Blackmore DK. Prevalence of *Streptococcus suis* types 1 and 2 in domestic pigs in Australia and New Zealand. *Vet Rec*; 124: 391–94 (1989).

106. Gottschalk M, Segura M. The pathogenesis of the meningitis caused by *Streptococcus suis*: the unresolved questions. *Vet Microbiol*; 76: 259–72 (2000).

107. Arends JP, Hartwig N, Rudolphy M, Zanen HC. Carrier rate of *Streptococcus suis* capsular type 2 in palatine tonsils of slaughtered pigs. *J Clin Microbiol*; 20: 945–47 (1984).

108. Clifton-Hadley FA, Enright MR. Factors aff ecting the survival of Streptococcus suis type 2. *Vet Rec*; 114: 584–86 (1984).

109. Gottschalk M, Turgeon P, Higgins R, Beaudoin M, Bourgault AM. Susceptibility of *Streptococcus suis* to penicillin. *J Vet Diagn Invest*; 3: 170–72 (1991).

110. Prieto C, Garcia FJ, Suarez P, Imaz M, Castro JM. Biochemical traits and antimicrobial susceptibility of *Streptococcus suis* isolated from slaughtered pigs. *Zentralbl Veterinarmed B*; 41: 608–17 (1994).

111. Aarestrup FM, Jorsal SE, Jensen NE. Serological characterization and antimicrobial susceptibility of *Streptococcus suis* isolates from diagnostic samples in Denmark during 1995 and 1996. *Vet Microbiol*; 60: 59–66 (1998).

112. *Wellcome Trust Sanger Institute*. *Streptococcus suis*. http://www.sanger.ac.uk/Projects/S_suis (2006).

113. Smith HE, Wisselink HJ, Stockhofe-Zurwieden N, Vecht U, Smits MA. Virulence markers of *Streptococcus suis* type 1 and 2. *Adv Exp Med Biol*; 418: 651–55 (1997).

114. Serhir B, Dubreuil D, Higgins R, Jacques M. Purifi cation and characterization of a 52-kilodalton immunoglobulin G binding protein from *Streptococcus suis* capsular type 2. *J Bacteriol*; 177: 3830–36 (1995).

115. Smith HE, Damman M, Van Der Velde J, *et al*. Identifi cation and characterization of the cps locus of Streptococcus suis serotype 2: the capsule protects against phagocytosis and is an important virulence factor. *Infect Immun*; 67: 1750 56 (1999).

116. Smith HE, Buijs H, de Vries RR, Wisselink HJ, Stockhofe-Zurwieden N, Smits MA. Environmentally regulated genes of *Streptococcus suis*: identification by the use of ironrestricted conditions *in vitro* and by experimental infection of piglets. *Microbiology*; 147: 271–80 (2001).

117. Gruening P, Fulde M, Valentin-Weigand P, Goethe R. Structure, regulation, and putative function of the arginine deiminase system of *Streptococcus suis*. *J Bacteriol*; 188: 361–69 (2006).

118. Winterhoff N, Goethe R, Gruening P, *et al*. Identification and characterization of two temperature-induced surface associated proteins of *Streptococcus suis* with high homologies to members of the arginine deiminase system of *Streptococcus pyogenes*. *J Bacteriol*; 184: 6768–76 (2002).

119. Ma YZ, Fang WH, Ke CL, Zhang XF. Biological characteristics of isolates of *Streptococcus suis* type 2. *Chin J Vet Sci*; 23: 326–28 (2003).

120. Davies PR, Ossowicz CJ. Evaluation of methods used for detecting *Streptococcus suis* type 2 in tonsils, and investigation of the carrier state in pigs. *Res Vet Sci*; 50: 190–94 (1991).

121. Paterson RA, Robertson ID, Sanders RC, Siba PM, Clegg A, Hampson DJ. The carriage of *Streptococcus suis* type 2 by pigs in Papua New Guinea. *Epidemiol Infect*; 110: 71–78 (1993).

122. Robertson ID, Blackmore DK. Occupational exposure to *Streptococcus suis* type 2. *Epidemiol Infect*; 103: 157–64 (1989).

123. del Campo Sepulveda EM, Altman E, Kobisch M, D' Allaire S, Gottschalk M. Detection of antibodies against *Streptococcus suis* capsular type 2 using a purifi ed capsular polysaccharide antigenbased indirect ELISA. *Vet Microbiol*; 52: 113–25 (1996).

124. Higgins R, Gottschalk M. An update on *Streptococcus suis* identifi cation. *J Vet Diagn Invest*; 2: 249–52 (1990).

125. Guillaume Goyette-Desjardins, Jean-Philippe Auger, Jianguo Xu, Mariela Segura and Marcelo Gottschalk. *Streptococcus suis*, an important pig pathogen and emerging zoonotic agent—an update on the worldwide distribution based on serotyping and sequence typing. *Nature, Emerging Microbes and Infections* 3, e45; doi:10.1038/emi.2014.45 (2014).

126. Wilson TL, Jeffers J, Rapp-Gabrielson VJ *et al.* A novel signature-tagged mutagenesis system for *Streptococcus suis* serotype 2. *Vet. Microbiol.* 122(1–2), 135–145 (2007).

127. Marie J, Morvan H, Berthelot-Herault F *et al*. Antimicrobial susceptibility of *Streptococcus suis* isolated from swine in France and from humans in different countries between 1996 and 2000. *J Antimicrob Chemother*; 50: 201–209 (2002).

128. Alarcon P, Araya P, Aguayo C *et al*. [Laboratory confirmation of *Streptococcus suis* in Chile.] *Rev Chilena Infectol*; 30: 539–540 (2013).

129. Vela AI, Goyache J., Tarradas C. *et al*. Analysis of genetic diversity of *Streptococcus suis* clinical isolates from pigs in Spain by pulsed-field gel electrophoresis. *J Clin Microbiol*; 41: 2498–2502 (2003).

130. Tarradas C, Perea A, Vela AI *et al*. Distribution of serotypes of *Streptococcus suis* isolated from diseased pigs in Spain. *Vet Rec*; 154: 665–666 (2004).

131. Luque I, Blume V, Borge C *et al*. Genetic analysis of *Streptococcus suis* isolates recovered from diseased and healthy carrier pigs at different stages of production on a pig farm. *Vet J*; 186: 396–398 (2010).

132. Schultsz C, Jansen E, Keijzers W *et al*. Differences in the population structure of invasive *Streptococcus suis* strains isolated from pigs and from humans in The Netherlands. *PLoS One*; 7: e33854 (2012).

133. Fittipaldi N, Xu J, Lacouture S *et al*. Lineage and virulence of *Streptococcus suis* serotype 2 isolates from North America. *Emerg Infect Dis*; 17: 2239–2244 (2011).

134. King SJ, Leigh JA, Heath PJ *et al*. Development of a multilocus sequence typing scheme for the pig pathogen *Streptococcus suis*: identification of virulent clones and potential capsular serotype exchange. *J Clin Microbiol*; 40: 3671–3680 (2002).

135. Blume V, Luque I, Vela AI *et al.* Genetic and virulence-phenotype characterization of serotypes 2 and 9 of *Streptococcus suis* swine isolates. *Int Microbiol*; 12: 161– 166 (2009).

136. Chen L, Song Y, Wei Z, He H, Zhang A, Jin M. Antimicrobial susceptibility, tetracycline and erythromycin resistance genes, and multilocus sequence typing of *Streptococcus suis* isolates from diseased pigs in China. *J Vet Med Sci* ; 75: 583–587 (2013).

137. Zhu W, Wu C, Sun X *et al*. Characterization of *Streptococcus suis* serotype 2 isolates from China. *Vet Microbiol*; 166: 527–534 (2013).

138. Onishi H, SugawaraM, OkuraM, Osaki M, Takamatsu D. Prevalence of *Streptococcus suis* genotypes in isolates from porcine endocarditis in East Japan. *J Vet Med Sci*; 74: 1681–1684 (2012).

139. Princivalli MS, Palmieri C, Magi G *et al*. Genetic diversity of *Streptococcus suis* clinical isolates from pigs and humans in Italy (2003–2007). *Euro Surveill*; 14: pii: 19310 (2009).

140. Kerdsin A, Dejsirilert S, Puangpatra P *et al.* Genotypic profile of *Streptococcus suis* serotype 2 and clinical features of infection in humans, Thailand. *Emerg Infect Dis*; 17: 835–842 (2011).

141. Li W, Ye C, JingH *et al. Streptococcus suis* outbreak investigation using multiple-locus variable tandem repeat number analysis. *Microbiol Immunol*; 54: 380–388 (2010).

142. Luey CK, Chu YW, Cheung TK *et al.* Rapid pulsed-field gel electrophoresis protocol for subtyping of *Streptococcus suis* serotype 2. *J Microbiol Methods*; 68: 648–650 (2007).

143. Takamatsu D, Nishino H, Ishiji T *et al.* Genetic organization and preferential distribution of putative pilus gene clusters in *Streptococcus suis*. *Vet Microbiol*; 138: 132–139 (2009).

144. Chang B, Wada A, Ikebe T *et al.* Characteristics of *Streptococcus suis* isolated from patients in Japan. *Jpn J Infect Dis*; 59: 397–399 (2006).

145. Takamatsu D, Wongsawan K, Osaki M *et al*. *Streptococcus suis* in humans, Thailand. *Emerg Infect Dis*; 14: 181–183 (2008).

146. Kerdsin A, Dejsirilert S, Sawanpanyalert P *et al*. Sepsis and spontaneous bacterial peritonitis in Thailand. *Lancet*; 378: 960 (2011).

147. Nghia HD, Hoa NT, Linh le D *et al*. Human case of *Streptococcus suis* serotype 16 infection. *Emerg Infect Dis*; 14: 155–157 (2008).

148. Berthelot-Herault F, Gottschalk M, Morvan H, Kobisch M. Dilemma of virulence of *Streptococcus suis*: Canadian isolate 89-1591 characterized as a virulent strain using a standardized experimental model in pigs. *Can. J. Vet. Res.* 69(3), 236–240 (2005).

149. Nahuel Fittipaldi, Mariela Segura, Daniel Grenier & Marcelo Gottschalk. Virulence factors involved in the pathogenesis of the infection caused by the swine

pathogen and zoonotic agent *Streptococcus suis*. *Future Microbiol.* 7(2), 259–279 (2012).

150. Esgleas M, Lacouture S, Gottschalk M. *Streptococcus suis* serotype 2 binding to extracellular matrix proteins. *FEMS Microbiol. Lett.* 244(1), 33–40 (2005).

151. De Greeff A, Buys H, Verhaar R, Dijkstra J, Van Alphen L, Smith HE. Contribution of fibronectin-binding protein to pathogenesis of *Streptococcus suis* serotype 2. *Infect. Immun.* 70(3), 1319–1325 (2002).

152. Zhang A, Chen B, Mu X *et al.* Identification and characterization of a novel protective antigen, Enolase of *Streptococcus suis* serotype 2. *Vaccine* 27(9), 1348–1353 (2009).

153. Esgleas M, Dominguez-Punaro Mde L, Li Y, Harel J, Dubreuil JD, Gottschalk M. Immunization with SsEno fails to protect mice against challenge with *Streptococcus suis* serotype 2. *FEMS Microbiol. Lett.* 294(1), 82–88 (2009).

154. Ge J, Feng Y, Ji H *et al*. Inactivation of dipeptidyl peptidase IV attenuates the virulence of *Streptococcus suis* serotype 2 that causes streptococcal toxic shock syndrome. *Curr. Microbiol.* 59(3), 248–255 (2009).

155. Vanier G, Sekizaki T, Dominguez-Punaro MC *et al*. Disruption of *srtA* gene in *Streptococcus suis* results in decreased interactions with endothelial cells and extracellular matrix proteins. *Vet. Microbiol*. 127(3–4), 417–424 (2008).

156. Jobin MC, Brassard J, Quessy S, Gottschalk M, Grenier D. Acquisition of host plasmin activity by the swine pathogen *Streptococcus suis* serotype 2. *Infect. Immun.* 72(1), 606–610 (2004).

157. Brassard J, Gottschalk M, Quessy S. Cloning and purification of the *Streptococcus suis* serotype 2 glyceraldehyde-3-phosphate dehydrogenase and its involvement as an adhesin. *Vet. Microbiol.* 102(1–2), 87–94 (2004).

158. Wang K, Lu C. Adhesion activity of glyceraldehyde-3-phosphate dehydrogenase in a Chinese *Streptococcus suis* Type 2 strain. *Berl. Munch. Tierarztl. Wochenschr.* 120(5–6), 207- 209 (2007).

159. Tan C, Liu M, Jin M *et al.* The key virulence-associated genes of *Streptococcus suis* Type 2 are upregulated and differentially expressed *in vivo*. *FEMS Microbiol. Lett.* 278(1), 108–114 (2008).

160. Tan C, Fu S, Liu M *et al.* Cloning, expression and characterization of a cell wall surface protein, 6-phosphogluconate-dehydrogenase, of *Streptococcus suis* serotype 2. *Vet. Microbiol.* 130(3–4), 363–370 (2008).

161. Ferrando ML, Fuentes S, De Greeff A, Smith H, Wells JM. ApuA, a multifunctional α - glucan-degrading enzyme of *Streptococcus suis*, mediates adhesion to porcine epithelium and mucus. *Microbiology* 156(Pt 9), 2818–2828 (2010).

162. Wu T, Chang H, Tan C, Bei W, Chen H. The orphan response regulator RevSC21 controls the attachment of *Streptococcus suis* serotype-2 to human laryngeal epithelial cells and the expression of virulence genes. *FEMS Microbiol. Lett.* 292(2), 170–181 (2009).

163. Li J, Tan C, Zhou Y *et al.* The two-component regulatory system CiaRH contributes to the virulence of *Streptococcus suis* 2. *Vet. Microbiol.* 148(1), 99–104 (2011).

164. Pan X, Ge J, Li M *et al.* The orphan response regulator CovR: a globally negative modulator of virulence in *Streptococcus suis* serotype 2. *J. Bacteriol.* 191(8), 2601–2612 (2009).

165. Valentin-Weigand P. Intracellular invasion and persistence: survival strategies of *Streptococcus suis* and *Mycobacterium avium* ssp. paratuberculosis. *Berl. Munch. Tierarztl. Wochenschr.* 117(11–12), 459–463 (2004).

166. Gottschalk MG, Lacouture S, Dubreuil JD. Characterization of *Streptococcus suis* capsular type 2 haemolysin. *Microbiology* 141(Pt 1), 189–195 (1995).

167. Palmer M. The family of thiol-activated, cholesterol-binding cytolysins. *Toxicon* 39(11), 1681–1689 (2001).

168. Zhang A, Mu X, Chen B *et al.* Identification and characterization of IgA1 protease from *Streptococcus suis. Vet. Microbiol.* 140(1–2), 171–175 (2010).

169. Zhang A, Mu X, Chen B, Han L, Chen H, Jin M. IgA1 protease contributes to the virulence of *Streptococcus suis. Vet. Microbiol.* 148(2–4), 436–439 (2011).

170. Fittipaldi N, Sekizaki T, Takamatsu D *et al.* Significant contribution of the *pgdA* gene to the virulence of *Streptococcus suis. Mol. Microbiol.* 70(5), 1120–1135 (2008).

171. Fittipaldi N, Sekizaki T, Takamatsu D *et al.* D-alanylation of lipoteichoic acid contributes to the virulence of *Streptococcus suis. Infect. Immun.* 76(8), 3587–3594 (2008).

172. Vanier G, Segura M, Lecours MP, Grenier D, Gottschalk M. Porcine brain microvascular endothelial cell-derived interleukin-8 is first induced and then degraded by *Streptococcus suis. Microb. Pathog.* 46(3), 135–143 (2009).

173. Fontaine MC, Perez-Casal J, Willson PJ. Investigation of a novel DNase of *Streptococcus suis* serotype 2. *Infect. Immun.* 72(2), 774–781 (2004).

174. Wartha F, Beiter K, Normark S, Henriques- Normark B. Neutrophil extracellular traps: casting the NET over pathogenesis. *Curr. Opin. Microbiol.* 10(1), 52–56 (2007).

175. Aranda J, Garrido ME, Fittipaldi N *et al.* The cation-uptake regulators AdcR and Fur are necessary for full virulence of *Streptococcus suis. Vet. Microbiol.* 144(1–2), 246–249 (2010).

176. Li M, Wang C, Feng Y *et al.* SalK/SalR, a two-component signal transduction system, is essential for full virulence of highly invasive *Streptococcus suis* serotype 2. *PLoS ONE* 3(5), e2080 (2008).

177. Winterhoff N, Goethe R, Gruening P, Valentin-Weigand P. Response of *Streptococcus suis* to iron-restricted growth conditions at high and low oxygen availability. *Berl. Munch. Tierarztl. Wochenschr.* 117(7–8), 266–270 (2004).

178. Niven DF, Ekins A, Al-Samaurai AA. Effects of iron and manganese availability on growth and production of superoxide dismutase by *Streptococcus suis. Can. J. Microbiol.* 45(12), 1027–1032 (1999).

179. Wichgers Schreur PJ, Rebel JM, Smits MA, Van Putten JP, Smith HE. TroA of *Streptococcus suis* is required for manganese acquisition and full virulence. *J. Bacteriol*. 193(19), 5073–5080 (2011).

180. Aranda J, Teixedo L, Fittipaldi N *et al.* Inactivation of the gene encoding zincbinding lipoprotein 103 impairs the infectivity of *Streptococcus suis. Can. J. Vet. Res*. 76(1), 72–76 (2012).

181. Langford P, Williams AE, Kroll JS. Superoxide dismutases of pathogenic and non-pathogenic *Streptococcus suis* Type 2 isolates. *FEMS Microbiol. Lett.* 61(2–3), 347–350 (1991).

182. Tsiotou AG, Sakorafas GH, Anagnostopoulos G, Bramis J. Septic shock; current pathogenetic concepts from a clinical perspective. *Med. Sci. Monit.* 11(3), RA76–RA85 (2005).

183. Schreur PJ, Rebel JM, Smits MA, Van Putten JP, Smith HE. Differential activation of the Toll-like receptor 2/6 complex by lipoproteins of *Streptococcus suis* serotypes 2 and 9. *Vet. Microbiol.* 143(2–4), 363–370 (2010).

184. Wichgers Schreur PJ, Rebel JM, Smits MA, Van Putten JP, Smith HE. Lgt processing is an essential step in *Streptococcus suis* lipoprotein mediated innate immune activation. *PLoS ONE* 6(7), e22299 (2011).

185. Segura M, Vanier G, Al-Numani D, Lacouture S, Olivier M, Gottschalk M. Pro-inflammatory cytokine and chemokine modulation by *Streptococcus suis* in a whole-blood culture system. *FEMS Immunol. Med. Microbiol.* 47(1), 92–106 (2006).

186. Lun S, Perez-Casal J, Connor W, Willson PJ. Role of suilysin in pathogenesis of *Streptococcus suis* capsular serotype 2. *Microb. Pathog.* 34(1), 27–37 (2003).

187. Tanabe S, Gottschalk M, Grenier D. Hemoglobin and *Streptococcus suis* cell wall act in synergy to potentiate the inflammatory response of monocyte-derived macrophages. *Innate. Immun.* 14(6), 357–363 (2008).

188. Bonifait L, Grenier D. The SspA subtilisinlike protease of *Streptococcus suis* triggers a pro-inflammatory response in macrophages through a non-proteolytic mechanism. *BMC Microbiol.* 11, 47 (2011).

189. Rubin LL, Staddon JM. The cell biology of the blood–brain barrier. *Ann. Rev. Neurosci.* 22, 11–28 (1999).

190. Vanier G, Segura M, Gottschalk M. Characterization of the invasion of porcine endothelial cells by *Streptococcus suis* serotype 2. *Can. J. Vet. Res.* 71(2), 81–89 (2007).

191. Esgleas M, Li Y, Hancock MA, Harel J, Dubreuil JD, Gottschalk M. Isolation and characterization of α-enolase, a novel fibronectin-binding protein from *Streptococcus suis*. *Microbiol.* 154(Pt 9), 2668–2679 (2008).

192. Vanier G, Segura M, Friedl P, Lacouture S, Gottschalk M. Invasion of porcine brain microvascular endothelial cells by *Streptococcus suis* serotype 2. *Infect. Immun.* 72(3), 1441–1449 (2004).

193. Tenenbaum T, Papandreou T, Gellrich D *et al.* Polar bacterial invasion and translocation of *Streptococcus suis* across the blood–cerebrospinal fluid barrier *in vitro. Cell. Microbiol.* 11(2), 323–336 (2009).

194. Si Y, Yuan F, Chang H *et al.* Contribution of glutamine synthetase to the virulence of *Streptococcus suis* serotype 2. *Vet. Microbiol.* 139(1–2), 80–88 (2009).

195. Baums CG, Kaim U, Fulde M, Ramachandran G, Goethe R, Valentin- Weigand P. Identification of a novel virulence determinant with serum opacification activity in *Streptococcus suis. Infect. Immun.* 74(11), 6154–6162 (2006).

196. Li P, Liu J, Zhu L *et al. VirA*: a virulencerelated gene of *Streptococcus suis* serotype 2. *Microb. Pathog.* 49(5), 305–310 (2010).

197. Zhang H, Fan H, Lu C. Identification of a novel virulence-related gene in *Streptococcus suis* Type 2 strains. *Curr. Microbiol.* 61(6), 494–499 (2010).

198. De Greeff A, Buys H, Van Alphen L, Smith HE. Response regulator important in pathogenesis of *Streptococcus suis* serotype 2. *Microb. Pathog.* 33(4), 185–192 (2002).

199. Wang Y, Zhang W, Wu Z, Zhu X, Lu C. Functional analysis of *luxS* in *Streptococcus suis* reveals a key role in biofilm formation and virulence. *Vet. Microbiol.* 152(1–2), 151–160 (2011).

200. Willenborg J, Fulde M, De Greeff A *et al.* Role of glucose and CcpA in capsule expression and virulence of *Streptococcus suis. Microbiology* 157(Pt 6), 1823–1833 (2011).

201. Zheng F, Ji H, Cao M *et al.* Contribution of the Rgg transcription regulator to metabolism and virulence of *Streptococcus suis* serotype 2. *Infect. Immun.* 79(3), 1319–1328 (2011).

202. Li W, Hu X, Liu L, Chen H, Zhou R. Induction of protective immune response against *Streptococcus suis* serotype 2 infection by the surface antigen HP0245. *FEMS Microbiol. Lett.* 316(2), 115–122 (2011).

203. Chen B, Zhang A, Li R *et al.* Evaluation of the protective efficacy of a newly identified immunogenic protein, HP0272, of *Streptococcus suis. FEMS Microbiol. Lett.* 307(1), 12–18 (2010).

204. Zhang A, Chen B, Mu X *et al.* Identification of three novel *in vivo*-induced expressed antigens during infection with *Streptococcus suis* serotype 2. *FEMS Microbiol. Lett.* 295(1), 17–22 (2009).

205. Zhang A, Chen B, Li R *et al.* Identification of a surface protective antigen, HP0197 of *Streptococcus suis* serotype 2. *Vaccine* 27(38), 5209–5213 (2009).

206. Li Y, Martinez G, Gottschalk M *et al.* Identification of a surface protein of *Streptococcus suis* and evaluation of its immunogenic and protective capacity in pigs. *Infect. Immun.* 74(1), 305–312 (2006).

207. Shao Z, Pan X, Li X *et al.* HtpS, a novel immunogenic cell surface-exposed protein of *Streptococcus suis*, confers protection in mice. *FEMS Microbiol. Lett.* 314(2), 174–182 (2011).

208. Norton PM, Rolph C, Ward PN, Bentley RW, Leigh JA. Epithelial invasion and cell lysis by virulent strains of *Streptococcus suis* is enhanced by the presence of suilysin. *FEMS Immunol. Med. Microbiol.* 26(1), 25–35 (1999).

209. Lalonde M, Segura M, Lacouture S, Gottschalk M. Interactions between *Streptococcus suis* serotype 2 and different epithelial cell lines. *Microbiology* 146 (Pt 8), 1913–1921 (2000).

210. Benga L, Goethe R, Rohde M, Valentin-Weigand P. Non-encapsulated strains reveal novel insights in invasion and survival of *Streptococcus suis* in epithelial cells. *Cell. Microbiol.* 6(9), 867–881 (2004).

211. Tisoncik, J.R., Korth, M.J., Simmons, C.P., Farrar, J., Martin, T.R., Katze, M.G. Into the eye of the cytokine storm. *Microbiol. Mol. Biol. Rev*. 76, 16–32 (2012).

212. Berthelot-Herault F, Cariolet R, Labbe A, Gottschalk M, Cardinal JY, Kobisch M. Experimental infection of specific pathogen free piglets with French strains of *Streptococcus suis* capsular Type 2. *Can. J. Vet. Res.* 65(3), 196–200 (2001).

213. Kim KS. Microbial translocation of the blood–brain barrier. *Int. J. Parasitol.* 36(5), 607–614 (2006).

214. Benga L, Friedl P, Valentin-Weigand P. Adherence of *Streptococcus suis* to porcine endothelial cells. *J. Vet. Med. B. Infect. Dis. Vet. Pub. Health* 52(9), 392–395 (2005).

215. Wewer C, Seibt A, Wolburg H *et al.* Transcellular migration of neutrophil granulocytes through the blood–cerebrospinal fluid barrier after infection with *Streptococcus suis*. *J. Neuroinflammation* 8, 51 (2011).

216. Dominguez-Punaro MC, Segura M, Plante MM, Lacouture S, Rivest S, Gottschalk M. *Streptococcus suis* serotype 2, an important swine and human pathogen, induces strong systemic and cerebral inflammatory responses in a mouse model of infection. *J. Immunol.* 179(3), 1842–1854 (2007).

217. Zheng H, Punaro MC, Segura M *et al.* Toll-like receptor 2 is partially involved in the activation of murine astrocytes by *Streptococcus suis*, an important zoonotic agent of meningitis. *J. Neuroimmunol.* 234(1–2), 71–83 (2011).

218. Vadeboncoeur N, Segura M, Al-Numani D, Vanier G, Gottschalk M. Pro-inflammatory cytokine and chemokine release by human brain microvascular endothelial cells stimulated by *Streptococcus suis* serotype 2. *FEMS Immunol. Med. Microbiol.* 35(1), 49–58 (2003).

219. Dominguez-Punaro Mde L, Segura M, Contreras I *et al. In vitro* characterization of the microglial inflammatory response to *Streptococcus suis*, an important emerging zoonotic agent of meningitis. *Infect. Immun.* 78(12), 5074–5085 (2010).

220. Jobin MC, Gottschalk M, Grenier D. Upregulation of prostaglandin E2 and matrix metalloproteinase 9 production by human macrophage-like cells: synergistic effect of capsular material and cell wall from *Streptococcus suis*. *Microb. Pathog.* 40(1), 29–34 (2006).

221. Al-Numani D, Segura M, Dore M, Gottschalk M. Up-regulation of ICAM-1, CD11a/CD18 and CD11c/CD18 on human THP-1 monocytes stimulated by *Streptococcus suis* serotype 2. *Clin. Exp. Immunol.* 133(1), 67–77 (2003).

222. Madsen LW, Bak H, Nielsen B, Jensen HE, Aalbaek B, Riising HJ. Bacterial colonization and invasion in pigs experimentally exposed to *Streptococcus suis* serotype 2 in aerosol. *J. Vet. Med. B. Infect. Dis. Vet. Pub. Health* 49(5), 211–215 (2002).

223. Wilson SM, Norton P, Haverson K, Leigh J, Bailey M. Interactions between *Streptococcus suis* serotype 2 and cells of the myeloid lineage in the palatine tonsil of the pig. *Vet. Immunol. Immunopathol.* 117(1–2), 116–123 (2007).

224. Charland N, Kellens JT, Caya F, Gottschalk M. Agglutination of *Streptococcus suis* by sialic acid-binding lectins. *J. Clin. Microbiol.* 33(8), 2220–2221 (1995).

225. Van Calsteren MR, Gagnon F, Lacouture S, Fittipaldi N, Gottschalk M. Structure determination of *Streptococcus suis* serotype 2 capsular polysaccharide. *Biochem. Cell Biol.* 88(3), 513–525 (2010).

226. Andresen LO, Tegtmeier C. Passive immunization of pigs against experimental infection with *Streptococcus suis* serotype 2. *Vet. Microbiol.* 81(4), 331–344 (2001).

227. Charland N, Kobisch M, Martineau-Doize B, Jacques M, Gottschalk M. Role of capsular sialic acid in virulence and resistance to phagocytosis of *Streptococcus suis* capsular Type 2. *FEMS Immunol. Med. Microbiol.* 14(4), 195–203 (1996).

228. Geng H, Zhu L, Yuan Y *et al.* Identification and characterization of novel immunogenic proteins of *Streptococcus suis* serotype 2. *J. Proteome Res.* 7(9), 4132–4142 (2008).

229. Li Y, Gottschalk M, Esgleas M *et al.* Immunization with recombinant Sao protein confers protection against *Streptococcus suis* infection. *Clin. Vacc. Immunol.* 14(8), 937–943 (2007).

230. Chabot-Roy G, Willson P, Segura M, Lacouture S, Gottschalk M. Phagocytosis and killing of *Streptococcus suis* by porcine neutrophils. *Microb. Pathog.* 41(1), 21–32 (2006).

231. Segura M, Gottschalk M. *Streptococcus suis* interactions with the murine macrophage cell line J774: adhesion and cytotoxicity. *Infect. Immun.* 70(8), 4312–4322 (2002).

232. Graveline R, Segura M, Radzioch D, Gottschalk M. TLR2-dependent recognition of *Streptococcus suis* is modulated by the presence of capsular polysaccharide which modifies macrophage responsiveness. *Int. Immunol.* 19(4), 375–389 (2007).

233. Lecours MP, Segura M, Lachance C *et al.* Characterization of porcine dendritic cell response to *Streptococcus suis. Vet. Res.* 42(1), 72 (2011).

234. Scheld WM, Koedel U, Nathan B, Pfister HW. Pathophysiology of bacterial meningitis: mechanism(s) of neuronal injury. *J. Infect. Dis.* 186(Suppl. 2), S225–S233 (2002).

235. Tang Y, Zhao H, Wu W, Wu D, Li X, Fang W. Genetic and virulence characterization of *Streptococcus suis* type 2 isolates from swine in the provinces of Zhejiang and Henan, China. *Folia Microbiol* (Praha); 56: 541–548 (2011).

236. Chen C, Tang J, Dong W *et al.* A glimpse of streptococcal toxic shock syndrome from comparative genomics of *S. suis* 2 Chinese isolates. *PLoS One*; 2: e315 (2007).

237. Vecht U, Wisselink HJ, Jellema ML, Smith HE. Identification of two proteins associated with virulence of *Streptococcus suis* type 2. *Infect Immun*; 59: 3156–3162 (1991).

238. Vela AI, Aspiroz C, Fortuno B *et al*. Meningitis caused by an unusual genotype (ST3) of *Streptococcus suis*. *Infection*; 41: 701–703 (2013).

239. Medzhitov, R. Origin and physiological roles of inflammation. *Nature* 454, 428-435 (2008).

240. Osamu Takeuchi, Shizuo Akira. Pattern Recognition Receptors and Inflammation. *Cell* 140, 805–820 (2010).

241. Akira, S., Uematsu, S., and Takeuchi, O. Pathogen recognition and innate immunity. *Cell* 124, 783–801 (2006).

242. Takeuchi, O., and Akira, S. Innate immunity to virus infection. *Immunol. Rev.* 227, 75–86 (2009).

243. Inohara, Chamaillard, McDonald, C., and Nunez, G. NOD-LRR proteins: role in host-microbial interactions and inflammatory disease. *Annu. Rev. Biochem*. 74, 355–383 (2005).

244. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell* 140: 805–820 (2010).

245. Kawai T, Akira S. The roles of TLRs, RLRs and NLRs in pathogen recognition. *Int Immunol* 21: 317–337 (2009).

246. Shida K, Kiyshima-Shibata J, Kaji R, Nagaoka M, Nanno M. Pepdidoglycan from lactobacilli inhibits interleukin-12 production by macrophages induced by *Lactobacillus casei* through Toll-like receptor 2- dependent and independent mechanisms. *Immunology* 128: e858- 869 (2009).

247. Lachance C. *et al*. Exacerbated Type II Interferon Response Drives Hypervirulence and Toxic Shock by an Emergent Epidemic Strain of *Streptococcus suis*. *Infection and Immunity* 1928–1939, Volume 81, Number 6 (2013).

248. Greer, J. M., and M. B. Less. Myelin proteolipid protein- the first 50 years. *Int. J. Biochem. Cell. Biol.* 34: 211–215 (2002).

249. T.T. Tien le, T. Nishibori, Y. Nishitani, R. Nomoto, R. Osawa. Reappraisal of the taxonomy of *Streptococcus suis* serotypes 20, 22, 26, and 33 based on DNA–DNA homology and *sodA* and *recN* phylogenies. *Vet. Microbiol*., 162, pp. 842–849 (2013).

250. R. Nomoto, F. Maruyama, S. Ishida, M. Tohya, T. Sekizaki, R. Osawa. Reappraisal of the taxonomy of *Streptococcus suis* serotypes 20, 22 and 26: *Streptococcus parasuis* sp. *Nov. Int. J. Syst. Evol. Microbiol*., 65, pp. 438–443 (2015).

251. Ishida *et al*. Development of an appropriate PCR system for the reclassification of *Streptococcus suis*. *Journal of Microbiological Methods*, Vol.107, Pages 66–70 (2014).

252. Liu *et al*. Development of Multiplex PCR Assays for the Identification of the 33 Serotypes of *Streptococcus suis. PLOS ONE,* Vol. 8, Issue 8, e72070 (2013).

253. Lili Bi *et al.* Toll-likereceptor 4 confers inflammatory response to Suilysin. *Frontiers in Microbiology*, Vol. 6, Art. 644 (2015).

254. Pandey *et al*. Microbial sensing by Toll-Like Receptors and Intracellular Nucleic Acid Sensors. *Cold Spring Harbor Laboratory Press,* 7:a016246 (2015).

255. Tian *et al.* Poly I:C enhances susceptibility to secondary pulmonary infections by Gram-Positive bacteria. *PLOS ONE,* Vol.7, Issue 9, e41879 (2012).

256. D. Parker, A. Prince. *Staphylococcus aureus* induces type I interferon signaling in dendritic cells via TLR9. *J. Immunol*. 189(8): 4040-4046. doi: 10.4049/jimmunol.1201055 (2012).

257. Sotolongo *et al.* Host innate recognition of an intestinal bacterial pathogen induces TRIF-dependent protective immunity. *J. Exp. Med*, Vol 208, No. 13, 2705-2716 (2011).

258. Lecours *et al*. Immune receptors involved in Streptococcus suis recognition by dendritic cells. *PLOS ONE*, Vol. 7, Issue 9, e44746 (2012).

259. Zheng *et al.* The rol of Toll-like receptors in the pathogenesis of *Streptococcus suis*. *Veterinary Microbiology* 156; 147- 156 (2012).

260. Lachance *et al*. Toll-like Receptor 2- Independent host innate immune response against an epidemic strain of *Streptococcus suis* that causes a toxic shock-like syndrome in humans. *PLOS ONE,* Vol. 8, Issue 5, e65031 (2013).

261. Yuhai Bi *et al.* Assessment of the pathogenesis of *Streptococcus suis* type 2 infection in piglets for understanding streptococcal toxic shock-like syndrome, meningitis and sequelae. *Veterinary Microbiology* 173; 299-309 (2014).

262. Lecours MP *et al*. Critical Role for *Streptococcus suis* Cell Wall Modifications and Suilysin in Resistance to Complement-Dependent Killing by Dendritic Cells. *J Infect Dis* 204: 919–929 (2011).

263. Integrated DNA Technologies. [http://www.idtdna.com/Scitools/Applications/Primerquest/] (2012).

264. Slater JD, Allen AG, May JP, Bolitho S, Lindsay H and Maskell DJ. Mutagenesis of *Streptococcus equi* and *Streptococcus suis* by transposon Tn917. *Vet Microbiol* 93:197-206 (2003).

265. Kobisch M, Gottschalk M, Morvan P, Cariolet G, Bénévent G and Joly JP. Experimental infection of SPF piglets with *Streptococcus suis* serotype 2. *J Rech Porcine Fr* 27:97-102 (1995).

266. Quessy S, Dubreuil JD, Caya M, Higgins R. Discrimination of virulent and avirulent *Streptococcus suis* capsular type 2 isolates from different geographical origins. *Infect Immun.* 63:1975–1979 (1995).

267. Ye C *et al. Streptococcus suis* sequence type 7 outbreak, Sichuan, China. *Emerg. Infect. Dis*. 12:1203–1208 (2006).

268. Kaisho *et al*. Regulation of dendritic cell function through Toll-Like receptors. *Current Molecular Medicine* 3: 373-385 (2003).

269. Lecours *et al*. Sialylation of *Streptococcus suis* serotype 2 is essential for capsule expression but is not responsible for the main capsular epitope. *Elsevier, Microbes and Infection*, Vol. 14, Issue 11, 941-950 (2012).

270. Zheng *et al*. Eight Novel Capsular Polysaccharide Synthesis Gene Loci Identified in Nontypeable Streptococcus suis Isolates. *Applied and Environmental Microbiology*, Vol. 81, Num. 12 (2015).

271. Gottschalk *et al*. Production and characterization of two *Streptococcus suis* capsular type 2 mutants. *Elsevier, Veterinary Microbiology*, Vol. 30, 59-71 (1992).

272. Athey *et al*. Determining *Streptococcus suis* serotype from short-read whole-genome sequencing data. *BMC Microbiology* 16 :162 (2016).

273. Kerdsin *et al.* Emergence of *Streptococcus suis* serotype 9 infection in humans. *Journal of Microbiology, Immunology and Infection* (2015).

274. Segura *et al*. Initial steps of the pathogenesis of the infection caused by *Streptococcus suis* : fighting against nonspecific defenses. *FEBS Letters* (2016).

275. Segura M. Fisher scientific award lecture - the capsular polysaccharides of group B *Streptococcus* and *Streptococcus suis* differently modulate bacterial interactions with dendritic cells. *Can J Microbiol*.; 58(3):249-60 (2012).

276. Trinchieri G. Type I interferon: friend or foe? *J Exp Med*.; 207(10):2053-63 (2010).

277. Honda K, Taniguchi T. IRFs: master regulators of signalling by Toll-like receptors and cytosolic pattern-recognition receptors. *Nat Rev Immunol*.; 6(9):644-58 (2006).

278. Gruenberg J, van der Goot FG. Mechanisms of pathogen entry through the endosomal compartments. *Nat Rev Mol Cell Biol*.;7(7):495-504 (2006).

279. Macia E, Ehrlich M, Massol R, Boucrot E, Brunner C, Kirchhausen T. Dynasore, a cell-permeable inhibitor of dynamin. *Dev Cell*.;10(6):839-50 (2006).

280. Hertzog PJ. Overview. Type I interferons as primers, activators and inhibitors of innate and adaptive immune responses. *Immunol Cell Biol*.; 90(5):471-3 (2012).

281. Cavaillon J-M, Adib-Conquy M. The pro-inflammatory cytokine cascade. In: Marshall JC, Cohen J, editors. Immune Response in the Critically Ill. Berlin, Heidelberg: *Springer Berlin Heidelberg*; p. 37-66 (2002).

282. Dorhoi A, Yeremeev V, Nouailles G, Weiner J, 3rd, Jorg S, Heinemann E *et al*. Type I IFN signaling triggers immunopathology in tuberculosis-susceptible mice by modulating lung phagocyte dynamics. Eur J Immunol.;44(8):2380-93 (2014).

283. LeMessurier KS, Hacker H, Chi L, Tuomanen E, Redecke V. Type I interferon protects against pneumococcal invasive disease by inhibiting bacterial transmigration across the lung. *PLoS Pathog*.;9(11):e1003727 (2013).

284. Auger J-P, Fittipaldi N, Benoit-Biancamano M-O, Segura M, Gottschalk M. Virulence studies of different sequence types and geographical origins of *Streptococcus suis* serotype 2 in a mouse model of infection. *Pathogens* ;5(3):48 (2016).

285. Ye C, Zheng H, Zhang J, Jing H, Wang L, Xiong Y *et al*. Clinical, experimental, and genomic differences between intermediately pathogenic, highly pathogenic, and epidemic *Streptococcus suis*. *J Infect Dis*.;199(1):97-107 (2009).

286. Holden MT, Hauser H, Sanders M, Ngo TH, Cherevach I, Cronin A *et al*. Rapid evolution of virulence and drug resistance in the emerging zoonotic pathogen *Streptococcus suis*. *PLoS One*.;4(7):e6072 (2009).

287. Schmitz F, Heit A, Guggemoos S, Krug A, Mages J, Schiemann M *et al*. Interferon-regulatory-factor 1 controls Toll-like receptor 9-mediated IFN-beta production in myeloid dendritic cells. *Eur J Immunol.*;37(2):315-27 (2007).