Mechanisms of Vaccine-Induced Protective Immunity against *Coxiella burnetii* Infection in BALB/c Mice

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Mechanisms of Vaccine-Induced Protective Immunity against Coxiella burnetii Infection in BALB/c Mice

Guoquan Zhang, Kasi E. Russell-Lodrigue, Masako Andoh, Yan Zhang, Laura R. Hendrix, and James E. Samuel

To elucidate the mechanisms of vaccine-induced protective immunity against Coxiella burnetii infection, we compared the protective efficacy and immunogenicity between formalin-inactivated phase I vaccine (PI-V) and phase II vaccine (PII-V) in BALB/c mice. PI-V generated significant protection while PII-V did not confer measurable protection. Analysis of cytokine and subclass Ab responses indicated that both PI-V and PII-V were able to induce a Th1-dominant immune response but did not identify the component of host response that distinguished their ability to induce protective immunity. Interestingly, immunoblot analysis identified a difference between PI-V and PII-V vaccines in antigenic recognition by specific Ab isotypes. The observation that PI-LPS elicited significant protection but PII-LPS did not confer measurable protection suggests PI-LPS may play a key role in PI-V-induced protection. Adoptive transfer of either immune sera or splenocytes mediated significant protection in naive BALB/c mice, supporting the hypothesis that T cell-mediated immunity is critical for host defense against C. burnetii infection. This report presents novel evidence to highlight the importance of PI-LPS and Abs in protective immunity and has important implications for the design of new generation vaccines against Q fever. The Journal of Immunology, 2007, 179: 8372–8380.

Coxiella burnetii is an obligate intracellular bacterium that causes the worldwide zoonotic disease, Q fever. Infection in most animals is mainly subclinical, but abortion and infertility are common manifestations in ruminants (1). Domestic animals, especially cattle, sheep, and goats, are important reservoirs of the agent and are mainly responsible for infection of humans (2, 3). Human Q fever usually manifests as a flu-like, self-limiting, or treatable acute illness, although some infections develop into a severe and sometimes fatal chronic disease (3). Infection in humans commonly occurs via the respiratory route by inhalation of infectious aerosols produced by domestic livestock (4). It is considered an occupational hazard among livestock workers, veterinarians, research laboratory workers, and personnel of research animal facilities. Recent epidemiological evidence suggests that infected pet animals, such as cats and dogs, play an important role in the transmission of C. burnetii infection to humans (5, 6). More importantly, the highly infectious nature of C. burnetii and its hardness in adverse environmental conditions, make the organism potentially useful in bioterrorism and biological warfare. Because human Q fever can develop into severe chronic disease, vaccination is a logical approach to protect individuals at risk for contact with naturally infected animals or other exposures to the agent. Formalin-inactivated whole cell vaccine provides near complete protection in animal models as well as human vaccinees, and a licensed vaccine (Q-Vax) is available in Australia (7–9). Unfortunately, vaccines produced from whole cell Ag can induce severe local or systemic adverse reactions, especially when administered to individuals with prior exposure to the agent (10, 11). Therefore, creation of a safe and effective vaccine to control Q fever remains an important public health and biosecurity goal.

C. burnetii undergoes a phase variation in which smooth-LPS virulent phase I (PI3) converts to rough-LPS avirulent phase II (PII) upon serial passage in a nonimmunologically competent host (12). One early study suggested that phase I vaccine (PI-V) was more protective than phase II vaccine (PII-V) against virulent C. burnetii challenge in a guinea pig model (13). Hackstadt et al. (14) demonstrated that the LPS structurally and antigenically varied between PI and PII cells, but the protein components were shared. The only characterized difference between PI and PII organisms is LPS polysaccharide content in the core and O side chain, supporting the hypothesis that PI-LPS plays a critical role in the development of protective immunity. However, because there is no direct evidence to demonstrate either PI-LPS or PI unique protein Ags are involved in the development of protective immunity, it remains unclear why the protective efficacy is distinct between PI-V and PII-V. Previous studies have demonstrated that PI-V was able to induce both Ab- and cell-mediated immune responses and was effective in preventing Q fever in animals and humans (15–19). However, the mechanisms of PI-V-induced protective immunity are not well-understood and it remains unknown what components of host defense are responsible for control of C. burnetii replication and clearance of the organisms. The protective nature of PI-V is unique for intracellular bacteria because vaccination with most killed whole cell Ags of intracellular bacterial (such as...
Mycobacterium tuberculosis, Francisella tularensis, Chlamydia trachomatis, and Brucella abortus) do not elicit long-lasting and significant protective immunity (20, 21). Therefore, elucidation of the mechanism of protective immunity elicited by PI-V may provide critical information for understanding the mechanisms of host defense against intracellular bacterial pathogens.

Early studies suggested that both humoral and cell-mediated immune responses are important for host defense against C. burnetii infection, while cell-mediated immunity probably plays the critical role in eliminating the organisms. Abinanti and Marmion (22) first reported that mixtures of Ab and C. burnetii organisms were not infectious in experimental animals, suggesting Ab may play a role in the control of C. burnetii infection. Several in vitro studies indicated that treatment of C. burnetii with immune sera made the organisms more susceptible to phagocytosis and to destruction by normal polymorphonuclear leukocytes or macrophages (23–25). These studies provided strong support for the notion that humoral immunity is important in the development of the acquired resistance to C. burnetii infection. However, the observation that treatment of athymic mice with immune sera 24 h before challenge with C. burnetii had no effect on bacterial multiplication within the spleens of the T cell-deficient animals (15) suggests that T cell-mediated immunity plays a critical role for elimination of C. burnetii. Because passive transfer of immune sera did not confer clear protection in an early study (26), and careful characterization of the role of Ab in protective immunity has been lacking, Ab-mediated protective immunity has been forgotten in development of a new generation vaccine against C. burnetii infection.

In this study, we compared the protective efficacy and immunogenicity between PI-V and PII-V in BALB/c mice. Subsequently, we examined the protective ability of PI-LPS and PII-LPS. We also investigated whether immune sera or splenocytes isolated from PI-V-vaccinated mice can transfer protective immunity to naive recipient mice. In addition, we confirmed whether IgG is the active component in immune sera that is responsible for immune sera-induced protection. Furthermore, we tested whether immune sera, splenocytes, B cells, and T cells from PI-V-vaccinated mice can protect SCID mice against C. burnetii infection. This is the first report to clearly show that Ab plays an important role in vaccine-induced protective immunity against C. burnetii infection.

Materials and Methods

Animals

Specific-pathogen-free 6-wk-old female BALB/c mice and 12-wk-old female SCID mice (in BALB/c background) were purchased from The Jackson Laboratory. All mice were housed in sterile microisolator cages under specific-pathogen-free conditions at the Texas A&M University (TAMU) laboratory animal facility according to the Guide for the Care and Use of Laboratory Animals. The research protocols described in this report were approved by the Animal Care and Use Committee of TAMU. All C. burnetii infection experiments were performed in a B3L facility at TAMU.

C. burnetii strains

Nine Mile phase I (NMI) clone 7 (RSA493) and phase II (NMII) clone 4 (RSAA439) were grown in embryonated yolk sacs and purified as described previously (27). NMI and NMII Ags used in vaccination and ELISA were inactivated by 1% formaldehyde solution as described elsewhere (28). The protein concentrations of inactivated NMI and NMII Ags were measured by a Micro BCA Protein Assay kit (Pierce). The NMI and NMII Ags used in this study were prepared from the same lot of formalin inactivation.

PI-V and PII-V vaccination and challenge

BALB/c mice were immunized with 20, 2, and 0.2 µg of PI-V or PII-V three times at 14-day intervals. Mice immunized with PBS and IFA served as unvaccinated controls. At each immunization, one mouse was s.c. injected with a mixture of 50 µl of Ag and 50 µl of IFA. Vaccinated and unvaccinated control mice were challenged at 14 days postinfluenza infection by i.p. injection with 107 organisms of NMI as described previously (29). Mice were sacrificed at 14 days post challenge. Serum samples were collected from each vaccination group of mice at prechallenge and 14 days postchallenge, and stored at −80°C until used. Splenomegaly was used as an indicator to evaluate the protective efficacy of PI-V and PII-V.

ELISA

Mouse sera from PI-V- or PII-V-vaccinated mice at prechallenge and 14 days postchallenge were tested for total IgG, IgG1, and IgG2a to PI or PII Ag by ELISA as modified from the method described previously (30). Briefly, 50 µl of inactivated PI or PII Ag at 500 ng/ml in 0.05 M carbonate/bicarbonate coating buffer (pH 9.6) were added to each well of a 96-well microtiter plate and coated at 4°C for 48 h. The plates were blocked with 1% BSA in PBST buffer (0.05% Tween 20 in PBS) and then incubated with 100 µl of serially diluted mouse sera at room temperature for 2 h. After washing four times with PBST buffer, the plates were incubated with 100 µl of HRP-conjugated goat anti-mouse IgG, IgG1, or IgG2a (1:5000 dilution) at room temperature for 2 h. The Sigma Fast O-Phenyldiamine Dihydrochloride Tablet Sets (Sigma-Aldrich) were used as substrates, and OD was measured at 490 nm by the Spectra Max M2 system using the SoftMax program (Molecular Devices).

Immunoblotting

To identify IgG1 and IgG2a recognized Ags, mouse sera from PI-V- and PII-V-vaccinated mice at prechallenge were compared for IgG1 and IgG2a responses to PI and PII Ags by immunoblotting. To determine whether IgG1 and IgG2a recognize protein or LPS Ags, the IgG1 and IgG2a Abs to proteinase K-digested PI and PII Ags were also analyzed. Ag preparation and immunoblotting were performed as described previously (31). The reactions were detected by using ECL Western blot Detection kit (Amer sham Pharmacia).

Analysis of cytokine responses

Mice were immunized with 4 µg of PI-V or 4 µg of PII-V and challenged with C. burnetii at 28 days post vaccination as described above. Three mice from PI-V- or PII-V-vaccinated and unvaccinated control groups were sacrificed at 7 days postvaccination and at 3, 7, and 14 days postchallenge. Serum samples were collected from each individual mouse and stored at −80°C until use. Mouse spleen weight was measured and a portion of spleen from each mouse was taken for real-time PCR analysis. The protective activities of PI-V and PII-V at different time points were evaluated by measuring both splenomegaly and C. burnetii com1 gene copy numbers in whole spleen. The serum concentrations of 18 cytokines were measured from each mouse by a Bio-Plex cytokine assay using the mouse cytokine 18-Plex Panel (Bio-Rad).

PI-LPS and PII-LPS vaccination and challenge

PI-LPS and PII-LPS were extracted from purified NMI and NMII organisms, respectively, by hot phenol as described elsewhere (32). Mice were immunized with 3.5 µg of PI-LPS, 3.5 µg of PII-LPS, 4 µg of PI-V plus 3.5 µg of PI-LPS or 4 µg of PII-LPS twice at 4-wk intervals. Mice vaccinated with PBS, 4 µg of PI-V, or 4 µg of PII-V served as unvaccinated, protected, or unprotected controls, respectively. Vaccination and challenge methods were similar to those described above, except mice were challenged at 5 wk postvaccination. Mice were sacrificed at 14 days postchallenge and the efficacy of PI-LPS and PII-LPS was evaluated by measuring splenomegaly.

Adaptive transfer of immune sera and splenocytes

Immune sera were collected from PI-V-vaccinated (4 µg/mouse, once) mice at 28 days postvaccination and pooled as equal amounts from each mouse. Each recipient naive mouse received 700 µl of pooled sera i.p. 24 h before challenge. The spleens from the same donor mice were harvested and used for isolation of splenocytes. Splenocyte suspensions were prepared by homogenization of spleens, passage through nylon mesh, and lysis of erythrocytes by lysing buffer (containing 0.15 M NaCl, 0.01 M KHCO3, and 0.01 mM EDTA (pH 7.2)). Splenocytes were resuspended in 1× PBS at a concentration of 3.0 × 106 cells/ml. The cell viability of splenocytes was examined by trypan blue staining and only live cells were counted. Adoptive transfer was performed by i.p. injection of 1.2 × 107 spleen cells in 0.4 ml of PBS to each naive recipient BALB/c mouse at 24 h before challenge. Mice were challenged with 107 NMI by i.p. injection at 24 h after adoptive transfer. PI-V-vaccinated and unvaccinated control mice were challenged in the same manner and served as positive and negative controls. Mice were sacrificed at 14 days postchallenge. Mouse
spleen weight was measured, and a portion of spleen from each mouse was taken for real-time PCR analysis. The protective efficacy of immune sera and splenocytes was evaluated by measuring both splenomegaly and C. burnetii bacterial loads in spleen.

**Passive transfer of purified IgG from immune sera**

Immune sera were isolated from PI-V-vaccinated mice at 28 days post-vaccination in the same manner as described above. IgG was purified from pooled immune sera by protein G-Sepharose affinity chromatography (Pharmacia Biotech). The concentration of purified IgG was assessed by measurement of absorption in a UV spectrophotometer at 280 nm. The purity of the IgG was analyzed by SDS-PAGE. Passive transfer of IgG was performed by i.p. injection (500 μg/mouse) in naive BALB/c mice. In addition to unvaccinated and PI-V-vaccinated mice control groups, mice receiving 0.5 ml of inactivated normal mouse sera or immune sera were also used as controls. All groups of mice were challenged with 10^7 NMI by i.p. injection at 48 h postpassive transfer. The ability of IgG to confer protection was evaluated by comparing splenomegaly and bacterial loads in spleens at 14 days postchallenge with controls as described above.

**Adaptive transfer of immune sera, splenocytes, B cells, and T cells to SCID mice**

Immune sera from PI-V-vaccinated mice were collected in the same manner as described above. The spleens from the same donor mice were harvested and used for isolation of splenocytes, B cells, and T cells. Splenocyte suspensions were prepared in the same manner as described above. B cells and T cells were purified from splenocyte suspension by negative selection using B cell and T cell isolation kits (Miltenyi Biotec), respectively. The purities of B cells and T cells were 97 and 99%, respectively, as examined by FACS analysis with FITC-conjugated rat anti-mouse CD45RB/PE-conjugated rat anti-mouse CD90.2 (Thy-1.2) mAbs (BD Biosciences). The cell viabilities of splenocytes, B cells, and T cells were examined by trypan blue staining. Adoptive transfer was performed by i.p. inoculation of naive recipient SCID mice with 3 × 10^7 splenocytes, 1.5 × 10^7 B cells, or 1.5 × 10^7 T cells in 0.4 ml of PBS. Mice were challenged with 10^7 NMI at 24 h after adoptive transfer. SCID mice receiving normal mouse sera or splenocytes were also challenged and served as controls. The abilities of immune sera, splenocytes, B cells, and T cells to confer protection in SCID mice were evaluated by comparing body weight change, splenomegaly and bacterial loads in spleens at postchallenge with controls as described above.

**Quantitative PCR assay**

The High Pure PCR Template Preparation kit (Roche Molecular Biochemicals) with modifications was used for extraction of DNA templates from spleen samples. Briefly, a small piece of spleen sample was homogenized into a 1:20 solution with lysis buffer using a HandyPestle. After centrifugation at 2000 × g for 5 min, 200 μl of supernatant was transferred into a fresh tube to which 10 μl of proteinase K (20 mg/ml) was added and incubated at 60°C for 4 h. Subsequently, the procedure as described in the High Pure PCR Template Preparation kit was followed. Real-time PCR was performed as described previously (33) with modification using Applied Biosystems 7300/7500 Real-Time PCR System. The recombinant plasmid DNA (coml gene ligated into pET23a vector) (29) was used as standard DNA to quantify coml gene copy numbers in spleen samples.

**Statistical analysis**

A two-tailed Student t test was used to compare the significance between different immunization groups. Values of p < 0.05 were considered significant.

**Results**

**Comparison of the protective efficacy between PI-V and PII-V**

Three days postchallenge, unvaccinated and all doses of PII-V-vaccinated mice developed an observable clinical disease, including reduced movement, ruffled fur, and body weight loss, while unchallenged controls and all doses of PI-V-vaccinated mice did not show clinical signs. However, by 10 days postchallenge, unvaccinated and PII-V-vaccinated mice had recovered from clinical disease, and there were no significant clinical signs in mice of any group. After euthanasia, splenomegaly was the only observed difference between vaccination groups. Fig. 1 shows a comparison of splenomegaly between PI-V- and PII-V-vaccinated mice using percent spleen weight per body weight as an indicator. All doses of PI-V protected mice from development of significant splenomegaly (p < 0.05 with unvaccinated control), while all doses of PII-V-vaccinated mice developed levels of severe splenomegaly similar to unvaccinated mice (p > 0.05). These results suggest that PI-V was able to elicit significant protection from the development of clinical signs and splenomegaly against challenge, while PII-V did not confer measurable protection regardless of vaccination dose.

**Comparison of total IgG, IgG1, and IgG2a responses to PI-V and PII-V**

Table I shows IgG, IgG1, and IgG2a titers of PI-V- or PII-V-vaccinated mice postchallenge with 10^7 NMI. Spleen weight as percentage of body weight was used to measure splenomegaly. The data presented in each group is the average with SD of three mice. * p < 0.05; ** p < 0.01; *** p < 0.001.

<table>
<thead>
<tr>
<th>Vaccine groups</th>
<th>Against phase I Ag</th>
<th>Against phase II Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>PI-V-20</td>
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<td>PI-V-2</td>
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</tr>
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<td>PI-V-2</td>
<td>6.4</td>
<td>12.8</td>
</tr>
<tr>
<td>PI-V-0.2</td>
<td>3.2</td>
<td>1.0</td>
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The Ab titers are the highest dilution giving a mean OD (490 nm) of at least 0.1 and twice that of negative controls.

**Table II**

Mice vaccinated with either PI-V or PII-V with 20, 2, or 0.2 μg/injection with three injections.
IgG1, and IgG2a titers of naive, PI-V-, or PII-V-vaccinated mice at 14 days postchallenge. The ratio of IgG2a to IgG1 was \(16\) in naive mice, suggesting a Th1-skewed immune response was engendered in response to \textit{C. burnetii} infection. Interestingly, both PI-V- and PII-V-vaccinated mice developed similar levels of secondary Ab responses against challenge and the ratios of IgG2a to IgG1 were \(4\) in all vaccination groups, suggesting the secondary Ab response against \textit{C. burnetii} infection was characterized by a Th1-dominant immune response in both protected and unprotected groups after infectious challenge.

IgG1 and IgG2a recognized different Ags between PI and PII

Fig. 2 shows reactivity of IgG1 and IgG2a to PI and PII Ags in immune sera from PI-V- or PII-V-vaccinated mice. IgG1 in immune sera from PI-V-vaccinated mice recognized proteinase K-sensitive 28- and 32-kDa immunodominant Ags which were identical between PI and PII (Fig. 2A). Interestingly, IgG2a in immune sera from PI-V-vaccinated mice recognized 21-kDa proteinase K-sensitive and 25-kDa proteinase K-resistant Ags in PI but did not react to these Ags in PII (Fig. 2B). IgG1 and IgG2a in immune sera from PI-V-vaccinated mice also recognized different proteinase K-sensitive Ags between PI and PII (Fig. 2, C and D). These results identify distinct subclass Ab responses between PI-V- and PII-V-vaccinated mice.

**Distinct protective activity between PI-V and PII-V in early control of \textit{C. burnetii}**

The protective efficacies of PI-V and PII-V were compared in BALB/c mice at different time points postchallenge by measuring splenomegaly and \textit{C. burnetii} loads in the spleens. Unvaccinated and PII-V-vaccinated mice developed similar levels of severe splenomegaly at 7 and 14 days postchallenge, while PI-V-vaccinated mice were significantly protected from the development of splenomegaly against challenge at all time points postchallenge (\(p < 0.01\) to unvaccinated mice) (Fig. 3A). Interestingly, the \textit{C. burnetii} genome copy was undetectable in PI-V-vaccinated mice, but \(\sim 10^4\) genome copies were detected in unvaccinated and PII-V-vaccinated mice at 3 days postchallenge (Fig. 3B), suggesting...
early control of *C. burnetii* contributed to PI-V-induced protection against *C. burnetii* challenge. In addition, although significantly higher *C. burnetii* genome copies were detected in unvaccinated and PII-V-vaccinated mice than PI-V-vaccinated mice at 7 and 14 days postchallenge (*p* < 0.05), \(10^3–10^4\) copies were also detected in PI-V-vaccinated mice. These results suggested that late clearance of the organisms was necessary for complete control of the infection.

**Cytokine responses**

To test the hypothesis that PI-V and PII-V differ in ability to skew Th1 responses as suggested by isotype analysis of Ab response, vaccinated mice were analyzed by Bio-Plex cytokine assay for serum cytokine and chemokine levels. However, there were no significant differences for serum cytokine and chemokine levels at 7, 14, 21, and 28 days postvaccination, except for a modestly higher level of IL-12p40 in PI-V compared with PII-V at 7 days postvaccination (Fig. 4). This data supports the interpretation that vaccination with both PI-V and PII-V was able to elicit a Th1 immune response. Fig. 5 shows cytokine responses in PI-V- and PII-V-vaccinated and naive mice at different time points postchallenge. Unvaccinated and PII-V-vaccinated mice generated significantly higher levels of IL-12p40, IFN-γ, and G-CSF than PI-V-vaccinated mice at 7 days postchallenge as a result of their unprotected status to infection. These results demonstrate that a Th1 immune response was generated in response to *C. burnetii* challenge.

**PI-LPS provided significant protection**

Because the serum cytokine levels did not clearly identify a distinct mechanism for differential protective immunity, an alternative hypothesis was tested that the major antigenic difference between PI-V and PII-V, LPS O side chain, is essential for inducing protective immunity. The protective activity of purified PI-LPS and PII-LPS was compared in BALB/c mice. Fig. 6 shows comparison of splenomegaly between different vaccination groups. Unvaccinated control, PII-V-, and PII-LPS-vaccinated mice developed similar levels of severe splenomegaly (*p* > 0.05), while PI-V, PI-LPS, PI-V plus PI-LPS, and PII-V plus PI-LPS-vaccinated mice exhibited significantly less severe splenomegaly at 14 days postchallenge (*p* < 0.001 compared with unvaccinated control mice). Interestingly, PI-LPS was able to rescue the lack of protection against challenge in PII-V-vaccinated mice, and vaccination with PI-LPS alone also generated a similar level of protection as PI-V. PII-LPS was unable to induce protection against virulent NMI challenge but did not affect the efficacy of PI-V. These results suggest PI-LPS is an important protective Ag responsible for PI-V-induced protection against *C. burnetii* infection.

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Both immune sera and splenocytes confer significant protection

To elucidate the role(s) of humoral and cellular immunity in PI-V-induced protection, we tested whether adoptive transfer of immune sera or splenocytes from PI-V-vaccinated mice can confer protection in naïve recipient mice. Splenomegaly and *C. burnetii* bacterial loads in the spleens with unvaccinated and PI-V-vaccinated control mice at 14 days postchallenge with $10^7$ NMI. A, Splenomegaly was measured by spleen weight as percentage of body weight. B, Bacterial loads in whole spleen as determined by real-time PCR. The data presented in each group are the average with SD of three mice. ND, Undetectable; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

FIGURE 7. Evaluation of the protective efficacy of immune sera and splenocytes by comparing splenomegaly and *C. burnetii* bacterial loads in the spleens with unvaccinated and PI-V-vaccinated control mice at 14 days postchallenge with $10^7$ NMI. A, Splenomegaly was measured by spleen weight as percentage of body weight. B, Bacterial loads in whole spleen as determined by real-time PCR. The data presented in each group are the average with SD of three mice. ND, Undetectable; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

IgG is the active component in immune sera that is responsible for protection

To confirm that Ig is responsible for immune sera-induced protection, the protective activity of IgG from PI-V-vaccinated mouse sera was evaluated in BALB/c mice. Compared with normal mouse sera control and unvaccinated mice, splenomegaly was significantly reduced in IgG and immune sera-transferred mice ($p < 0.01$). The levels of splenomegaly were similar between IgG and immune sera-transferred mice and more severe than PI-V-vaccinated mice ($p < 0.05$) (Fig. 8A). Bacterial loads in the spleen from IgG and immune sera-transferred mice were significantly lower than normal mouse sera control and unvaccinated mice ($p < 0.001$), but higher than PI-V-vaccinated mice ($p < 0.05$) (Fig. 8B). These results demonstrate that purified IgG was able to provide specific protection against *C. burnetii* challenge.

Splenocytes can transfer significant protection from PI-V-vaccinated donor mice to naïve recipient mice. The finding that passive transfer of immune sera provided significant protection suggests that Abs play an important role in PI-V-induced protection against *C. burnetii* challenge.
similar levels of protection as immune sera in naive recipient animals and suggest that IgG is the active component that is responsible for immune sera-induced protection.

*The role of immune sera and cells in protecting SCID mice against C. burnetii infection*

To determine whether Ab alone can control *C. burnetii* infection as well as to understand the key host components that are responsible for control of the infection, we tested whether immune sera, splenocytes, B cells, and T cells isolated from *PI-V*-vaccinated BALB/c mice can transfer protection to SCID mice against *C. burnetii* challenge. Although *C. burnetii* infection did not induce significant body weight loss in BALB/c mice, it does cause severe body weight loss in SCID mice (35), we used relative body weight change (body weight postinfection/body weight preinfection) to monitor the severity of clinical manifestation after infection (Fig. 9A). Compared with normal mouse sera and PBS control, immune sera and all immune cells protected SCID mice from development of clinical disease and body weight loss. Interestingly, immune sera and B cell-recipient mice developed levels of severe splenomegaly similar to normal mouse sera and PBS control (*p > 0.05*), while splenomegaly was significantly reduced in immune splenocyte and T cell-recipient mice (*p < 0.001*) (Fig. 9B). In support of the splenomegaly results, similarly higher *C. burnetii* loads were detected in PBS, normal mouse sera, immune sera, and B cell-recipient mice but bacterial loads were significantly reduced in immune splenocyte and T cell-recipient mice (*p < 0.001*) (Fig. 9C). In addition, although splenocytes from naïve BALB/c mice protected SCID mice against body weight loss, splenomegaly, and bacterial loads, the protection levels were significantly lower than the protection levels conferred by splenocytes and T cells from *PI-V*-vaccinated mice (*p < 0.01*). These results indicated that immune sera and B cells protected SCID against clinical disease but did not control the infection, while immune splenocytes and T cells were able to effectively protect against clinical disease and control the infection.

*Discussion*

Mice infected with virulent *C. burnetii* develop severe or fatal illness or remain relatively free of clinical signs depending upon the mouse strain (36–39). The BALB/c mouse strain is immediately sensitive to lethal challenge and has been used to estimate protective efficacy of *C. burnetii* Ags by several research groups (39–41). Our previous study demonstrated that the sublethal BALB/c mouse model, using splenomegaly as an indicator of disease, is useful for the evaluation of efficacy of vaccine candidates (29). This study confirms earlier observations in the guinea pig model and demonstrates that PI-V provides near complete protection against virulent *C. burnetii* challenge, but PII-V does not confer measurable protection in BALB/c mice. To support the splenomegaly data, a quantitative real-time PCR procedure to accurately measure the number of *C. burnetii* in spleens was used to evaluate the protective activities of PI-V and PII-V. The real-time PCR data correlated with splenomegaly and supports the interpretation that PI-V is more protective than PII-V, suggesting the measurement of *C. burnetii* loads in spleens by real-time PCR is useful for evaluation of the protective activity of vaccines.

Analysis of Ab responses indicated that the ability of PI-V and PII-V differed in inducing Ab responses. This result supports an earlier observation in goat vaccination with PI-V and PII-V (42) and suggests that PII-V is less immunogenic than PI-V. However, the ratio of IgG2a to IgG1 did not clearly distinguish between Th1 and Th2 responses in *PI-V*- and *PII-V*-vaccinated mice because the ratio of IgG2a to IgG1 was ≥2 in high-dose (20 μg) PII-V-vaccinated mice. These results suggest that both PI-V and PII-V were able to induce a Th1-dominant immune response. A comparison of cytokine responses also indicates that high levels of Th1 cytokines were detected similarly in both PI-V- and PII-V-vaccinated mice at prechallenge while Th2 cytokines were undetectable or detected at very low levels. The cytokine and subclass Ab responses at prechallenge support the hypothesis that both PI-V and PII-V were able to induce a Th1-dominant immune response but did not identify the component of host response that distinguishes their ability to induce protective immunity.

The ability of IgG2a to confer protection against intracellular pathogens has been demonstrated in *Cryptococcus neoformans* and *Ehrlichia chaffeensis*. Sanford et al. (43) reported that passive immunization with polysaccharide-recognizing IgG2a was more protective than IgG1 against *C. neoformans* infection. Recently, Li et al. (44) indicated that an IgG2a mAb specific to the *E. chaffeensis* major outer membrane protein conferred significant protection.
against fatal *E. chaffeensis* infection in SCID mice. These studies suggested that IgG2a was an important component in Ab-mediated protection against intracellular pathogens. It is noted that early studies reported that IgG2a was undetectable in *C. burnetii*-infected chronic Q fever patients (45, 46) but was significant in individuals postvaccination with PI-V (47). Our observation of the production of elevated levels of IgG2a in PI-vaccinated and infected mice supports the hypothesis that IgG2a may play an important role in the host defense against *C. burnetii* infection. Interestingly, immunoblot analysis demonstrated that IgG2a in immune sera from PI-V-vaccinated mice recognized PI-specific protein and nonprotein Ags, suggesting that specific Abs against both PI-protein and LPS may be involved in PI-V-induced protection.

An important question raised from our results is what antigenic components in PI-V are responsible for PI-V-induced protection against challenge. The difference in LPS core polysaccharide O side chain expression between PI and PII suggests that PI-LPS may play an important role in PI-V-induced protection. This hypothesis was supported by an early observation that PI-LPS was able to elicit Ab responses to PI and PII Ags and to confer protection against virulent *C. burnetii* challenge in a mouse model (48). Interestingly, our results demonstrated that PI-LPS was able to generate a level of protection similar to PI-V, but PII-LPS did not provide measurable protection. These results confirmed the earlier observation and support the notion that PI-LPS may be the key component that is responsible for PI-V-induced protection. However, current results do not show whether there are specific protective antigenic determinants on PI-LPS or whether contaminating molecules such as peptidoglycan, lipoprotein, or protein-LPS complex are involved in PI-LPS-induced protection. Further purification of LPS, as well as characterization of the immunologic functions of LPS, may determine the role of PI-LPS in the development of protective immunity against *C. burnetii* infection.

The observation that adoptive transfer of both immune sera and splenocytes conferred similar levels of protection to PI-V vaccination suggests both humoral and cellular immunity contributed to PI-V-induced protection. The finding that purified IgG from immune sera conferred a level of protection similar to immune sera demonstrated that IgG is the protective component in immune sera, suggesting Ab-mediated immunity plays an important role in PI-V-induced protection. This hypothesis was supported by several studies demonstrating that Abs can mediate resistance to a variety of intracellular bacterial and fungal pathogens (49, 50). Ab-mediated protection has been described in host defense against *M. tuberculosis* (51), *Listeria monocytogenes* (52), *Candida albicans* (53), *Histoplasma capsulatum* (54), *C. neoformans* (49, 50), and *E. chaffeensis* (44, 55). However, our results also indicate that immune sera and B cells protected SCID mice against body weight loss but did not significantly reduce splenomegaly and bacterial burdens, suggesting that Ab alone cannot control *C. burnetii* infection. In contrast, we found that adoptive transfer of either splenocytes or T cells can protect SCID mice against body weight loss and significantly reduce splenomegaly and bacterial burdens. These results suggest that Ab-mediated protection may be dependent on T cells, and T cell-mediated immunity is critical for host defense against *C. burnetii* infection. In addition, our recent studies demonstrate that mice with T cell or IFN-γ deficiency displayed significantly increased susceptibility to *C. burnetii* infection, but B cell deficiency did not significantly decrease host resistance against the infection (56). These data suggest that a T cell-mediated Th1 immune response played a major role while Ab-mediated immune response may provide a minor role in host defense against *C. burnetii* infection. This hypothesis was supported by an earlier report that treatment of athymic mice with immune sera 24 h before challenge did not affect *C. burnetii* multiplication in the spleens of T cell-deficient animals (15). In addition, our results indicated that mice receiving Ab were unable to completely control *C. burnetii* infection, suggesting T cell-mediated immunity was necessary for complete clearance of the organisms. Our results, together with early studies, suggest that anti-PI specific Abs play an important role in protection from the development of clinical disease at an early stage against *C. burnetii* challenge, while the T cell-mediated Th1 immune response is critical for clearance and complete elimination of the organisms at the late stage of the infection. Therefore, novel vaccine approaches for Q fever should be focused on boosting both humoral and cellular immune responses.

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**Disclosures**

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**References**


