Eur J Clin Microbiol Infect Dis
DOI 10.1007/s10096-012-1581-2

ARTICLE

Severe cochlear inflammation and vestibular syndrome in an experimental model of *Streptococcus suis* infection in mice

M. C. Domínguez-Punaro · U. Koedel · T. Hoegen · C. Demel · M. Klein · M. Gottschalk

Received: 22 October 2011 / Accepted: 4 February 2012

Abstract Hearing impairment is a common and frequently permanent sequel of *Streptococcus suis* meningitis in humans. Nevertheless, mechanisms underlying the development of cochlear damage have not been addressed so far. In the present work, we characterized a mouse model of suppurative labyrinthitis and meningitis induced by a systemic infection with *S. suis* and studied the impact of the injected bacterial dosage on the progression of such inflammatory events. We observed that high infection doses of bacteria lead to sustained bacteremia, with an increase in the permeability of the blood–labyrinth and blood–brain barriers, causing suppurative labyrinthitis and meningitis, respectively. However, in mice infected with a low dose of *S. suis*, bacteria disappeared quickly from blood, hence, cochlear inflammation and meningitis were not consistent features. This model of *S. suis* infection seems ideal to evaluate novel drugs that may help alleviate the negative consequences of such important sequelae of *S. suis*-induced meningitis and labyrinthitis.

Electronic supplementary material The online version of this article (doi:10.1007/s10096-012-1581-2) contains supplementary material, which is available to authorized users.

M. C. Domínguez-Punaro · M. Gottschalk
Groupe de Recherche sur les Maladies Infectieuses du Porc and Centre de Recherche en Infectiologie Porcine, Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada

U. Koedel · T. Hoegen · C. Demel · M. Klein
Department of Neurology, Klinikum Grosshadern, Ludwig Maximilians University Munich, Munich, Germany

M. Gottschalk (✉)
Faculté de Médecine Vétérinaire, Université de Montréal, 3200 Sicotte, Saint-Hyacinthe, Quebec J2S 2M2, Canada
e-mail: marcelo.gottschalk@umontreal.ca

Present Address:
M. C. Domínguez-Punaro
Immunology-Oncology Section, Research Center, Maisonneuve-Rosemont Hospital, Montreal, Québec, Canada

Introduction

Streptococcus suis is currently considered as one of the most relevant disease-evoking pathogens in the swine industry worldwide, and also an important emerging agent of zoonosis. In pigs, the most frequent pathologies of *S. suis* infections include meningitis, septicemia, arthritis, pneumonia, and endocarditis [1]. As a human pathogen, *S. suis* is responsible for meningitis, labyrinthitis, septicemia, toxic shock-like syndrome, and endocarditis [2, 3]. Traditionally, *S. suis* infections have been documented in people working in close contact with pigs or raw pork products, thus, it was considered to be a risk in countries where pig rearing is common [4, 5]. However, the classical picture of *S. suis* infection as an occupational disease has drastically changed, as large outbreaks have taken place in Asian countries [2, 6]. In fact, *S. suis* is currently the primary cause of adult meningitis in Vietnam, the second in Thailand, as well as the third most common cause of community-acquired bacterial meningitis in Hong Kong [7, 8]. People who survive *S. suis* infection may be handicapped, as severe post-infection sequels, mainly permanent hear impairing, frequently develop [9, 10].

Although research on *S. suis* pathogenesis has increased in recent years, there are still many unsolved questions, rendering the control of infection and prevention of sequels difficult. Whereas it is believed that *S. suis* enters through

Published online: 01 March 2012

the respiratory route with further colonization of the tonsils in swine, infections occur through skin cuts and/or the oral route in humans [2, 11, 12]. For research purposes, infection of adult mice of *S. suis* via the intraperitoneal route has been established as a model exhibiting typical features of the disease. This model has successfully allowed to study the development of septicemia and meningitis and to test the in vivo relevance of *S. suis* virulence factors [13–15]. Using this model, it has been established that, once in the bloodstream, virulent *S. suis* will colonize different tissues and organs, with high mortality associated to septic shock characterized by an exaggerated systemic production of proinflammatory cytokines [13–15]. In cases where the host survives septicemia, *S. suis* may still invade the central nervous system (CNS), where it generally induces the upregulation of different proinflammatory genes, including Toll-like receptor 2 (TLR2), CD14, IκBα (an index of NF-κB expression), interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α), and monocyte chemoattractant protein 1 (MCP-1). It has recently been confirmed that microglia and astrocytes are largely responsible for the orchestration of such an inflammatory response [15–17]. As a consequence, meningitis and encephalitis develop, leading to clinical signs of neurologic disease.

Similar to other important meningitis-associated pathogens, sensorineural hearing loss is the most frequent long-term complication of *S. suis* infection in humans [5, 18]. Meningitis is most severe in the high-frequency hearing range. Retrospective studies documented that 47.7% to 73% of the surviving meningitis cases suffer persistent sensorineural hearing loss and that 50% demonstrate vestibular impairment [5, 19]. Although the cochlea has been recognized as the primary site of damage, the mechanisms involving labyrinthitis and vestibular damage are uncertain, and it has been hypothesized that they are caused by some ototoxins from bacteria [18].

Here, we studied the histomorphological alterations that occur in the cochlea in our mouse model of systemic infection of *S. suis*. We evaluated the integrity of the blood–labyrinth barrier, verified neuronal injury in spiral ganglions, and assessed the impact of the infective bacterial loads in the development of cochlear damage and its association with CNS inflammation.

Materials and methods

Bacterial strain and growth conditions

S. suis serotype 2 strain 31533 was used for all experimental infections. This encapsulated, hemolysin (suilysin)-positive virulent strain was originally isolated from a case of porcine meningitis and has been used in previous studies [15, 20, 21]. Bacteria were grown overnight on sheep blood agar plates at 37°C and isolated colonies were inoculated into 5 ml of Todd–Hewitt broth (THB) (Difco Laboratories, Detroit, MI, USA), which was incubated for 8 h at 37°C with agitation. Working cultures were prepared by transferring 10 μl of 1/1,000 dilutions of 8-h cultures into 30 ml of THB, which was incubated for 16 h at 37°C with agitation. Stationary-phase bacteria were washed twice in phosphate-buffered saline (PBS; pH 7.3). The bacterial pellet was then resuspended and adjusted to a concentration of 5×10^8 CFU/ml. For experimental infections, inoculums were diluted in THB to obtain the different final concentrations specified below. Final suspensions were plated onto blood agar to accurately determine the CFU/ml.

Mice and experimental models of *S. suis* meningitis

Female 6- to 8-week-old CD1 mice (Charles River Laboratories, Saint-Constant, Québec, Canada) were acclimatized to standard laboratory conditions of 12-h light/12-h dark cycles with free access to rodent chow and water. All experiments involving mice were conducted in accordance with the guidelines and policies of the Canadian Council on Animal Care and the principles set forth in the Guide for the Care and Use of Laboratory Animals, and approved by the Animal Welfare Committee of the Université de Montréal. For the high-dose (HD) intraperitoneal systemic model of meningitis (i.p.), 50 mice were infected with 1 ml of 5×10^7 CFU/ml of *S. suis*. For the low-dose (LD) i.p. group, 30 mice were infected with 1×10^7 CFU/ml of *S. suis* [15, 20]. Animals infected i.p. were monitored daily and sacrificed at specific time-points (see the Results section). Sampling included mice with no clinical signs that were randomly chosen and mice with clinical signs of CNS disease. In the case of the transcutaneal intracisternal model of meningitis (i.c.), five mice were anaesthetized with inhaled isofluorane (Halocarbon Products Corporation, River Edge, NJ, USA) and they then received 20 μl of 1×10^7 CFU/ml of *S. suis* (final concentration of 2×10^5 CFU/mouse). Animals were allowed to wake up and were sacrificed 24 h later. Controls were injected with 1 ml (i.p.) or 20 μl (i.c.) of the vehicle solution (sterile THB) [22].

Determination of viable bacteria in the blood

At each designated time, mice were anesthetized with CO_2 and blood was collected by cardiac puncture. Then, 50 μl of 10^{-1} to 10^{-6} dilutions of blood in PBS were plated onto blood agar plates. All samples were plated using an Autoplate 400 Automated Spiral Plater (Spiral Biotech). Blood agar plates were incubated overnight at 37°C. Colonies were counted and expressed as CFU/ml.

Histology

Temporal bones and brains were dissected and fixed in formalin 4% for four days. Once fixed, temporal bones were decalcified in PBS containing 10% EDTA. Seven-μm sections of formalin-fixed, paraffin-embedded mouse temporal bones and brains were deparaffinized, rehydrated, and stained with Mayer's hematoxylin. The severity of the inflammatory changes in different cochlear structures and brain sections was evaluated on a scale of 0 (no changes) to 4 (severe changes), as previously described [23]. The area of each section of the spiral ganglion in the basal turn of the cochlear was measured using the UTHSCSA ImageTool software version 3.0 (University of Texas, San Antonio, TX, USA) and intact-appearing spiral ganglion neuron (criteria: round cell body containing a nucleus and homogenous cytoplasm) were counted within. The spiral ganglion neuronal density was expressed as neurons/0.01 mm^2 [23]. Three sections were investigated for each cochlea and the mean was calculated for each readout parameter.

Determination of blood–labyrinth barrier permeability

One hour before sacrifice, 300 μl of Evans Blue dye (EB) (Sigma-Aldrich, St. Louis, MO, USA) in PBS (pH 7.3) was applied by intravenous (i.v.) injections into the dorsal veins of the tail. EB extravasation in the cochlea (spiral limbus, spiral ligament, stria vascularis, and cochlear bone) was detected by fluorescence microscopy (excitation filter 545 nm, barrier filter 590 nm). Images of 7-μm temporal bone sections were digitized using a black and white VarioCam video system (PCO CDD Imaging, Kelheim, Germany).

Statistical analysis

EB extravasations, inflammatory changes, and spiral ganglion neuronal density were evaluated in a blinded fashion. Survival curves for the different treatment groups were compared using the log-rank test. The Mann–Whitney test served to compare bacterial loads in blood and neuron counts in spiral ganglions between HD and LD groups. A p-value of <0.05 was considered to be statistically significant. All analyses were performed using the SigmaPlot system (v.11; Systat Software, San Jose, CA, USA).

Results

Presence of *S. suis* in blood

The numbers of *S. suis* in blood differed between the HD or LD groups (Fig. 1). In the HD group, the bacterial blood counts stayed elevated from the first 5 days post-infection (PI), ranging from a mean of 5×10^8 CFU/ml during the first 2 days PI) to 1×10^7 CFU/ml at day 3 PI and 2×10^4 CFU/ml at day 5 PI, thus, independently of the presence or not of clinical disease. HD mice sampled at later time-points presented significantly lower bacterial counts (less than 10^3 CFU/ml), which was also not related to the severity of their clinical status (data not shown). On the other hand, *S. suis* blood counts in LD mice were considerably lower, have similar kinetics, with a mean of 1×10^5 CFU/ml at day 1 PI, ~1×10^4 CFU/ml between days 2 and 3 PI, and 1×10^1 CFU/ml at day 5 PI (Fig. 1). Statistical differences in bacterial loads in blood between the HD and LD mouse groups were found at 1, 2, 3, and 5 days PI ($p<0.05$). No bacteria were recovered in any of the mice from the HD or LD groups considered as healthy at the end of the experiment (15 days PI).

Clinical behavior in mice infected with high and low doses of *S. suis*

Clinical behavior differed, especially in intensity, between the group of mice i.p. infected with HD or LD of *S. suis*. Table 1 summarizes the main clinical results from both groups. Clinical signs of septicemia, meningitis, or neurological disease in mice infected with *S. suis* have been previously described [15]. In the HD group, eight mice had died (16% mortality). From days 1 to 3 PI, all mice from the HD group presented clear clinical signs associated to septic shock, consisting of depression, piloerection, hunchback, and swollen/closed eyelids [15]. Despite the fact that a further ten mice died at days 2 and 3 PI, no exact percentage of mortality was assessed, as the sampling of mice started at day 1 PI, with a total of 17 HD mice used for

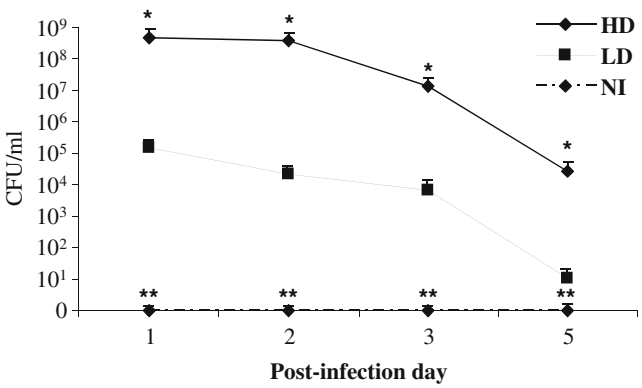

Fig. 1 Bacterial loads in blood from mice infected intraperitoneally with a high dose (*HD*) or a low dose (*LD*) of *Streptococcus suis* or non-infected controls (*NI*). Bacterial loads are expressed as CFU/ml. The results are expressed as mean (± standard error of the mean [SEM]) and represent at least three infected mice per post-infection (PI) time-point. * indicates significant differences between the HD and LD groups ($p<0.05$), while ** indicates significant differences between the NI and HD and LD groups ($p<0.001$)

Table 1 Summary of the clinical signs and sampling from mice infected with high and low doses of *Streptococcus suis*

Number of mice	Group	
	High dose (HD)	Low dose (LD)
Total per group	50	30
Dead on day 1 PI	8 (16%)	0 (0%)
Main clinical features of septicemia (days 1–3 PI)	Death, depression, piloerection, hunchback, and swollen/closed eyelids	Piloerection and swollen eyelids
Number of sampled mice (day 1 to end of experiment)	17	25
Sampled mice with clinical signs of M/VS	8 (47%)	5 (20%)
Sampled mice with cochlear damage	12 (71%)	10 (40%)
Sampled mice with cochlear and CNS damage	13 (76%)	2 (8%)
Day of appearance of clinical signs of M/VS	Day 3 PI	Day 5 PI
Main clinical features of M/VS	Hyperexcitation, opisthotonus, tilted head, walking in circles, spinning while in recumbency	
Number of remaining mice (end of experiment)	14	4 (plus one mouse found dead on day 12)

CNS, central nervous system; PI, post-infection; M, meningitis; VS, vestibular syndrome

this purpose. From days 3 to 15 PI (47% of sampled mice), eight mice from the HD group developed clear clinical signs of CNS disease and/or vestibular syndrome. These signs consisted mainly of hyperexcitation, hyperextension of the neck (opisthotonus), tilted head, walking in circles, and spinning while in recumbency (see supplemental video 1) [15]. At the end of the experiment, 14 HD mice remained, all of them considered as healthy based on clinical signs (Table 1). Contrary to the HD group, the LD group did not present deaths associated to septicemia during the first 24 h PI ($p<0.022$). Moreover, during the first 3 days PI, clinical signs were present, although milder in the LD group compared to the HD group, consisting mainly of slight and transitory piloerection and swollen eyelids. A total of 25 LD mice were taken for sampling throughout the experiment, though only five animals (20% of sampled mice) developed clear neurological signs from days 5 to 15 PI [15]. At the end of the experiment, there were four remaining mice, all of which were considered as healthy (Table 1).

S. suis infection induces strong inflammatory changes in the cochlea and blood–labyrinth barrier disruption

In order to evaluate the evolution of *S. suis* infection in the cochlea, inflammatory and degenerative changes were arbitrarily classified into four categories according to the severity of the cochlear damage (no damage, light, medium, and severe damage). In the HD group, 12 out of 17 samples presented different degrees of inner ear inflammation. At day 1 PI, neither inflammatory nor degenerative lesions (spiral ganglion neuronal damage) were observed in any of the three mice sampled. The first inflammatory changes were observed on day 2 PI, still during the acute phase of septicemia, 2 out of 3 HD sampled mice had cochlear damage that was considered as light, with fibrinous exudate, some neutrophils, and bacteria in the lumen of the different scalae (vestibuli, media, and tympani), and involved basal to apical turns of the cochlea, though no signs of neurological disease were recorded in any of these mice (Fig. 2A). At day 3 PI, 1 of 4 HD sampled mice showed clinical signs associated to meningitis, though cochlea damage was present in 3 of 4 mice. At day 5 PI, 5 of 5 sampled mice presented with clinical meningitis and labyrinthitis. In mice sampled on days 3 and 5 PI, inner ear damages varied from medium to severe. Histopathological analysis revealed a strong suppurative infiltration (mainly granulocytes) at the different scalae (Fig. 2B). Inflammatory changes affected the spiral prominence and stria vascularis, the latter structure with evident damage to epithelial cells (Fig. 2D). The pattern of inflammation suggested that infection spread from the scala tympani into the scala media, affecting firstly the organ of Corti, causing loss of hair cells (Fig. 2C). From days 7 to 15 PI, two more HD mice showed clear clinical signs of meningitis, which progressed with time and, when sampled, ear infection was severe and chronic, with polymorphonuclear cells replaced by macrophages and proliferation of a well-irrigated fibrotic matrix, causing partial to total occlusion of the perilymphatic spaces (early stage of labyrinthitis ossificans). Interestingly, scalae vestibuli and media presented cyst-like structures filled with proteinaceous material, which seemed to originate from the Reissner's membrane. Moreover, damage was not restrained to the cochlea, as there was infiltration by inflammatory cells at the perilymphatic spaces of the vestibular system (Fig. 2E).

The time until the manifestation of inner ear lesions in the LD group differed from the HD group, although the morphology of lesions was similar. Only 10 out of 25 sampled mice throughout the experiment presented inner ear lesions.

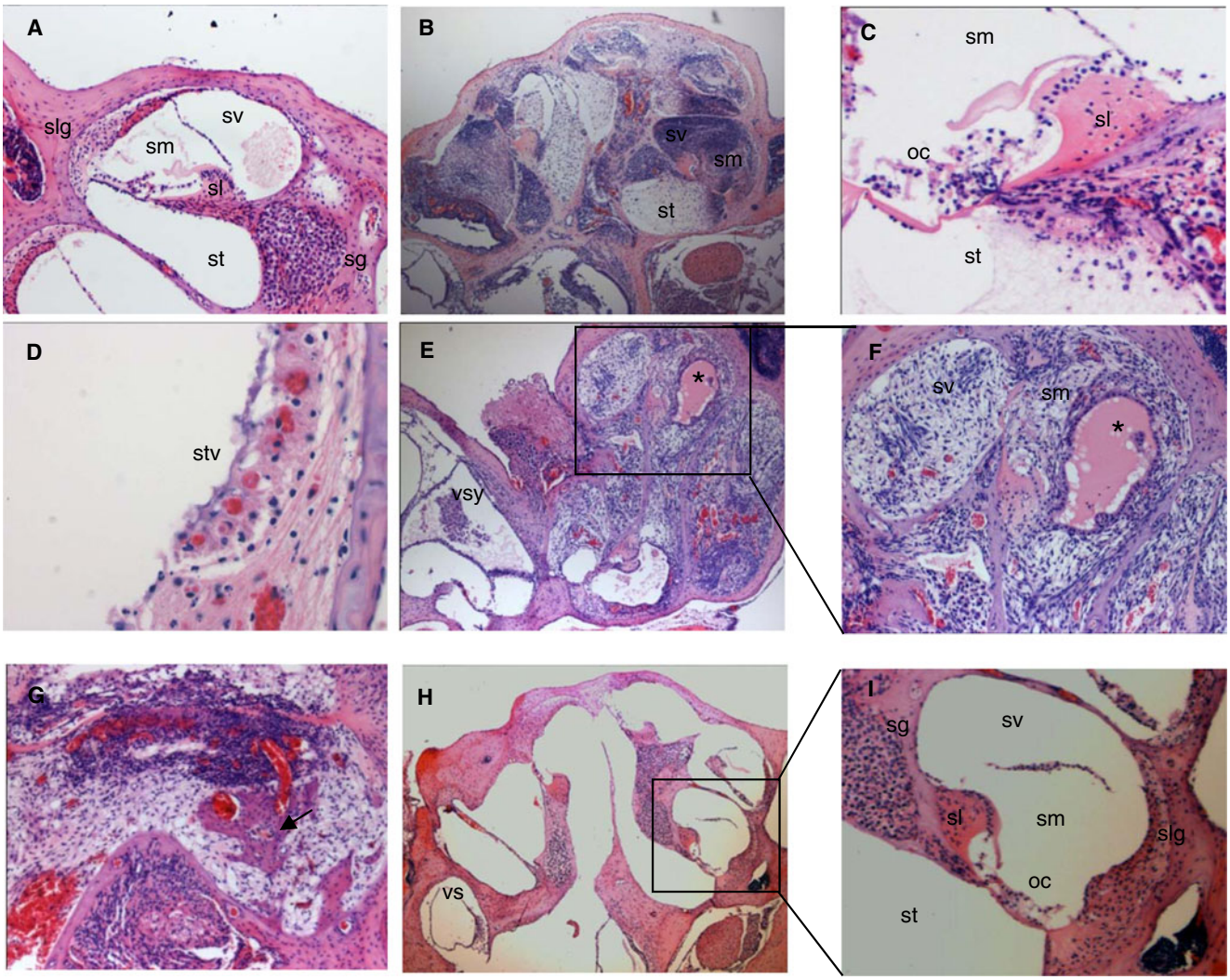

Fig. 2 Images illustrating the inflammatory score in the cochlea in mice infected intraperitoneally with *S. suis*: **A** 2 days post-infection (PI), light inflammation towards the pinna of the cochlea (×25); **B** 5 days PI, medium inflammatory changes (×70); **C** 5 days PI, medium inflammation, detail of the spiral limbus and organ of Corti with loss of hair cells (×1,000); **D** 5 days PI, medium inflammation, diminishment of epithelial cells in the stria vascularis (×1,000); **E** 15 days PI, severe inflammation, loss of cochlear structures and fibrous obliteration, a pseudocyst and inflammation at the vestibular system (×25); **F** Detail of **E** showing the pseudocyst and fibroblast matrix (×70); **G** 15 days PI, severe inflammation, proliferation of fibroblasts and bone tissue (labyrinthitis ossificans) (×70); **H** and **I** non-infected mouse with no inflammatory or degenerative changes (×25 and ×70). *oc* organ of Corti, *sg* spiral ganglion, *sl* spiral limbus, *slg* spiral ligament, *sm* scala media, *st* scala tympani, *sv* scala vestibuli, *stv*, stria vascularis, *vsy* vestibular system, * pseudocyst, *arrow* bone tissue

No histopathological lesions could be observed in the cochleae of any of the four mice examined on day 1 and only 1 of 4 of the mice sampled on day 2 PI presented medium cochlear damage. By day 3 PI, no inner ear damage was recorded in any of the four sampled mice. However, by day 5 PI, 3 of 5 sampled mice presented inflammatory changes classified as light to medium. As infection progressed, by days 7 to 10 PI, 4 of 6 sampled mice had developed inner ear changes considered as severe (data not shown). At 2 weeks PI, two more LD mice were sampled, which presented clear clinical signs of meningitis and vestibular syndrome. These animals had developed severe degenerative changes leading to the destruction of scalae, proliferation of a bone matrix mixed with fibroblasts, macrophages, and lymphocytes, and important loss of spiral ganglions (Fig. 2G). No inflammatory or degenerative changes were observed in uninfected controls (Fig. 2H, I).

LD and HD mice that developed cochlear damage also presented increased vascular permeability of the blood–labyrinth barrier in comparison to non-infected controls. This was evidenced by EB extravasation, in particular at the spiral ligament, the stria vascularis, and the spiral limbus (Fig. 3A, B). Vascular damage persisted up to 2 weeks after infection.

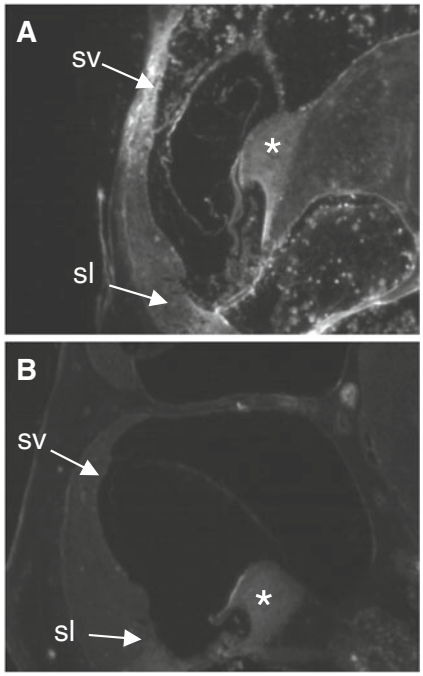

Fig. 3 Evans Blue (EB) extravasation as detected by fluorescence microscopy, indicating blood–labyrinth barrier disruption. **A** Mouse infected with HD *S. suis*, 5 days PI, medium inflammation animal with medium inflammation in the cochlea **B** Non-infected control. * spiral limbus, *sl* spiral ligament, *sv* stria vascularis

S. suis-associated damage to the cochlea is accompanied by neuronal ganglion loss

Inflammatory changes at the different levels of the cochlea were accompanied by damage to spiral ganglions, independently of the bacterial dose. These structures presented a considerable loss of neurons that increased as inflammation and destruction of inner ear structures progressed. Indeed, the neuron numbers in animals sampled that did not present cochlear damage were similar to those of uninfected controls (Table 2). However, those mice with cochlear damage classified from light (Fig. 4A) to severe (Fig. 4B) presented significantly reduced ganglion neuron counts in comparison to uninfected controls (Fig. 4C) ($p<0.05$ and $p<0.001$, respectively).

Table 2 Spiral ganglion neuronal count in the basal turn of the cochlea in mice intraperitoneally infected with a high dose or a low dose of *S. suis*. Data are shown as mean (± standard error of the mean [SEM])

Severity of damage	Mean count (neurons/frame)		
	High dose	Low dose	Controls
No damage	36±6.5	33±6.5	34±4
Light	24±1.2	25±4.3	
Medium	17±5.3	18±2.3	
Severe	18±6.18	17±5.4	

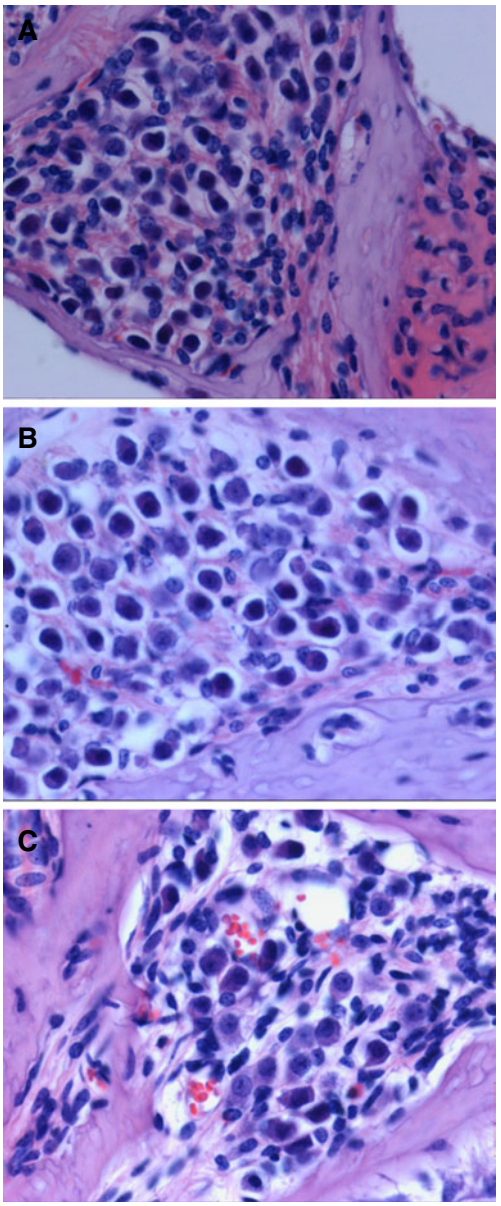

Fig. 4 Images (×500) from normal spiral ganglions from: **A** an uninfected control with no cochlear damage and normal neuronal density, **B** a mouse infected with a high dose of *S. suis* at 5 days PI with sensible neuronal loss, and **C** a mouse infected with a high dose of *S. suis* 2 weeks PI with severe neuronal loss

Cochlear damage in *S. suis* infection may be independent of central nervous system damage

One of the most interesting findings in this mouse model of *S. suis* infection is the fact that, in the HD group, sustained bacteremia lead to meningitis and/or encephalitis and suppurative labyrinthitis in most of the cases. Indeed, between days 1 and 2 PI, two mice suffered from both CNS damage and labyrinthitis, while another two presented inflammation only at the CNS. Lesions consisted of discrete foci of neutrophils infiltrating the meninges and small foci of necrosis. As

infection progressed (from day 3 PI onwards), 11 of 11 HD sampled mice had clinical signs of cochlear inflammation and meningitis/encephalitis. CNS lesions consisted of evident foci of gliosis and areas of necrosis of the brain parenchyma, mainly at the corpus callosum, accompanied by infiltration of the meninges and choroid plexus by polymorphonuclear cells and macrophages. On the other hand, in the LD group, in striking contrast to the HD group, CNS damage was a sporadic feature in LD mice suffering from labyrinthitis. In detail, only 1 of 14 sampled mice at days 1–3 PI presented with cochlea inflammation and a discrete inflammation of the meninges. However, from day 5 PI onwards, 9 of 11 mice had developed labyrinthitis, but only one of these mice also presented discrete meningoencephalitis.

Comparison of the intracisternal and intraperitoneal routes of infection for the development of cochlear inflammation by *S. suis*

The pattern of cochlear inflammation observed in mice infected with *S. suis* by the i.p. route suggested that labyrinthitis was the result of direct damage to labyrinth blood vessels during the septicemic phase of the infection. In order to verify this hypothesis, studies were conducted to compare cochlear damage obtained using the i.p. route with that obtained using the i.c. route of infection, in which bacteria is delivered directly into the subarachnoid space [23]. To achieve this objective, five mice were anesthetized and infected by the i.c. route with a dose of 2×10^5 CFU [24] and kept for only 24 h. At 12 h PI, all mice infected by the i.c. route were depressed, with rough hair coat, suggesting impaired consciousness. At 24 h PI, all of them were lethargic and tried to rest their heads against the floor of their cages. Histopathological examination at 24 h showed inflammatory changes in the cochleas of all i.c. infected animals. Protein precipitations and bacteria were observed predominantly in the turns towards the apex (Fig. 5A, B). As for the degree of inflammation, three animals presented light inflammatory changes (Fig. 5A), while another two showed medium damage (Fig. 5B). However, and in striking contrast with cochlear damage induced by *S. suis* inoculated by the i.p. route, inflammation was more pronounced towards the basal turns of the cochlea, and the scala tympani was more affected than the scala media and scala vestibuli (Fig. 5A, B), suggesting that infection spread through the cochlear aqueduct [23]. Interestingly, no inflammatory changes were observed in the vestibular system of any mice infected by the i.c. route.

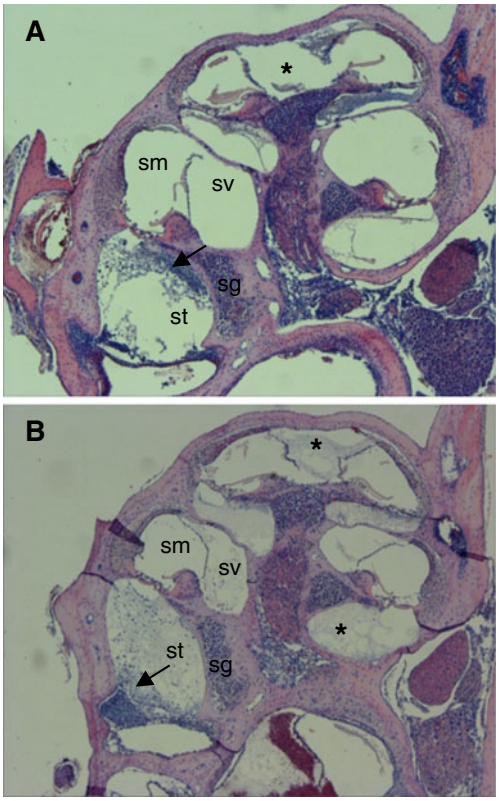

Fig. 5 Images from the cochleas of mice infected with *S. suis* using the intracisternal (i.c.) route. Inflammation is more accentuated at the basal turns of the cochlea (×25). **A** Light inflammatory infiltrate. **B** Medium inflammatory infiltrate. *sg* spiral ganglion, *sm* scala media, *st* scala tympani, *sv* scala vestibuli, * pseudocyst, *arrow* bone tissue

Discussion

The main purpose of this study was to evaluate the inflammatory changes in the cochlea during systemic *S. suis* infection using a mouse model of infection leading to septicemia and meningitis. We observed that the amount of bacteria injected intraperitoneally has a direct impact on clinical outcome and associated pathologies, including CNS inflammation and cochlear damage. In mice infected with high amounts of bacteria, *S. suis* loads remained high in the blood during the first 5 days PI, while in the group that was infected with a low dose, bacteria diminished quickly from the circulation. High loads of *S. suis* in the blood were associated with stronger clinical signs of septicemia and death of mice during the first 24 h PI. Interestingly, the difference of the challenge dose was half a log only. Moreover, below a certain bacterial threshold used to infect mice (1×10^6 CFU/animal), no clinical signs or lesions are observed and bacteria are quickly eliminated from the bloodstream by the innate immune system (unpublished observations). Only in a small number of mice infected with LD was *S. suis* able to reach the CNS and cause very limited local inflammation, while in mice that received high doses of bacteria, CNS inflammation and clinical signs of associated disease were a constant feature. This supports the hypothesis that high and sustained numbers of *S. suis* in blood circulation are necessary to efficiently trespass the

blood–brain barrier during septicemia and cause relevant CNS inflammation [15].

Interestingly, in mice infected with HD of *S. suis*, CNS inflammation was accompanied in most of the cases by cochlear inflammation. On the contrary, meningoencephalitis associated with cochlear damage was an infrequent finding in animals infected with LD of *S. suis*. This would suggest that cochlear invasion by *S. suis* and further local inflammation may occur during septicemia and that it may be independent of meningitis. In fact, in humans infected with *S. suis*, although labyrinthitis and deafness are traditionally considered as a consequence of meningitis, there are reports indicating that, at least in some cases, cochlear inflammation by this pathogen may occur without CNS inflammation [25]. In both HD and LD groups, cochlear and CNS inflammation was documented between 1 and 15 days PI. The most consistent clinical signs during this period were the sudden appearance of hyperexcitation, opisthotonus, and rolling, which have been associated to *S. suis* invasion of CNS structures [15] and vestibular syndrome in mice [26].

These findings are in accordance with humans recovering from *S. suis* meningitis, that is concomitant to cochlear inflammation, also suffer from vestibular damage, characterized by ataxia and vertigo [19, 27]. Infected mice developed suppurative labyrinthitis that comprised even from the earliest lesions, the perilymphatic spaces at different turns of the cochlea, with the presence of proteinaceous material, suppurative inflammatory cells and bacteria. These inflammatory changes are very similar to those observed in pigs experimentally infected with *S. suis*, which develop otitis interna characterized by a mixed inflammatory exudate comprising neutrophils, lymphocytes, and, less often, macrophages in the perilymphatic ducts of the cochlea and perineuritis along the vestibulocochlear nerve [28].

Inflammatory events at the cochlea were accompanied by blood–barrier disruption and affected highly vascularized structures [23], which may increase the passage of more bacteria and leukocytes into the cochlear perilymphatic structures, thus, exacerbating inflammation and affecting the delicate structures related to hearing, such as the organ of Corti, associated hair cells, and the spiral limbus. Cytokine production during septicemia, including TNF-α and IL-1β [15], may increase the permeability of endothelial walls, affecting the labyrinth blood–barrier and causing cochlear tissue invasion. Cell wall and/or secreted bacterial virulence factors, such as lipoteichoic acid, peptidoglycan, and suilysin, may also facilitate bacterial colonization of different tissues [13, 14, 29], including the cochlea. Rats experimentally infected with *S. pneumoniae* develop a similar pathology in inner ear structures, as there is disruption of the blood–labyrinth barrier and extravasation of inflammatory cells in different structures of the cochlea [23]. As inflammation progressed, the density of spiral ganglion neurons diminished considerably. It has been proposed that neuronal injury may be the result of the local production of reactive nitrogen species and reactive oxygen radicals [30]. Exacerbated inflammation may hamper the conduction of impulses of cochlear nerves via synapses between hair cells and axons of spiral ganglion neurons, and, finally, to central processing in the auditory cortex, thus, causing hearing impairment [31]. With time, perilymphatic spaces were occupied by the proliferation of fibroblasts and blood vessels characteristic of irreversible labyrinthitis ossificans, which has already been diagnosed in humans infected by *S. suis* [19].

In the present model of i.p. labyrinthitis by *S. suis*, damage involved different turns of the cochlea. This represents a remarkable difference to the pattern of labyrinthitis observed in rodents infected by the i.c. route with other meningitis-associated bacteria, such as *S. pneumoniae*. In such cases, damage is usually restricted to the perilymphatic spaces and accentuated in the basal turn of the scala tympani, suggesting that inflammation spreads through the cochlear aqueduct, which connects the middle cranial fossa to the basal turn of the scala tympani [23, 24]. In order to test if the pattern of damage observed in animals infected with *S. suis* by the i.p. route was the result of the inoculation route and not due to a particular characteristic of this pathogen, mice were also infected with *S. suis* by the i.c. route. Similarly to the observations from the same model with *S. pneumoniae*, *S. suis* infected mice developed suppurative labyrinthitis that was accentuated in the basal turns of the cochlea, with particular involvement of the scala tympani [23, 24]. These differences in the pattern of cochlear inflammatory changes after intraperitoneal and intracisternal infection suggests two different ways of the spread of infection: during septicemia, infection seems to spread hematogenously, leading to an evenly distributed cochlear inflammation; in contrast, intracisternal infection and resulting primary inflammation of the subarachnoid space seems to spread to the cochlea via the perilymphatic duct, which opens into the basal turn of the cochlea. In consequence, inflammation is aggravated in the basal turn of the cochlea and hearing loss is most severe in the high-frequency hearing range.

The use of the i.p. route of infection to study the pathogenesis of *S. suis* infection may provide some advantages to models of bacterial labyrinthitis/meningitis, as it better mimics a natural infection with increased permeability of the blood–brain barrier and possible damage to the epithelial cells from the choroid plexus (blood–cerebrospinal fluid barrier) [32, 33], leading to the development of clear clinical signs of CNS disease. Moreover, animals challenged with meningeal pathogens using the intravenous or i.p. routes usually develop bacteremia and septicemia, with the rapid death of infected animals with modest, if any, brain pathology [34, 35].

All the clinical and histopathological data of inner ear damage by *S. suis* in CD1 mice documented in our results

provide additional evidence for the clinical relevance of this experimental model. Furthermore, it seems to be an excellent tool for future research on *S. suis*-induced hearing loss and deafness. Besides serving to study the participation of different inflammatory pathway mechanisms of injury in *S. suis* infection, it seems useful for the investigation of novel drugs that may help alleviate the negative consequences of *S. suis* septicemia and meningitis.

Acknowledgments We thank Prof. H. Pfister for his interest and helpful comments and Barbara Angele for the technical support (Klinikum Grosshadern, Ludwig Maximilians University Munich, Germany). This work was supported by grants from the National Science and Engineering Research Council of Canada (NSERC) to M.G. (grant 154280 and Discovery Accelerator Supplement). The financial aid from the Deutsche Forschungsgemeinschaft (DFG), the University of Munich (FöFoLe), the Friedrich Baur Stiftung, and the Else Kroener Fresenius Foundation (EKFS) to M.K. and U.K. is highly appreciated. M.C.D.P. is the recipient of an International Training Program Award from Fonds Québécois de la Recherche sur la Nature et les Technologies (FQRNT).

References

1. Higgins R, Gottschalk M (2006) Streptococcocal diseases. In: Straw BE, D'Allaire S, Mengeling WL, Taylor DJ (eds) Diseases of swine. Blackwell Publishing, Ames, IA, pp 769–783
2. Gottschalk M, Segura M, Xu J (2007) *Streptococcus suis* infections in humans: the Chinese experience and the situation in North America. Anim Health Res Rev 8:29–45
3. Tang J, Wang C, Feng Y, Yang W, Song H, Chen Z, Yu H, Pan X, Zhou X, Wang H, Wu B, Wang H, Zhao H, Lin Y, Yue J, Wu Z, He X, Gao F, Khan AH, Wang J, Zhao GP, Wang Y, Wang X, Chen Z, Gao GF (2006) Streptococcal toxic shock syndrome caused by *Streptococcus suis* serotype 2. PLoS Med 3:e151
4. Arends JP, Zanen HC (1988) Meningitis caused by *Streptococcus suis* in humans. Rev Infect Dis 10:131–137
5. Wertheim HFL, Trung Nghia HD, Taylor W, Schultsz C (2009) *Streptococcus suis*: an emerging human pathogen. Clin Infect Dis 48:617–625
6. Segura M (2009) *Streptococcus suis*: an emerging human threat. J Infect Dis 199:4–6
7. Mai NT, Hoa NT, Nga TV, Linh LD, Chau TT, Sinh DX, Phu NH, Chuong LV, Diep TS, Campbell J, Nghia HD, Minh TN, Chau NV, de Jong MD, Chinh NT, Hien TT, Farrar J, Schultsz C (2008) *Streptococcus suis* meningitis in adults in Vietnam. Clin Infect Dis 46:659–667
8. Hui AC, Ng KC, Tong PY, Mok V, Chow KM, Wu A, Wong LK (2005) Bacterial meningitis in Hong Kong: 10-years' experience. Clin Neurol Neurosurg 107(5):366–370
9. Wangkaew S, Chaiwarith R, Tharavichitkul P, Supparatpinyo K (2006) *Streptococcus suis* infection: a series of 41 cases from Chiang Mai University Hospital. J Infect 52:455–460
10. Wangsomboonsiri W, Luksananun T, Saksornchai S, Ketwong K, Sungkanuparph S (2008) *Streptococcus suis* infection and risk factors for mortality. J Infect 57:392–396
11. Bahloul H, Mofredj A, Mrabet A, Gineyt G, Rousselier P (2008) *Streptococcus suis* meningitis after oral contamination? Med Mal Infect 38:281–282
12. Gottschalk M, Xu J, Calzas C, Segura M (2010) *Streptococcus suis*: a new emerging or an old neglected zoonotic pathogen? Future Microbiol 5:371–391
13. Fittipaldi N, Sekizaki T, Takamatsu D, Harel J, Domínguez-Punaro MC, Von Aulock S, Draing C, Marois C, Kobisch M, Gottschalk M (2008) D-alanylation of lipoteichoic acid contributes to the virulence of *Streptococcus suis*. Infect Immun 76:3587–3594
14. Fittipaldi N, Sekizaki T, Takamatsu D, Domínguez-Punaro MC, Harel J, Bui NK, Vollmer W, Gottschalk M (2008) Significant contribution of the *pgdA* gene to the virulence of *Streptococcus suis*. Mol Microbiol 70:1120–1135
15. Domínguez-Punaro MC, Segura M, Plante MM, Lacouture S, Rivest S, Gottschalk M (2007) *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 179:1842–1854
16. Domínguez-Punaro MC, Segura M, Contreras I, Lachance C, Houde M, Lecours MP, Olivier M, Gottschalk M (2010) In vitro characterization of the microglial inflammatory response to *Streptococcus suis*, an important emerging zoonotic agent of meningitis. Infect Immun 78(12):5074–5085
17. Zheng H, Domínguez-Punaro MC, Segura M, Lachance C, Rivest S, Xu J, Houde M, Gottschalk M (2011) Toll-like receptor 2 is partially involved in the activation of murine astrocytes by *Streptococcus suis*, an important zoonotic agent of meningitis. J Neuroimmunol 234:71–83
18. Rusmeechan S, Sribusara P (2008) *Streptococcus suis* meningitis: the newest serious infectious disease. J Med Assoc Thai 91:654–658
19. Navacharoen N, Chantharochavong V, Hanprasertpong C, Kangsanarak J, Lekagul S (2009) Hearing and vestibular loss in *Streptococcus suis* infection from swine and traditional raw pork exposure in northern Thailand. J Laryngol Otol 123:857–862
20. Domínguez-Punaro MC, Segura M, Radzioch D, Rivest S, Gottschalk M (2008) Comparison of the susceptibilities of C57BL/6 and A/J mouse strains to *Streptococcus suis* serotype 2 infection. Infect Immun 76:3901–3910
21. Kobish M, Gottschalk M, Morvan P, Cariolet R, Bénévent G, Joloy JP (1995) Experimental infection of SPF piglets with *Streptococcus suis* serotype 2. J Rech Porc Fr 27:97–101
22. Koedel U, Rupprecht T, Angele B, Heesemann J, Wagner H, Pfister HW, Kirschning CJ (2004) MyD88 is required for mounting a robust host immune response to *Streptococcus pneumoniae* in the CNS. Brain 127:1437–1445
23. Klein M, Koedel U, Pfister HW, Kastenbauer S (2003) Morphological correlates of acute and permanent hearing loss during experimental pneumococcal meningitis. Brain Pathol 13:123–132
24. Klein M, Schmidt C, Kastenbauer S, Paul R, Kirschning CJ, Wagner H, Popp B, Pfister HW, Koedel U (2007) MyD88-dependent immune response contributes to hearing loss in experimental pneumococcal meningitis. J Infect Dis 2007(195):1189–1193
25. Tambyah PA, Kumarasinghe G, Chan HL, Lee KO (1997) *Streptococcus suis* infection complicated by purpura fulminans and rhabdomyolysis: case report and review. Clin Infect Dis 24:710–712
26. Kohn DF, MacKenzie WF (1980) Inner ear disease characterized by rolling in C3H mice. J Am Vet Med Assoc 177:815–817
27. McNeil NI, Gordon T (1986) Meningitis caused by *Streptococcus suis* type II. Postgrad Med J 62:743–744
28. Madsen LW, Svensmark B, Elvestad K, Jensen HE (2001) Otitis interna is a frequent sequela to *Streptococcus suis* meningitis in pigs. Vet Pathol 38:190–195
29. Lun S, Perez-Casal J, Connor W, Willson P (2003) Role of suilysin in pathogenesis of *Streptococcus suis* capsular serotype 2. Microb Pathog 34:27–37

30. Klein M, Koedel U, Pfister HW (2006) Oxidative stress in pneumococcal meningitis: a future target for adjunctive therapy? Prog Neurobiol 80:269–280
31. Wakabayashi K, Fujioka M, Kanzaki S, Okano HJ, Shibata S, Yamashita D, Masuda M, Mihara M, Ohsugi Y, Ogawa K, Okano H (2010) Blockade of interleukin-6 signaling suppressed cochlear inflammatory response and improved hearing impairment in noise-damaged mice cochlea. Neurosci Res 66:345–352
32. Koedel U, Scheld WM, Pfister HW (2002) Pathogenesis and pathophysiology of pneumococcal meningitis. Lancet Infect Dis 2:721–736
33. Tenenbaum T, Papandreou T, Gellrich D, Friedrichs U, Seibt A, Adam R, Wewer C, Galla HJ, Schwerk C, Schroten H (2009) Polar bacterial invasion and translocation of *Streptococcus suis* across the blood-cerebrospinal fluid barrier in vitro. Cell Microbiol 11:323–336
34. Koedel U, Pfister HW (1999) Models of experimental bacterial meningitis. Role and limitations. Infect Dis Clin North Am 13:549–577
35. Tsao N, Chang WW, Liu CC, Lei HY (2002) Development of hematogenous pneumococcal meningitis in adult mice: the role of TNF-alpha. FEMS Immunol Med Microbiol 32:133–140