

Pulmonary and serum antibody responses elicited in zebu cattle experimentally infected with *Mycoplasma mycoides* subsp. *mycoides* SC by contact exposure

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Abstract – The purpose of the present study was to characterize the *Mycoplasma mycoides* subsp. *mycoides* small colony (*MmmSC*)-specific humoral immune response at both systemic and local levels in cattle experimentally infected with *MmmSC*, for a better understanding of the protective immune mechanisms against the disease. The disease was experimentally reproduced in zebu cattle by contact. Clinical signs, postmortem and microbiological findings were used to evaluate the degree of infection. Serum and bronchial lavage fluids (BAL) were collected sequentially, before contact and over a period of one year after contact. The kinetics of the different antibody isotypes to *MmmSC* was established. Based on the severity of the clinical signs, post mortem and microbiological findings, the animals were classified into three groups as acute form with deaths, sub-acute to chronic form and resistant animals. Seroconversion was never observed for the control animals throughout the duration of the experiment, nor for those classified as resistant. Instead, seroconversion was measured for all other cattle either with acute or sub-acute to chronic forms of the disease. For these animals, IgM, IgG1, IgG2 and IgA responses were detected in the serum and BAL samples. The kinetics of the IgM, IgG1 and IgG2 responses was nearly similar between both groups of animals. No evident correlation could thus be established between the levels of these isotypes and the severity of the disease. Levels of IgA were high in both BAL and serum samples of animals with sub-acute to chronic forms of the disease, and tended to persist throughout the entire experimental period. In contrast, animals with acute forms of the disease showed low levels of IgA in their BAL samples with none or very transient but low levels of IgA in the serum samples. Our results thus demonstrated that IgA is produced locally in *MmmSC* experimentally infected cattle by contact and may play a role in protection against contagious bovine pleuropneumonia.

contagious bovine pleuropneumonia / experimental infection / humoral response / *MmmSC*-specific Ig isotypes / protective immune mechanism

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1. INTRODUCTION

Contagious bovine pleuropneumonia (CBPP) is a respiratory disease representing a serious threat to cattle raising in Africa but also in some regions of Asia. It is caused by *Mycoplasma mycoides* subsp. *mycoides* small colony (*MmmSC*), which is a member of the *mycoides* cluster. CBPP is part of the List "A" of diseases of the Office International des Epizooties, which includes several other highly contagious diseases¹.

CBPP has been eradicated from most developed countries through a rigorous policy of restriction of cattle movement, slaughter and financial compensation. Unfortunately, for socio-cultural and economical reasons, such measures cannot be implemented in most African countries. For these reasons, it is believed that the only realistic way of controlling CBPP in the third world including African countries is by widespread and repeated vaccination [17, 21, 22]. However, the currently used vaccines against CBPP throughout Africa are not as effective as they should be, since the immunity induced is not only of short duration but also not all vaccinated animals are protected [12, 21–23].

Therefore, there is a real need to develop a more efficient vaccine for CBPP control in Africa. To achieve this goal, some prerequisites are required including characterization of the immune responses involved in protection and identification of the components of the pathogen eliciting these responses.

Since no small animal model for CBPP is available so far, these studies have to be performed on the natural bovine host and require naturally infected cattle displaying

different clinical stages of the disease. Furthermore, since in most field cases, the history of infection is unknown, experimental infections by contact exposure are required in order to mimic the natural conditions of field infections.

Understanding the characteristics of the protective immune responses is essential for the development of improved vaccines against diseases. In CBPP, while short term immunity is elicited by vaccination and long term immunity after recovery, very little is known regarding the mechanisms by which this protection is mediated [16, 22]. In a recent study we demonstrated the involvement of cellular immunity in the *MmmSC*-specific protective immune response [5, 6]. However, since CBPP is a respiratory disease, the local humoral response should also play a key role in protection by inhibiting *MmmSC* growth and potential attachment to host cells. Studies with some other respiratory mycoplasmas highlight the important role of local humoral immunity in host defense [8, 19].

The course of immune reactions of the manifold antigens of *MmmSC* has been studied in serum and bronchial lavage samples of cattle infected under experimental conditions. The western-blot analysis indicated a strong induction of local IgA antibodies (Abs) against lipoprotein surface antigens of the organism [1]. However, this study did not investigate the kinetics of the various immunoglobulin (Ig) isotypes involved in the local and systemic humoral responses nor the relationship between isotype and protection against the disease.

The purpose of the present study was to characterize the *MmmSC*-specific humoral immune response at both systemic and local levels in cattle experimentally infected with *MmmSC*, for a better understanding of the protective immune mechanisms against the disease.

¹ OIE, Manual of diagnostic tests and vaccines for terrestrial animals, 5th edition [on line] (2004) http://www.oie.int/eng/norms/A_summary.htm [consulted 14 March 2006].

2. MATERIALS AND METHODS

2.1. Experimental infection

The experimental infection protocol was designed according to the French national legislation for animal experimentation and was performed, in accordance with the same guidelines, at the Central Veterinary Laboratory (CVL) in Bamako. Details of the experimental in-contact *MmmSC* infection have already been published [13].

Eighteen clinically healthy zebu cattle, 3–6 years old, were selected from several herds and transported to CVL facilities. The cattle were never exposed to *MmmSC* infection nor vaccinated against CBPP. These data were based on information given by the owners and by the local animal health services. Serological analysis had confirmed that the selected animals were negative for Abs to *MmmSC* by slide agglutination, competitive Enzyme-linked immunosorbent assay (cELISA) and by the complement fixation (CFT). These tests are recommended by the OIE as the standard serological methods for CBPP diagnosis [4]. The animals were also free of brucellosis and tuberculosis as judged by slide agglutination and skin tests, respectively.

For procurement of *MmmSC*-infected animals, 12 sick animals (N'dama) were collected in the field during an active CBPP outbreak and were brought to the CVL facilities. The confirmation of *MmmSC* infection was made by isolation and identification of *MmmSC* from samples of lung tissues and pleural liquid taken from the animals that died during the outbreak.

For contact transmission of the disease, *MmmSC*-infected animals were housed together with 14 healthy-naïve animals. Animals were tagged with numbers from C1 to C14 for the contact animals and I1 to I12 for the infected animals. The four remaining healthy-naïve cattle were housed,

far away from the contact group, as control animals and tagged with numbers from T1 to T4.

The animals were observed daily for clinical signs of illness including cough, nasal discharge, prostration, respiratory distress, anorexia, weight loss and/or lachrymation. Rectal temperatures and respiratory pulls were recorded twice a week. Post mortem (PM) analysis was performed on all animals. The lungs were thoroughly examined for gross pathological lesions of CBPP and detailed findings were recorded.

Based on the severity of the clinical signs, *MmmSC*-infected animals were assigned to three groups: (1) acute form with two deaths (C3, C8, C9, C12 and C14), (2) sub-acute to chronic form (C4, C5, C6, C7, C11 and C13) and (3) animals which never demonstrated any clinical signs of CBPP (C1 and C2). Lung lesions observed at necropsy confirmed this trend and showed that C1 and C2 had no CBPP lesions, the two animals that died from acute CBPP (C12 and C14) had acute lesions and that the 9 remaining and surviving animals had resolved lesions or sequesters. It has to be mentioned that animal C10 died 2 weeks after the beginning of the experiment from digestive problems.

The first evident clinical signs of illness were observed in C4 (51 days post contact (pc)), followed in chronological order by C9 (56 days pc), C13 (62 days pc), C11 (66 days pc), C8 (67 days pc), C12 (69 days pc), C14 (95 days pc), C3 (104 days pc), C5 (144 days pc), C7 (160 days) and C6 (197 days).

MmmSC was isolated in pure cultures from the PM samples, including hepatized lung and sequestral contents, taken from C12, C14, and C3, C6, C11, respectively.

The control animals (T1, T2, T3 and T4) remained clinically healthy throughout the experimental period and at necropsy, no pathological changes characteristic of CBPP were noted and no mycoplasma was isolated.

2.2. Bronchoalveolar lavages and serum collection

Bronchoalveolar lavages (BAL) and blood were collected weekly, starting 7 weeks before the contact and ending at week 52 pc when the surviving animals were slaughtered.

Blood samples were allowed to clot at room temperature and then centrifuged to collect the serum samples that were aliquoted and stored at -20°C until testing. BAL samples were centrifuged and supernatants were aliquoted and stored at -20°C until testing.

2.3. Follow up of the *MmmSC*-specific antibody response

The *MmmSC*-specific antibody (Ab) response was assessed in serum samples, using the CFT (CIRAD-EMVT, Montpellier, France) and the cELISA (Institut Pourquier, Montpellier, France), according to the protocols provided with the kits. Both tests are recommended by the OIE as the standard serological methods for CBPP diagnosis [4].

For the cELISA, 96-well pre-coated microplates with *MmmSC* antigens were used for the assay. One in ten diluted sera were incubated with the monoclonal antibody. The reaction was then revealed by a peroxidase antimouse conjugate and a Tetramethyl Benzidine (TMB) substrate. The reaction was stopped by addition of sulfuric acid solution and optical densities were read at 450 nm with a Titretek Multiscan Plus MKII microplate reader (Flow Laboratories, Finland) and recorded by the ELISA Data interchange (EDI) version 2.2 software connected to the reader.

The CFT was done in microtiter plates by use of twofold serial dilutions of serum samples (starting at 1:5), 2.5 units of complement and 2 units of *MmmSC* antigen. Plates were incubated at 37°C for 30 min

before 3% of sensitized sheep red blood cells were added. The plates were then incubated at 37°C for 30 min, spun for 5 min at 1500 rpm, and the reading was carried out based on the percentage of complement fixation observed. Prior to the test, all the reagents (hemolysin, complement, antigen and sheep red blood cells) were standardized to determine optimal concentrations.

2.4. Determination of *MmmSC*-specific IgM, IgG1, IgG2 and IgA antibody responses

An indirect ELISA (iELISA) was used to measure the relative levels of the *MmmSC*-specific Ab isotypes in serum and BAL collected samples. In preliminary studies the quantity of reagents and incubation times were determined by a checkerboard titration. The *MmmSC* isolate used for this purpose was isolated from tissue samples collected from the experimental animals. Mycoplasma organisms were grown in Hayflick medium and cultures were harvested in log phase of growth by centrifugation at 10 000 g for 20 min at 4°C and washed 2 times in phosphate buffered saline (PBS). The *MmmSC* total protein concentration was measured by the bicinchoninic acid method [20]. Mycoplasmas were heat-killed by 1 h incubation at 60°C before coating plates.

Ninety six-well flat-bottom microtiter plates (Nunc Laboratories, USA) were coated with heat-inactivated-whole-*MmmSC* (2 $\mu\text{g}/\text{mL}$) in coating buffer (PBS, pH 7.4). After overnight incubation at 4°C , the plates were washed twice with PBS containing 0.05% Tween 20 (wash solution) and incubated for 1 h 45 min at 37°C in a blocking buffer (0.1% casein hydrolysate, 0.1% Tween 20 in PBS). After washing as before, 100 μL of serum (1:500 for IgM detection, 1:200 for IgA, 1:400 for IgG1 or IgG2) or BAL sample (1:2 for IgM, IgG1 or IgG2 detection, 1:16 for IgA), diluted in blocking solution,

were added in duplicate wells. Positive and negative control sera were included in each plate. These were determined in preliminary studies using sera selected among the experimental animals that seroconverted and sera taken from the experimental control animals, respectively. The plates were incubated for 1 h, at 37 °C and washed 3 times with wash solution. For serum analysis, 100 µL of horseradish peroxidase (HRP)-conjugated sheep anti-bovine Ab isotype (Bethyl Laboratories, USA): IgA (1:2000), IgG1 (1:1000), IgG2 (1:1000), or IgM (1:4000), diluted in blocking solution, were added into each well and then incubated for 1 h at 37 °C. For BAL samples, 100 µL of HRP-conjugated sheep anti-bovine IgA (1:4000), IgG1 (1:1000), IgG2 (1:1000) or IgM (1:4000) in blocking solution were used. One hundred µL/well of TMB liquid substrate system for ELISA (Sigma, St. Quentin, France) was added and the plates were incubated for 15 min at room temperature. The reaction was stopped by addition of 50 µL/well of 0.5 M sulfuric acid and optical densities (OD) were read at 450 nm with a Titretrek Multiscan Plus MKII microplate reader (Flow Laboratories, Finland). The mean OD value of each sample was calculated and corrected by subtracting the mean OD value of the negative control.

3. RESULTS

3.1. *MmmSC*-specific serological antibody responses

The *MmmSC*-specific serological response was assessed for each animal in the experiment using the CFT and the cELISA. The four control animals as well as C1 and C2 did not seroconvert. In contrast, all animals with acute or sub-acute to chronic forms of the disease seroconverted. This seroconversion occurred at

different periods pc regardless of the onset of the clinical signs. Titers were high and varied for both the CFT and cELISA test (Figs. 1 A and 1 B).

No obvious correlation could be established between the Ab titers and the severity of the clinical signs or lung lesions.

3.2. Serum and pulmonary antibody isotype responses

The control animals, as well as C1 and C2, were excluded from this study since they never showed an *MmmSC*-specific Ab response during the entire experiment. For all other animals, a kinetic analysis of the various Ab isotypes (IgM, IgG1, IgG2 and IgA) was carried out in sera and BAL samples by iELISA.

3.2.1. *MmmSC*-specific IgM response

Figures 2 A and 2 B present the serological *MmmSC*-specific IgM response of the *MmmSC*-infected cattle. They show that the *MmmSC* infection induced a low IgM response. The response was either observed for a short period of time for C3 (5 weeks), C5 (2 weeks), C6 (5 weeks) or more sustained but with intermittence for C7, C9 and C11. The onset of this IgM serological response corresponded to the onset of the clinical signs (± 2 weeks) for the majority of animals except for C11 (6 weeks before). The two non surviving cattle presented either an early and slight IgM serological response (C14), observed 5 weeks before appearance of clinical signs, or a significant but transient IgM response (C12) noticed 12 weeks after the onset of clinical signs.

In parallel, the local IgM analysis revealed no *MmmSC*-specific IgM response detectable in BAL, except at very low levels for three animals, C9, C12 and C14 (Figs. 2 C and 2 D). C12 showed a slight local IgM response initiated the same week

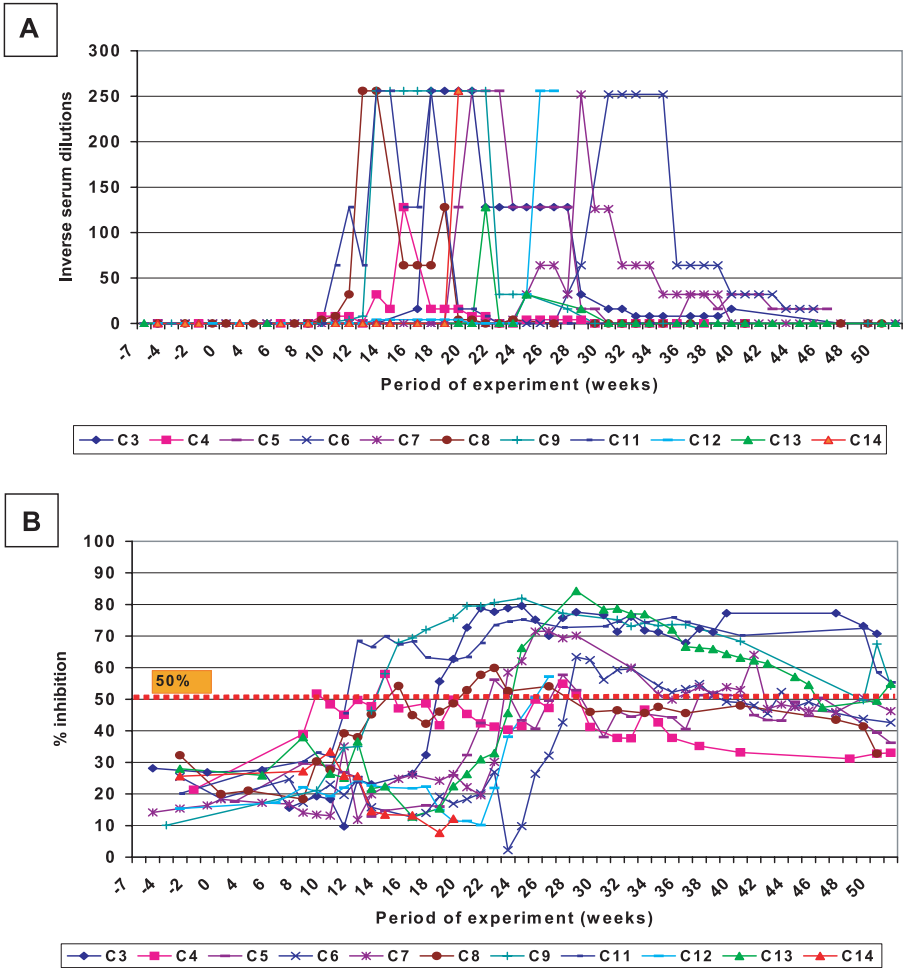


Figure 1. Results of the *MmmSC*-specific serological responses in CBPP contact-exposed animals with acute and sub-acute to chronic forms of the disease. Serum samples, collected weekly, starting at week -7 and ending at week 52 pc, were tested for each animal using the CFT (A) and the cELISA (B). (A color version of this figure is available at www.edpsciences.org.)

as the clinical symptoms whereas C9 presented a moderate and short response beginning three weeks following the onset of clinical signs. A rise of local IgM was observed for C14 in the last sample taken before death of the animal, in parallel to the serological IgM response.

No significant difference in the IgM response was observed between the two groups of animals.

3.2.2. *MmmSC*-specific IgG1 response

A significant *MmmSC*-specific IgG1 response was detected in the sera (Figs. 3 A and 3 B) and BAL (Figs. 3 C and 3 D) of all *MmmSC*-infected animals except C14 which presented strong variations in the OD values before *MmmSC* infection.

The general pattern of the IgG1 kinetic response in the serum was similar for all

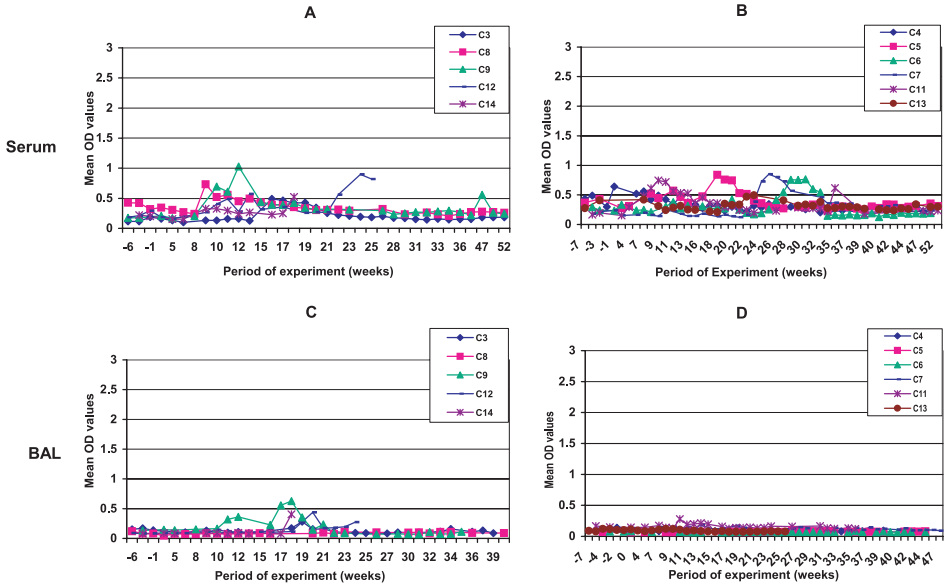


Figure 2. Kinetic analysis of the *MmmSC*-specific IgM Ab responses in cattle with acute (A, C) and sub-acute to chronic (B, D) forms of CBPP. Serum and BAL samples, collected weekly, starting at week -7 and ending at week 52 pc, were tested by an iELISA test. (A color version of this figure is available at www.edpsciences.org.)

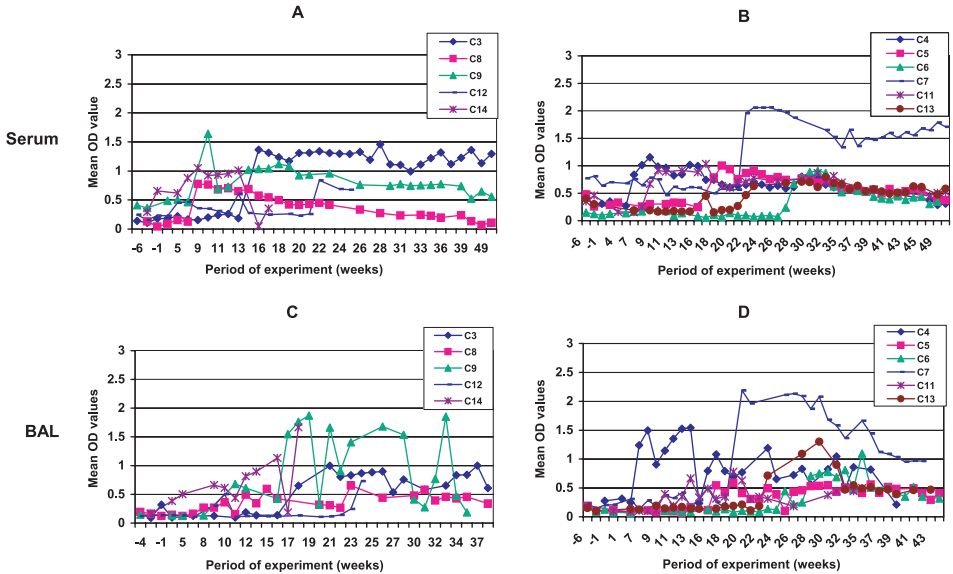


Figure 3. Kinetic analysis of the *MmmSC*-specific IgG1Ab responses in cattle with acute (A, C) and sub-acute to chronic (B, D) forms of CBPP. Serum and BAL samples, collected weekly, starting at week -7 and ending at week 52 pc, were tested by an iELISA test. (A color version of this figure is available at www.edpsciences.org.)

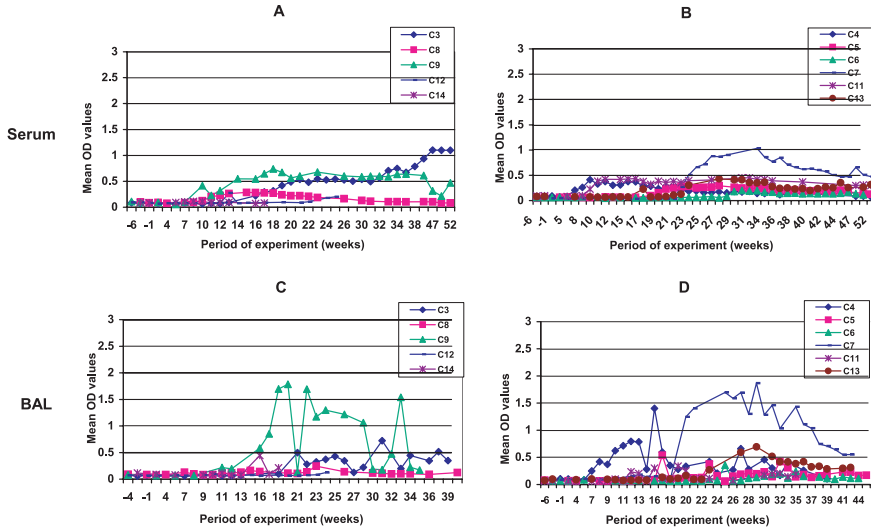


Figure 4. Kinetic analysis of the *MmmSC*-specific IgG2 Ab responses in zebu cattle with acute (A, C) and sub-acute to chronic (B, D) forms of CBPP. Serum and BAL samples, collected weekly, starting at week -7 and ending at week 52 pc, were tested by an iELISA test. (A color version of this figure is available at www.edpsciences.org.)

animals, although with variable levels, except for C12 and C13. Indeed, for the majority of animals the IgG1 serological response was initiated in parallel to the onset of the clinical signs (± 1 week) and was sustained for at least 3 months. For C13, the seroconversion was noticed 14 weeks after the first clinical signs and was maintained for 15 weeks. In contrast, for C12 an increase of the IgG1 response was present 5 weeks before the clinical signs but was not maintained and a second rise of the IgG1 was observed 12 weeks after the appearance of clinical signs (2 weeks before death) and maintained until the death of the animal.

A strong local IgG1 response was observed for C3, C4, C7, C9 and C13, moderate for C6 and slight for C5, C8 and C11. The kinetic analysis revealed that for the 3 cattle, C5, C6 and C7, presenting a late infection (20 to 28 weeks pc), the pulmonary IgG1 increased 3 weeks before the onset of clinical signs. Concerning

C13, as described above for sera, the local IgG1 response was present 14 weeks post clinical signs whereas for C12 a local IgG1 increase was noticed 1 week before the onset of clinical signs, then decreasing and followed by a second rise 2 weeks before death. For all other cattle, the local IgG1 increase was noticed either at the same week as the onset of the clinical signs (C4) or 2 (C11, C8) to 3 (C3, C9) weeks later. For all recovered animals, this local IgG1 response was sustained almost until the end of the experiment.

3.2.3. *MmmSC*-specific IgG2 response

An *MmmSC*-specific serological IgG2 response was detected in all the cattle recovered from the *MmmSC* infection although with a lower intensity than the IgG1 serological response (Figs. 4 A and 4 B). Three animals showed a strong IgG2 serological response, C3, C9 and C7 whereas all other cattle presented a low but sustained IgG2 response. No IgG2 response

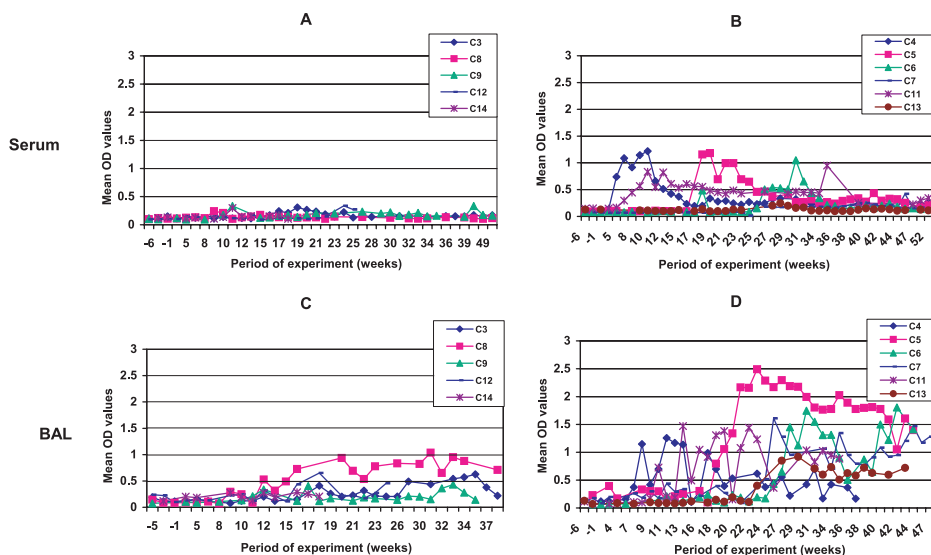


Figure 5. Kinetic analysis of the *MmmSC*-specific IgA Ab responses in zebu cattle with acute (A, C) and sub-acute to chronic (B, D) forms of CBPP. Serum and BAL samples, collected weekly, starting at week -7 and ending at week 52 pc, were tested by an iELISA test. (A color version of this figure is available at www.edpsciences.org.)

was observed for C14 while for C12, as was the case for IgG1, a slight increase of the IgG2 response was noticed in sera 2 weeks before death.

Analysis of the local IgG2 response revealed a strong response for C9, a moderate response for C3 and no response for the other animals with acute infection while for the animals with subacute or chronic infection, a strong response was observed for C4 and C7, moderate for C5 and C13 and low for C11 (Figs. 4 C and 4 D).

The IgG2 response, where detected, appeared generally 1 or 2 weeks after the onset of the IgG1 response but, thereafter almost paralleling the IgG1 response.

3.2.4. *MmmSC*-specific IgA response

Analysis of the *MmmSC*-specific IgA response revealed a strong difference

between the group of animals with acute infection and those with subacute to chronic infection. Indeed while no serological IgA response was detected in the first group (Fig. 5 A), 4 cattle from the second group presented a strong and sustained IgA response (C4, C5, C6 and C11) and 1 cattle (C7) presented a low but sustained IgA response (Fig. 5 B).

The local IgA response also showed a strong difference between the two groups (Figs. 5 C and 5 D). A moderate but sustained local IgA response was observed in all the cattle from the group of animals with acute infection but which recovered. The two non-surviving animals were characterized by an absence of IgA response (C14) or a very low, intermittent, IgA response (C12). All cattle with subacute to chronic infection that survived the *MmmSC* infection were characterized by a strong and sustained local IgA response.

4. DISCUSSION

The objective of the present study was to characterize the systemic and local humoral *MmmSC*-specific immune responses in zebu cattle experimentally infected with *MmmSC*. For this purpose, a natural in-contact *MmmSC* infection was implemented with 14 naïve cattle in order to reproduce the various clinical forms of the disease. Following *MmmSC* infection, the animals were assigned to three groups, acute form (leading to death of two cattle), sub-acute to chronic form and animals resistant to the disease. This was consistent with the observations already described in naturally occurring CBPP [16]. Samples (blood and BAL) were taken regularly allowing a kinetic analysis of detectable *MmmSC*-specific Ab isotypes.

The global serological response, assessed by CFT and cELISA revealed that all *MmmSC*-exposed animals with evidence of the disease presented *MmmSC*-specific circulating Abs. However, no correlation was evident between Ab titers and the severity of CBPP clinical signs or the types and intensity of lung lesions observed at necropsy, as already described [3,4,10,15,18]. In contrast, Abs to *MmmSC* never developed in two animals for which the absence of clinical and pathological lesions might suggest a resistant form to CBPP.

Up to now, no data have been available concerning the *MmmSC*-specific local Ab response although it is likely, since CBPP is a respiratory disease, that the local immunity would play a major role. Comparative analysis of the *MmmSC*-specific Ab isotype responses was thus focused, at the systemic and local levels, on animals with acute infection and those with sub acute to chronic infection.

The most salient finding in the present study was the presence of persistent and high levels of *MmmSC*-specific Ab of the IgA isotype in both BAL and serum

samples of all cattle presenting a subclinic or chronic infection and that recovered. In contrast, much lower levels of *MmmSC*-specific IgA, if any, were detected in the BAL samples of the acutely infected animals with absence of a seric IgA response. This finding suggests a correlation between the presence of *MmmSC*-specific IgA and an attenuation of the clinical impact of the *MmmSC* infection. Our results have also shown, in animals recovered from a subclinic to chronic *MmmSC* infection, the predominance of the local IgA response over the corresponding serological response, either in magnitude or in duration. It is likely that *MmmSC*-specific local IgA were transferred to serum by transudation. This is further supported by the absence of serological IgA in the acutely-infected animals while moderate levels of IgA were found in their BAL. It has to be pointed out that accurate comparative analysis of relative Ab levels between BAL and serum was complicated by the different dilution factors of the respective samples. However, this could be compensated with the variable dilution factor when collecting BAL fluids.

Our results also indicate the presence of other *MmmSC*-specific Ig isotypes in *MmmSC*-infected cattle. However, no significant difference in terms of isotype, kinetic or magnitude of the Ab response allowed significant distinction between the different CBPP clinical forms. These results may suggest that, in cattle experimentally infected with *MmmSC*, these Ig isotypes are not correlated to protection. This finding is in accordance with other studies of the Ig isotype responses following mycoplasma infections reviewed in [19]. Furthermore, since *MmmSC*, as do numerous other mycoplasmas, causes a respiratory, thus mucosal, infection, the absence of correlation between these isotypes and protection is not unlikely. Indeed, in mucosal infection, the local Ab response plays a major role but is typically

associated with IgA [2, 19]. Nevertheless, several other studies suggest a protective role for local antibodies other than IgA. Indeed, local immunity to *M. bovis* has been related to specific IgG Ab rather than IgA Ab [9]. Similarly, resistance to *M. pulmonis* in mice has been correlated with the presence of IgG1 and IgG2 Ab in lung washings [8].

The results of the present study suggest that the *MmmSC*-specific IgA produced locally may participate in protective immunity against CBPP. Indeed, our findings showed that all animals with higher levels of local IgA were characterized by reduced severity of the lung lesions. The PM analysis revealed, for all animals with sub-acute or chronic infection, either resolved lesions or small unique sequestras. Furthermore, among the group of cattle with acute infection, the animals with the highest titer of local IgA also presented resolved lung lesions. In contrast, the low level of local IgA observed in all other acutely-infected animals was correlated with acute lung lesions (complete lung hepatization or large sequestras).

The importance of IgA in the humoral defense at mucosal surfaces is well known. Several studies with other respiratory mycoplasmas have been reported, demonstrating the importance of local immunity in host defense [14, 19]. In mycoplasmal pneumonia of swine the importance of the local immune response has been evidenced by the number of immunoglobulin-producing cells in lung tissues, as well as the increased Ab titres to *M. hyopneumoniae* in tracheobronchial secretions during the course of induced infection [8]. In humans, *M. pneumoniae*-specific IgA responses in secretions correlate better with protection than serum Ab [8, 19]. Similarly, a positive correlation between *M. gallisepticum*-specific IgA titers in tracheal secretions and decreased lesions caused by the organism has been reported [2]. Our results which are consistent with these reports and

similar studies conducted on other mucosal infections [7, 11], strongly suggest a potential role for the local IgA in the protective mechanism against *MmmSC* infection. Whether this role is to block *MmmSC* attachment to host mucosal surface or to help killing *MmmSC* by enzymes or other substances within mucosal secretions remains to be determined [19].

In conclusion, the present study demonstrates that, although several Ig isotypes were elicited during *MmmSC* infection, only the *MmmSC*-specific locally produced IgA play a role in the protection against CBPP. Therefore, this may provide valuable guidance for decisions about designing effective vaccines against CBPP.

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