Delayed Invasion of the Kidney and Brain by *Borrelia crocidurae* in Plasminogen-Deficient Mice

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*Borrelia crocidurae* is an etiologic agent of relapsing fever in Africa and is transmitted to humans by the bite of soft ticks of the genus *Ornithodoros*. The role of the plasminogen (Plg) activation system for the pathogenicity of *B. crocidurae* was investigated by infection of Plg-deficient (plg<sup>−/−</sup>) and Plg wild-type (plg<sup>+/+</sup>) mice. No differences in spirochetaemia were observed between the plg<sup>−/−</sup> and plg<sup>+/+</sup> mice. However, signs indicative of brain invasion, such as neurological symptoms and/or histopathological changes, were more common in plg<sup>+/+</sup> mice. Quantitative immunohistochemical analysis demonstrated infection of spirochetes in kidney interstitium and brain as soon as 2 days postinoculation. Lower numbers of extravascular spirochetes in plg<sup>−/−</sup> mice during the first days of infection suggested a less efficient invasion mechanism in these mice than in the plg<sup>+/+</sup> mice. The invasion of the kidneys in plg<sup>−/−</sup> mice produced no significant inflammation, as seen by quantitative immunohistochimistry of the CD45 common leukocyte marker. However, significant kidney inflammation was observed with infection in the plg<sup>+/+</sup> mice. In brain, inflammation was more severe in plg<sup>+/+</sup> mice than in plg<sup>−/−</sup> mice, and the numbers of CD45<sup>+</sup> cells increased significantly with duration of infection in the plg<sup>+/+</sup> mice. The results show that invasion of brain and kidney occurs as early as 2 days after inoculation. Also, Plg is not required for establishment of spirochetaemia by the organism, whereas it is involved in the invasion of organs.

Lyme disease and relapsing fever (RF) are caused by distinct species of *Borrelia* (3, 9). Lyme disease, most common in the Northern hemisphere, is caused by infection by, e.g., *Borrelia burgdorferi*, *B. afzelii*, and *B. garinii*, which are transmitted by the bite of hard-shelled *Ixodes* ticks. There are two types of RF, louse-borne and tick-borne RF. Louse-borne RF is induced by transmission of *B. recurrentis* by the crushing of feeding lice, and tick-borne RF is induced by transmission of, e.g., *B. hermsii*, *B. duttoni*, or *B. crocidurae* by the bite of soft-shelled *Ornithodoros* ticks (9). Lyme disease has been more extensively studied than RF and may involve several organ systems, most prominently the skin, nervous system, heart, and joints. Disease manifestations are induced by spirochete invasion of organ tissues, where *Borrelia* lipoproteins are likely to be involved in the early mechanisms of immune cell activation (29, 32, 45).

Unlike in Lyme disease, patients with RF experience one or more cycles of spirochetaemia. Each cycle is characterised by a febrile period with microscopically visible spirochetaemia lasting for 3 to 7 days, followed by nonfebrile periods of increasing lengths (18, 19, 40). The relapsing nature of the infection depends on the ability of the RF spirochetes to undergo antigenic variation, which has been studied in depth in the North American RF species *B. hermsii* (4, 7, 41). Similar to Lyme disease *Borrelia*, RF species also disseminate from the blood to many distant organs. Symptoms of brain invasion can include meningitis, focal deficits, hemiplegia, paraplegia, epilepsy, parasthesias, pains, pupillary abnormalities, peripheral and cranial neuritis, and myelitis (5, 24, 27, 31, 34, 42).

The mechanism by which *Borrelia* species enter blood and invade organs is still largely unknown. In higher vertebrates, plasminogen (Plg), the key component of the fibrinolytic system, can be converted to plasmin, a broad-spectrum serine protease, by the tissue-type Plg activator and the urokinase-type Plg activator (uPA) (6). In addition to fibrinolysis, plasmin-mediated proteolysis has been associated with many other biological processes, e.g., macrophage migration, tumor cell invasion, angiogenesis, and atherosclerosis (6). In vitro, a number of invasive bacteria have the ability to interact with the Plg activation system by binding plasminogen to the bacterial surface and/or by expressing endogenous Plg activators (12, 26). The activation of surface-bound Plg to plasmin is suggested to be a mechanism that enhances their ability to penetrate endothelium and tissue barriers. In addition, Plg binding may result in direct pathological effects due to the proteolytic activity of plasmin. Interestingly, besides the implication of Plg binding in bacterial pathogenicity, a recent study by Fischer and colleagues suggests that the binding of a pathological prion protein to Plg is of importance for pathogenicity in transmissible spongiform encephalopathies (20).

*B. crocidurae* was first isolated from the blood of a musk shrew in Senegal and was identified as the cause of endemic RF in western Africa (2, 11, 19, 28, 44), where it is considered a major cause of morbidity and neurologic disease (43). *B. crocidurae* organisms have the uncommon (among *Borrelia* species) protein to Plg is of importance for pathogenicity in transmissible spongiform encephalopathies (20).
species) ability to bind and cover themselves with erythrocytes, a phenomenon called erythrocyte rosetting, which is thought to result in a delayed immune response in the host (8). However, B. crocidurae shares with Lyme disease agents (i.e., B. burgdorferi) the ability to activate the adhesion molecules on the endothelium (35, 36), which could be a key pathophysiologic mechanism in B. crocidurae-induced vascular damage (37, 38).

In this study, we investigated the role of host-derived Plg in the ability of B. crocidurae to produce spirochtemia and disseminate to organs.

MATERIALS AND METHODS

Animals and bacterial strain. B. crocidurae was obtained from Alan G. Barbour (Irvine, Calif.), cloned by limiting dilution to serotype 2, and subsequently used in the infection experiments (8). BALB/c mice (Bomholtgård, Ry, Denmark) were used for passage of spirochetes from ~80°C to C57BL/6J mice. Adult Plg-deficient (plg−/−) and Plg wild-type (plg+/+) mice, generated from Plg heterozygous (plg+/-) breeding pairs, were used for the infection experiments. The plg−/− mice were generated by Carmeliet et al. (11) and genotyped by a rapid chromogenic assay and PCR as described by Ny et al. (30).

Dose-dependent coating of B. crocidurae with plasmin. Plasmin labeling of spirochetes was done as described earlier (14). Briefly, B. crocidurae organisms were cultivated and passed at least four times in Barbour-Stoenner-Kelly medium supplemented with 10% gelatin but without rabbit sera. Spirochetes were removed from the medium by centrifugation at 7,000 × g for 20 min. The pellet was resuspended in Hanks' balanced salt solution (HBSS) and divided into aliquots. One tube received 0.2 mg of Plg (Biopool, Umeå, Sweden)/ml and 10 ml (30 IU) of uPA, purchased from Sigma (plasin labeled). A second tube received only Plg (Plg labeled), and a third tube received only uPA (uPA labeled). The fourth tube received HBSS only (untreated). Another tube (sham) contained Plg and uPA but no spirochetes. All samples were incubated 90 min at 32°C prior to three washes with HBSS and addition of FlavignPiPl (Biopool), a chromogenic plasmin substrate. After incubation for 60 min at 32°C, the samples were centrifuged and supernatants were placed in a 96-well plate. The absorbance was read immediately at 410 nm with an MR700 microplate reader (Dynatech Laboratories, Chantilly, Va.).

Experimental infection. For revival of frozen B. crocidurae, a passage in mice was performed prior to the animal experiments. Four BALB/c mice were infected intraperitoneally with approximately 10^7 spirochetes. Spirochetemias were determined by the inability to coordinate movements when lifted by the tail and/or swimming inability. The plg−/− mice were examined daily for visible neurological symptoms of B. crocidurae infection, as determined by the inability to coordinate movements when lifted by the tail and/or swimming inability (10, 38, 39). At day 0 postinoculation (p.i.), the uninjected mice were anaesthetized as described above and sacrificed by cardiac exsanguination. At days 2, 5, 8, 12, and 14 p.i., three plg−/− and three plg+/+ animals were sacrificed similarly. All mice had been randomly selected for day of infection experiments (8). BALB/c mice (Bomholtgård, Ry, Denmark) were used in the infection experiments (8).

Quantitative analysis of spirochetes and immune cells in kidney tissue. The quantitation of bacteria and immune cells in sequential sections of kidney and brain tissue sections, the Mann-Whitney test was used. Comparisons of proportions of mice with clinical and histopathological symptoms (Table 1) were performed according to a normal approximation of binomial distribution (17). The criterion for significance of differences throughout the material was that the probability for random occurrence was less than 0.05.

RESULTS

Dose-dependent coating of B. crocidurae with plasmin. Addition of Plg to in vitro-cultivated B. crocidurae resulted in

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TABLE 1. Number of mice with histopathological and clinical symptoms indicative of spirochete invasion after infection with B. crocidurae

<table>
<thead>
<tr>
<th>Infection group</th>
<th>Histopathological</th>
<th>Clinical (brain)*</th>
<th>Total no. of mice with signs of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>Heart</td>
<td>Brain</td>
<td></td>
</tr>
<tr>
<td>plg−/−</td>
<td>2</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>plg+/+</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
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* Neurological symptoms were defined as the inability to coordinate movements when lifted by the tail and/or swimming inability.

p < 0.05 compared to proportion of plg−/− mice.
patterns and numbers of spirochetemia (Fig. 2A), and there was no significant differences between the two groups on any day ($P > 0.05$). A test of the order of infection and no infection was also carried out, showing no significant differences. A somewhat higher percentage of the spirochetes were found in rosettes (i.e., were attached to at least one erythrocyte) in $\text{plg}^{-/-}$ mice than in $\text{plg}^{+/+}$ mice, as shown by mean (Fig. 2B) and median (data not shown) values. This indication was most prominent during the first spirochetemic peak, on days 3 to 7 p.i. Attachment of spirochetes to more than two erythrocytes occurred to the same degree in the two mouse groups (data not shown).

Development of neurological symptoms. Of the inoculated mice, three $\text{plg}^{-/-}$ mice and one $\text{plg}^{+/+}$ mouse developed neurological symptoms. The $\text{plg}^{-/-}$ mice failed both the coordination and swim tests, whereas the $\text{plg}^{+/+}$ mouse failed only the swim test (Table 1). In all cases, the neurological symptoms appeared on day 8 p.i. The symptoms persisted until the day of sacrifice, i.e., day 12 p.i. for two of the $\text{plg}^{-/-}$ mice. The other two symptomatic mice were sacrificed on the day of the appearance of symptoms, day 8 p.i., due to the method of random selection for day of sacrifice which was used. The spirochetemias did not differ between mice with or without neurological symptoms, as seen by comparison of both mean and median values.

Quantitative analysis of spirochetes and inflammation. Representative staining of spirochetes in kidney, brain, and CD45$^+$ cells, histopathology, and endothelial staining are illustrated in Fig. 3. The localization of spirochetes in the organs was similar to earlier findings (38). The overall pattern of detected extravascular spirochetes in kidney tissue paralleled the pattern of spirochete fluctuation observed in blood, with the highest number of spirochetes detected at day 5 p.i. also in the tissue, and with the next peak at day 14 p.i. (Fig. 4A). The corresponding pattern was indicated to also occur in brain (Fig. 4B). The spirochetes were most often demonstrated in the proximity of blood vessels on days 2 and 5 p.i., whereas they were detected further away and more homogeneously across the sections at later stages. Among $\text{plg}^{-/-}$ mice, the number of spirochetes detected in interstitium increased with time and at day 8 p.i. approached those observed on day 2 p.i. in $\text{plg}^{+/+}$ mice. The difference in spirochete numbers in kidney interstitium was significant early, i.e., days 2 to 5 p.i. ($P = 0.02$), and stayed significant throughout the first spirochetemic peak, i.e., days 2 to 8 p.i. ($P = 0.04$).

In brain, a more efficient invasion was indicated in $\text{plg}^{+/+}$ mice day 2 p.i. than in $\text{plg}^{-/-}$ mice. Similar numbers of spirochetes were demonstrated in the mouse groups on day 5 p.i., with a tendency toward higher numbers on days 8 and 12 in $\text{plg}^{+/+}$ mice. Spirochetes in the process of extravasating were frequently detected (data not shown). Double staining of spirochetes and PECAM-1, an endothelial marker, was performed to confirm the extravascular location of spirochetes. The numbers of spirochetes associated with endothelium were the same for $\text{plg}^{+/+}$ and $\text{plg}^{-/-}$ mice. On day 2 p.i., an average of 71% of all spirochetes detected in brains of $\text{plg}^{+/+}$ mice were located extravascularly, compared to 25% in $\text{plg}^{-/-}$ mice.

The numbers of CD45$^+$ cells were higher in both kidney interstitium and glomeruli of $\text{plg}^{+/+}$ mice than of $\text{plg}^{-/-}$ mice (Fig. 5). There was a significant inflammatory response in glomeruli of $\text{plg}^{+/+}$ mice during the entire process ($P = 0.02,$

FIG. 1. Dose-dependent coating of B. crocidurae (B.c.) by plasmin.
Different concentrations of B. crocidurae were incubated with Plg and uPA, forming active plasmin on the surface of the spirochetes. Some of the borrelian preparations received only Plg, uPA, or HBSS. The sham control received Plg and uPA but no spirochetes. Data are means plus standard deviations for three replicate samples.
days 2 to 14 p.i.), most pronounced on days 2 to 5 ($P < 0.01$), but not in plg$^{-/-}$ mice ($P > 0.05$). The difference in inflammation was significant early, i.e., days 2 to 5 p.i. ($P = 0.02$). Similarly, in interstitium, more CD45$^+$ cells were present in plg$^{+/+}$ mice than in plg$^{-/-}$ mice ($P = 0.03$, days 2 to 14 p.i.). The difference was established early, i.e., days 2 to 5 p.i. ($P = 0.03$). No significant inflammation was detected in interstitium of plg$^{-/-}$ mice ($P > 0.05$), although a slight increase was indicated on days 8 to 12 p.i.

In brain, inflammation was more severe in plg$^{+/+}$ than plg$^{-/-}$ mice ($P = 0.047$) (Fig. 6). From day 2 p.i., numbers of leukocytes increased, and throughout the infection process, a decline was indicated only in plg$^{-/-}$ mice on day 14 p.i. In contrast, regression analysis showed an increase in CD45$^+$ cells over time, from day 2 to 14 p.i., in the brains of plg$^{+/+}$ mice ($P = 0.001$).

**Histopathology.** Histopathological changes were detected in two plg$^{-/-}$ mice and eight plg$^{+/+}$ mice (Fig. 3F). Changes in
plg$^{+/+}$ mice were observed in kidney and central nervous system samples. Changes in plg$^{+/+}$ mice also included heart and were more common in all three organs (Table 1). Clinical signs of central nervous system involvement were detected in one additional plg$^{+/+}$ mouse (one total) and one additional plg$^{−/−}$ mouse (three total). Thus, signs of brain invasion, as indicated by histopathological and/or clinical symptoms, were more commonly found among plg$^{+/+}$ mice (7 of 15) than plg$^{−/−}$ mice (2 of 15) ($P < 0.05$). Whereas the symptoms in plg$^{+/+}$ mice were detected on all days of sacrifice from day 8 p.i. onward, except for a heart symptom on day 2 p.i., any symptoms in plg$^{−/−}$ mice were seen on day 8 p.i. and were absent on the later days of sacrifice. A larger proportion of mice with histopathological and/or clinical symptoms of organ invasion was found in the plg$^{+/+}$ mouse group than in the plg$^{−/−}$ group ($P = 0.03$).

The histopathological observations in brains of plg$^{+/+}$ mice were meningeal leukocytosis and leukocytosis in meningeal vessels on day 8 p.i. and, at the later stages, inflammation showing granulocytes, monocytes, macrophages, and lymphocytes, suggestive of subacute meningitis. The plg$^{−/−}$ mouse with histopathological changes in the brain showed focal cell infiltrates of mononuclear leukocytes in a single site in meninges.

Histopathological symptoms observed in kidneys in plg$^{+/+}$ mice included capillary thrombi in medullary rays as well as a perivascular infiltrate showing mononuclear leukocytes, with several protein casts in tubuli of the inner cortex. In the plg$^{−/−}$

FIG. 4. Numbers of spirochetes in kidney interstitium (A) and cerebrum (B) of B. crocidurae-infected plg$^{−/−}$ and plg$^{+/+}$ mice, as demonstrated by immunohistochemistry. Analyses included five mice of each category sacrificed on day 0 and three of each category for days 2, 5, 8, 12, and 14 p.i.

FIG. 5. Numbers of leukocytes in kidney interstitium (A) and glomeruli (B) after infection of plg$^{−/−}$ and plg$^{+/+}$ mice with B. crocidurae, as demonstrated by immunohistochemical detection of the CD45 antigen. Day 0 values are means for five mice from each group; others are values for individual mice, with three plg$^{−/−}$ and plg$^{+/+}$ mice represented at each time point.

FIG. 6. Numbers of leukocytes in cerebrum after infection of plg$^{−/−}$ and plg$^{+/+}$ mice with B. crocidurae, as demonstrated by immunohistochemical detection of the CD45 antigen. Day 0 values are means for five mice from each group; others are values for individual mice, with three plg$^{−/−}$ and plg$^{+/+}$ mice represented at each time point.
mouse, the symptoms were leukocyte infiltration of perirenal fat tissues.

Symptoms in hearts were acute focal myocardial degeneration and interstitial myocarditis on day 8 p.i. and chronic myocardial degeneration on days 2 and 14 p.i.

**DISCUSSION**

Binding of Plg to the surface of bacteria has been proposed to be of importance for the invasion capacity of a number of pathogenic bacterial species belonging to several genera, including *Borrelia* (13, 16, 22). So far, in vivo studies of the role of Plg binding in pathogenicity have been performed for few organisms. Two such studies have been performed for *Borrelia* species, i.e., the Lyme disease species *B. burgdorferi* and a hitherto-uncharacterized Spanish RF isolate, and have indicated a role for Plg binding at different stages of the pathogenic process for the two species (15, 23). Whereas Plg binding appeared to be important for tissue invasion but not for the capacity to cause spirochtemia for the RF species, the opposite was indicated for the Lyme disease species. A major hindrance to evaluating invasion has been the requirement to use PCR for detection, due to the low numbers of spirochetes in tissues. Thus, until now, studies of in vivo invasion have required analysis at later stages of the infection process, when the blood is free from spirochetes with contaminating DNA. Thus, it is not clear if differences observed in spirochete load at later stages were due to utilization of Plg in invasion or in spreading through tissue postinvasion. As *B. crocidurae* invades organs in unusually (for *Borrelia*) high numbers, quantitative immunohistochemistry could be used to evaluate invasion during the early phase of infection. In addition to brain, which is known to be inflamed after infection of mice with the Spanish RF isolate (1, 21) and *B. crocidurae* (38), kidney was analyzed for invasion and inflammation, as the organ is consistently inflamed during murine *B. crocidurae* infection (38).

In this study, we show that Plg is not required for *B. crocidurae* to cause spirochtemia, as no differences could be observed in spirochtemic patterns between *plg*+/+ and *plg*−/− mice. The findings that both the rosette-forming *B. crocidurae* and a nonrosetting Spanish RF isolate (1, 23) use a Plg-independent mechanism to establish spirochtemia is intriguing, as it indicates that RF species may use a different mechanism(s) than Lyme disease *Borrelia* to accomplish this step. Tick-borne RF spirochetes are transmitted quickly by species of *Ornithodoros* that feed for 2 h or less. In contrast, Lyme disease spirochetes generally are not transmitted until after 48 h or more by the slow-feeding species of *Ixodes* ticks (33). Whether these differences may be attributed to different mechanisms used by the bacteria to spread and/or cross the endothelium from the site of infection or are related to different feeding mechanisms of the tick species remains to be elucidated.

Erythrocyte rosettes have been proposed to be a mechanism for *B. crocidurae* spirochetes to evade the immune response to the organism (8, 38). Thus, the rosetting may provide a longer period during which the bacteria can reach and invade distant organs as well as be ingested by new ticks. A slightly but not significantly higher rate of rosette formation was observed in *plg*−/− mice than *plg*+/+ mice. A possible explanation for this finding may be that plasmin plays a role in the resolution of spirochete-erythrocyte complexes. However, if this is true, the importance of plasmin(ogen) for such a resolution appears to be limited, as no difference was observed between the mouse groups in formation of larger rosettes, i.e., when the criterion was binding to at least two erythrocytes per bacterium.

Despite similar spirochtemias, there was a higher incidence of symptoms of organ invasion in *plg*+/+ mice than in *plg*−/− mice.

Spirochetes were also demonstrated in greater numbers in kidney interstitium of *plg*+/+ than *plg*−/− mice, where the slowly increasing numbers in *plg*−/− kidneys on day 8 p.i. were similar to those reached on day 2 p.i. in *plg*+/+ mice. The interstitial spirochetes were most often demonstrated close to blood vessels during the early phase, days 2 and 5 p.i., of the infection period, whereas they were further away from vessels at later stages, day 8 p.i. and onward. Thus, the spirochetes have the ability to move rather rapidly through the interstitium. A corresponding delayed invasion was also indicated in brains of *plg*−/− mice. By the method used, spirochetes were detected on day 2 p.i. in brain, which is the earliest demonstration of *B. crocidurae* in this tissue. The findings of extravascular spirochetes on day 2 p.i. in both kidney and brain, despite the high integrity of the blood-brain barrier, points to a very high efficiency of barrier crossing by *B. crocidurae*.

All spirochetes detected on day 2 p.i. were unassociated with cells, which indicates that the mechanism of crossing the blood-brain barrier is not accomplished by “hitchhiking” with other cell types. The delayed invasion into both organs of *plg*−/− mice strongly indicates that Plg aids the bacterium to accomplish this step.

The frequency of spirochete dissemination from the blood to brain was high: 93.3% in *plg*+/+ and 53.3% in *plg*−/− mice with brain invasion and 86.7% in *plg*+/+ and 60% in *plg*−/− mice with kidney invasion. For brain, the numbers of mice with spirochetes associated with vessels on day 2 p.i. were the same, i.e., two in each group, and no apparent difference between the mouse groups was noted in the numbers of spirochetes associated with vessels at this stage. Despite this fact, a higher percentage of all spirochetes detected in brain were located extravascularly in *plg*+/+ mice than in *plg*−/− mice on day 2 p.i. This finding may indicate that the association with, and possibly adhesion to, vessels is the same whether Plg is present or not and that the Plg-related difference is to be found in the actual barrier crossing.

Spirochetes were competent at invading both kidney and brain of *plg*−/− mice, although seemingly by a less efficient mechanism than in *plg*+/+ mice. As opposed to the difference observed on day 2 p.i., no differences were seen in numbers of *Borrelia* organisms in brains of *plg*+/+ and *plg*−/− mice on day 5 p.i. In addition, the presence of RF *Borrelia* in tissues of *plg*−/− mice on day 28 to 30 p.i. has been reported by Gebbia and colleagues (23). Thus, at least one additional mechanism of invasion other than Plg binding is used by these RF species. Rosette formation may contribute to organ invasion, by a resulting burst of blood vessels, a mechanism that may explain the high invasion efficiency of this organism compared to nonrosetting species. However, as DNA of the Spanish RF isolate was demonstrated in heart and brain tissue of *plg*−/− mice although it does not form erythrocyte rosettes, rosetting is not the sole explanation for Plg-independent invasion among RF
Borrelia (23). Whether the Plg-dependent and Plg-independent invasion mechanisms are mutually exclusive or acting in synergy remains to be elucidated. Borrelia spirochetes have been shown to activate matrix metalloproteinases, and a differential activation pattern was indicated when plasmin was bound to the bacterial surface (22). Thus, the different capabilities of invading organs observed in the present study may in part be related to differential activation of matrix metalloproteinases in the presence or absence of Plg.

A general assessment of inflammation was accomplished by quantification of the number of CD45+ cells in situ in brain as well as kidney glomeruli and interstitium. Both kidney compartments were significantly inflamed in plg+/+ but not in plg−/− mice, although a slight, but not significant, increase in inflammatory cells was indicated over time in interstitium of the latter mice. During the first 5 days of infection, a slight increase in CD45+ cells was seen in brains of plg−/− and plg−/− mice. On day 8 p.i. and at later stages, the inflammation tended to be more pronounced in plg−/− than in plg−/− mice, which fits with the more commonly demonstrated occurrence of mice with histopathological and/or clinical symptoms and the indication of a higher total spirochete burden in brains of plg−/− mice. The inflammation was shown to be more severe in the brains of plg−/− mice on day 2 to 14 p.i. than in plg−/− mice.

Thus, the Plg-independent mechanism for invading tissues was not sufficient to gain numbers high enough to cause significant inflammation or other investigated symptoms of organ invasion.

Despite the lower degree of invasion which resulted in the plg−/− mice showing less severe symptoms, spirochetes that have low with low efficiency may have the capacity to persist in the tissues for an extended length of time, as indicated by the presence of spirochetes in brains and kidneys of plg−/− mice on day 14 p.i. in the present study and by the study by Gebbia and colleagues (23), where spirochetes were present in low numbers in plg−/− mice on days 28 to 30 p.i. after infection with a Spanish RF isolate. Thus, the findings indicate that spirochete invasion in low numbers may lead to absence of invasion sequelae, masking a process during which the asymptomatic individual may acquire Borrelia persistently residing in tissues. The extent to which spirochetes residing in low numbers are associated with low-grade inflammation that does not manifest as clinical symptoms and the extent to which such spirochetes may cause a more significant inflammation at a later stage are unclear at this point.

Whereas inflammation in plg−/− mice showed a tendency to decrease in glomeruli after the first spirochetemic peak, and possibly in interstitium day 14 p.i., no obvious indication of a decrease was observed in brain, despite decreased detection of spirochetes. In contrast, an increase of CD45+ cells over time was observed in the brains of these mice. Further investigations of the longer perspective of inflammatory changes in the brain, as a result of B. crocidurae infection, should be of great interest for evaluating the long-term effects of spirochete invasion.

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