Immune Responses in Cattle Inoculated with *Mycobacterium bovis*, *Mycobacterium tuberculosis*, or *Mycobacterium kansasii*[∇]

W. R. Waters,[1]* A. O. Whelan,[2] K. P. Lyashchenko,[3] R. Greenwald,[3] M. V. Palmer,[1] B. N. Harris,[4]
R. G. Hewinson,[2] and H. M. Vordermeier[2]

*National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa[1];
Veterinary Laboratories Agency, Addlestone, New Haw, Addlestone, Surrey, United Kingdom[2];
Chembio Diagnostic Systems, Inc., Medford, New York[3]; and U.S. Department of Agriculture,
Animal and Plant Health Inspection Service, Veterinary Services,
National Veterinary Services Laboratory, Mycobacteria and
Brucella Section, Ames, Iowa[4]*

Cattle were inoculated with *Mycobacterium bovis*, *Mycobacterium tuberculosis*, or *Mycobacterium kansasii* to compare the antigen-specific immune responses to various patterns of mycobacterial disease. Disease expression ranged from colonization with associated pathology (*M. bovis* infection) and colonization without pathology (*M. tuberculosis* infection) to no colonization or pathology (*M. kansasii* infection). Delayed-type hypersensitivity and gamma interferon responses were elicited by each mycobacterial inoculation; however, the responses by the *M. bovis*- and *M. tuberculosis*-inoculated animals exceeded those of the *M. kansasii*-inoculated animals. Specific antibody responses were detected in all *M. tuberculosis*- and *M. bovis*-inoculated cattle 3 weeks after inoculation. From 6 to 16 weeks after *M. tuberculosis* inoculation, the antibody responses waned, whereas the responses persisted with *M. bovis* infection. With *M. kansasii* inoculation, initial early antibody responses waned by 10 weeks after inoculation and then increased 2 weeks after the injection of purified protein derivative for the skin test at 18 weeks after challenge. These findings indicate that antibody responses are associated with the antigen burden rather than the pathology, cellular immune responses to tuberculin correlate with infection but not necessarily with the pathology or bacterial burden, and exposure to mycobacterial antigens may elicit an antibody response in a presensitized animal.

Tuberculosis (TB) in humans and animals may result from exposure to bacilli within the *Mycobacterium tuberculosis* complex (i.e., *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. pinnipedi*, *M. microti*, *M. caprae*, or *M. canetti* [8]). Despite their ~99.95% sequence identity (12), *M. bovis* and *M. tuberculosis* exhibit distinct differences in virulence and host adaptation. Compared to *M. tuberculosis*, experimental *M. bovis* infection of mice or rabbits results in a more severe pathology and shorter mean survival times (9, 17, 18). *Mycobacterium tuberculosis* is primarily a human pathogen that demonstrates a high level of attenuation in cattle (as reviewed by Francis in 1947 [10]), whereas *M. bovis* has a wider host range and affects many domesticated and free-ranging mammals as well as humans. Prior to the initiation of control and eradication campaigns in the early to mid-1900s, *M. bovis* infection accounted for up to 30% of human tuberculosis cases, with *M. bovis* being transmitted to humans primarily by the consumption of unpasteurized dairy products and contact with infected livestock. Control efforts, including slaughter surveillance and test/cull campaigns, have dramatically reduced the prevalence of *M. bovis* infection in domestic cattle herds, thereby reducing the spread of *M. bovis* to humans. However, in developing countries, *M. bovis* infection of humans persists as a serious and relatively common zoonosis (16).

Although *Mycobacterium kansasii* is not a member of the *M. tuberculosis* complex, it may cause disease in otherwise healthy humans, albeit infrequently, that is clinically indistinguishable from *M. tuberculosis* infection (1, 3). As with humans, *M. kansasii* infection of cattle is uncommon; however, it is occasionally associated with granulomatous lesions within lymph nodes and the respiratory tract of cattle (B. Harris, unpublished observations). Of particular relevance for the diagnosis of tuberculosis, *M. kansasii* infection/sensitization may elicit responses to antigens generally considered to be tuberculosis specific, such as ESAT-6, CFP-10, and MPB83 (2, 30, 35).

With experimental *M. bovis* infection of cattle, the levels of MPB83-specific antibody correlate with disease severity, bacterial burden, and specific cell-mediated immune responses (15, 33). With this particular scenario, disease severity (i.e., pathology) and bacterial burden are intimately linked; thus, it is difficult to define a potential correlation of a particular immune response to either readout independently. Prior studies have demonstrated that virulent and attenuated strains of *M. bovis* induce similar delayed-type hypersensitivity responses in cattle; however, only the virulent *M. bovis* strain induces a persistent gamma interferon (IFN-γ), interleukin-2 (IL-2), and antibody response (34). The objective of the present study was to compare mycobacterium-specific immune responses to the patterns of mycobacterial disease expression in which the mycobacterial burden is uncoupled from pathological changes. Disease expression patterns included persistent colonization with an associated pathology (i.e., *M. bovis* infection), colonization without an associated pathology (i.e., *M. tuberculosis*

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* Corresponding author. Mailing address: USDA, ARS, National Animal Disease Center, P.O. Box 70, Ames, IA 50010-0070. Phone: (515) 337-7756. Fax: (515) 663-7458. E-mail: ray.waters@ars.usda.gov.
∇ Published ahead of print on 9 December 2009.

infection), and no colonization or pathology (i.e., *M. kansasii* infection). Antigen-specific immune responses were evaluated for their correlation to manifestations of disease expression.

MATERIALS AND METHODS

Calves, challenge inoculum, and necropsy. Friesian or Friesian cross castrated male calves of ~6 months of age were obtained from bovine TB-free herds and were housed within either biosafety level 3 facilities at the Veterinary Laboratory Agencies (VLA), Addlestone, United Kingdom (*M. bovis*- and *M. tuberculosis*-inoculated animals) or biosafety level 2 facilities at the National Animal Disease Center (NADC), Ames, IA (*M. kansasii*-inoculated animals). All cattle experiments were cleared by local ethical review, and animal procedures were performed in accordance with either British Home Office requirements [i.e., A(SP)A, 1086; VLA studies] or according to institutional guidelines and approved animal care and use protocols (NADC studies). The treatment groups included *M. tuberculosis*-inoculated calves ($n = 5$), *M. bovis*-inoculated calves ($n = 5$), and *M. kansasii*-inoculated calves ($n = 4$). The inoculation of *M. tuberculosis* (2.8×10^6 CFU) and *M. bovis* (1.0×10^6 CFU) was by direct instillation of the challenge inoculum into the tracheas of sedated calves, as described previously (5, 27). The inoculation of *M. kansasii* (4×10^8 CFU) was by direct instillation of the inoculum into the tonsillar crypts of sedated calves, as described previously (19). In general, these two routes of inoculation of tuberculous mycobacteria to cattle (i.e., intratracheal and intratonsillar) result in similar disease severities and infections (5, 20, 27). Whole blood was collected at 0, 2, 4, 6, 8, 10, 12, and 16 weeks after challenge for IFN-γ assays and placed in tubes with heparin; and serum was collected at 0, 3, 6, 7, 9, 10, 12, 13, 16, and 18 weeks after challenge for antibody detection assays (the slight variations in collection time points were dependent upon the treatment groups and assay). The 100-fold larger dose of *M. kansasii* was used on the basis of the findings of prior studies (19) and the relatively low virulence of *M. kansasii* compared to that of *M. bovis* and *M. tuberculosis*.

M. bovis field isolate AF2122/97 was grown to mid-log phase in Middlebrook 7H9 medium (Becton, Dickinson and Company [BD], Oxford, United Kingdom) supplemented with 10% (vol/vol) Middlebrook acid-albumin-dextrose-catalase enrichment (BD), 4.16 g/liter sodium pyruvate (Sigma-Aldrich Co., Poole, United Kingdom), and 0.05% (vol/vol) Tween 80 (Sigma-Aldrich); and stocks were stored frozen at −80°C. Stocks of *M. tuberculosis* H₃₇Rᵥ were prepared in the same way, except that the 7H9 medium was supplemented with 0.2% (vol/vol) glycerol (Sigma-Aldrich) rather than sodium pyruvate. *M. kansasii* field isolate 03-6931 (subtype 1, based on the 16S-23S rRNA sequence [1, 30]) was grown to mid-log phase in Middlebrook 7H9 medium supplemented with 10% oleic acid-albumin-dextrose complex (Becton Dickinson, Franklin Lakes, NJ) plus 0.05% Tween 80 (Sigma Chemical Co., St. Louis, MO), and stocks were stored frozen at −80°C. Enumeration of the bacterial stocks (CFU/ml) was by serial dilution on modified Middlebrook 7H11 agar (11). In addition, the virulence of the *M. tuberculosis* and *M. bovis* strains was confirmed in guinea pigs prior to the start of the cattle challenge experiment (data not shown). The *M. kansasii* strain used in the present study was isolated from a tuberculous lesion (i.e., a pyogranuloma) detected within an adult cow upon routine slaughter surveillance. The *M. kansasii* isolate had 100% identity with the subtype 1 genotype, which is most commonly associated with disease in immunocompetent humans (30).

Postmortem examination. Approximately 16 to 18 weeks after inoculation, all cattle were euthanized by the intravenous injection of sodium pentobarbital and examined. Specimens of various tissues were collected (tonsil, lung, lung-associated lymph nodes, and head-associated lymph nodes) for bacteriologic culture (described below) and microscopic examination, including Ziehl-Neelsen staining for acid-fast bacilli as well as hematoxylin-eosin staining for morphological assessment of the tissues, as described previously (4, 31). All major visceral organs were visually inspected for lesions, and tissue specimens were collected for further microscopic and bacteriologic assessment, when it was warranted. The lungs were examined externally and were sliced into 0.5- to 1-cm-thick sections for the detection of visible lesions. Lymph node tissues were sliced into thin sections (1 to 2 mm thick) and were examined for the presence of visible lesions. The severity of the gross pathological changes was scored by using a semiquantitative scoring system, as described previously (29). In summary, each of seven lung lobes was scored from 0 to 5, depending on the number of lesions and the extent of the pathology observed, with 0 being no pathology and 5 being extensive gross coalescing lesions. The lymph nodes of the upper and lower respiratory tracts were similarly scored but by use of a score of from 0 to 3. The scores of the individual lung lobes were added to calculate the lung score, and the

scores of the individual lymph nodes were added to calculate the lymph node score. Both lymph node and pathology scores were combined to determine the total pathology score for each animal.

Bacterial enumeration. Tissue sections collected from lymph node and lung samples postmortem were individually homogenized in 5 ml of sterile phosphate-buffered saline (PBS) with a rotating-blade macerator system. Enumeration of the CFU in each sample was done by inoculating modified 7H11 agar plates with 50 μl of tissue homogenate and counting the colonies after incubation at 37°C for 4 to 6 weeks (11). A semiquantitative bacterial burden score of 0 to 4 was determined for each tissue sample, where 0 indicates no colonies, 1 indicates 1 to 10 CFU, 2 indicates 11 to 100 CFU, 3 indicates 101 to 1,000 CFU, and 4 indicates >1,000 CFU per sample. The score for all tissues was combined to provide a total bacteriology burden score per animal. All culture-positive samples from the *M. tuberculosis*-infected animals and a representative sample from four of the *M. bovis*-infected cattle were submitted to the VLA Molecular Strain Typing Laboratory to confirm the strain identity by spoligotyping. Strains were spoligotyped by the method of Kamerbeek et al. (14) with minor modifications (6).

Tuberculin skin test procedures. For the studies at VLA (i.e., for *M. bovis*- and *M. tuberculosis*-inoculated animals), skin tests were performed as specified in European Economic Community Directive 80/219EEC and amending directive 64/422/EEC, annex B. For the studies at NADC (i.e., for the *M. kansasii*-inoculated animals), skin tests were performed as specified in the circular on uniform methods and rules for the eradication of bovine tuberculosis of the Animal and Plant Health Inspection Service (APHIS), USDA (APHIS circular 91-45-011 [24]). At approximately 16 weeks after inoculation, the skin thickness was measured with calipers immediately prior to the administration of *M. bovis* purified protein derivative (PPD), and the skin thickness was again measured 72 h after injection. The skin tests were performed at the midcervical region and the caudal fold for the studies at VLA and NADC, respectively. Data are presented as the change in the skin thickness (mm) from the preinjection measurements (mean ± standard error of the mean [SEM]).

IFN-γ assay. Duplicate 250-μl heparinized whole-blood aliquots were distributed in 96-well plates with *M. bovis* PPD (10 μg/ml; VLA or Prionics Ag, Schlieren, Switzerland) or no antigen and were incubated at 37°C in a 5% CO₂ atmosphere for 20 h. The IFN-γ concentrations in stimulated plasma were determined by using a commercial enzyme-linked immunosorbent assay (ELISA)-based kit (Bovigam; Prionics Ag). The absorbances of the standards (recombinant bovine IFN-γ; Endogen, Rockford, IL) and the test samples were read at 450 nm by using an ELISA plate reader (Molecular Devices, Menlo Park, CA). Duplicate samples for individual treatments were analyzed, and the data are presented as the optical densities at 450 nm of the response to *M. bovis* PPD minus the response to no antigen (mean ± SEM).

Lateral-flow assay. The VetTB Stat-Pak lateral-flow assay (Chembio Diagnostic Systems, Inc., Medford, NY) was performed as previously described for use with samples from cattle (33). Briefly, the lateral-flow device consists of a plastic cassette containing a strip of nitrocellulose membrane impregnated with test antigen (i.e., MPB83, ESAT-6, and CFP-10) and laminated with several pads made of glass fiber and cellulose. Thirty microliters of serum and 3 drops of sample diluent are sequentially added to the sample pad. As the diluted test sample migrates to the conjugate pad, the latex particles conjugated to antigen bind to antibody (IgM, IgG, and IgA), if antibody is present, thus creating a colored immune complex. This complex flows laterally via capillary forces across the nitrocellulose membrane impregnated with specific antigen and binds to the immobilized antigen, producing a visible blue band. In the absence of specific antibody, no band is visible.

MAPIA. The multiantigen print immunoassay (MAPIA) was performed as previously described for use with samples from cattle (30, 33). Briefly, *M. tuberculosis* complex antigens were immobilized on nitrocellulose membrane strips, blocked for 1 h with 1% nonfat skim milk in PBS with 0.05% Tween 20, and then incubated for 1 h with serum samples diluted 1:40 in blocking solution. After the strips were washed, they were incubated overnight with peroxidase-conjugated protein G (Sigma) diluted 1:1,000, washed, and developed with 3,3′,5,5′-tetramethylbenzidine (TMB; Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD). Responses to individual *M. tuberculosis* complex antigens were identified by MAPIA.

ELISA for serum IgG to MPB83 and *M. bovis* culture filtrate. The amount of antigen-specific IgG in serum from clotted blood was measured as described previously (28). Briefly, Maxisorp ELISA plates (Nunc, Roskilde, Denmark) were coated with either recombinant MPB83 (0.1 μg/ml; Lionex Diagnostics and Therapeutics GmbH, Braunschweig, Germany) or an *M. bovis* AN5 culture filtrate previously prepared at VLA (1 μg/ml). Serum samples were diluted 1:100 prior to addition to precoated and preblocked assay plates. Wells not coated with

TABLE 1. Disease expression upon mycobacterial inoculation

Group	Gross pathology[a]	Culture score[b]
M. bovis (n = 5)	All positive	27.2 ± 7.3
M. tuberculosis (n = 5)	All negative	13.9 ± 5.5
M. kansasii (n = 4)	All negative	0 ± 0

[a] Gross lesions were confirmed to be tuberculous upon histologic evaluation and culture of lung tissue as well as lung- and head-associated lymph node tissues.

[b] Cumulative score (mean ± SEM) based on a ranking of the total numbers of CFU per plate of lung tissue and lung-associated lymph node tissue homogenates at the highest dilution. Mycobacteria were not detected in any tissues collected from *M. kansasii*-inoculated animals. Mean scores reflect the results from each animal within the treatment group, including culture-negative animals. The results differ ($P < 0.05$) according to mycobacterial treatment: *M. bovis*-inoculated animals > *M. tuberculosis*-inoculated animals > *M. kansasii*-inoculated animals.

antigen were used to ensure the absence of nonspecific responses, and the prechallenge responses were used for comparison to the specific responses after challenge. Total IgG was detected by using a horseradish peroxidase-conjugated sheep anti-bovine IgG (AbD Serotec, Oxford, United Kingdom), with the data being expressed as the optical densities at 450 nm (mean ± SEM).

ELISA for serum Ig to *M. bovis* LAM. An ELISA for serum Ig to *M. bovis* lipoarabinomannan (LAM) was performed as described previously (30, 33). Briefly, Immulon II 96-well microtiter plates (Dynatech, Chantilly, VA) were coated with LAM-enriched mycobacterial antigen (8 μg, prepared as described previously [32]). Serum samples were diluted 1:100 prior to addition to precoated and preblocked assay plates. Total Ig was detected by using horseradish peroxidase-conjugated goat anti-bovine IgG heavy and light chains (Kirkegaard & Perry Laboratories Inc.). Data are presented as sample/positive (S/P) values (mean ± SEM). S/P values of test samples were calculated from absorbency values by using the following formula: sample − negative control/positive control − negative control. Positive and negative control sera with known reactivities were obtained from previous studies.

Statistics. Data were analyzed by analysis of variance, followed by the Bonferroni multiple comparisons test or Student's *t* test, using a commercially available statistics program (Prism, version 4.0; GraphPAD Software, La Jolla, CA).

RESULTS AND DISCUSSION

Disease progression. Granulomatous lesions containing acid-fast bacilli were detected in the lungs as well as the lung- and head-associated lymph nodes from five of five, five of five, and three of five *M. bovis*-infected animals, respectively (Table 1). Lesions with acid-fast bacilli were not detected in any of the *M. tuberculosis*- or *M. kansasii*-inoculated animals (Table 1). *Mycobacterium bovis* was isolated by culture from five of five animals, whereas *M. tuberculosis* was isolated from three of five animals inoculated with the respective mycobacteria. For the *M. bovis*-infected cattle, *M. bovis* was isolated by culture from lung-associated lymph nodes and lungs from five of five animals and from medial retropharyngeal lymph nodes from three of five cattle. For the *M. tuberculosis*-infected cattle, *M. tuberculosis* was isolated by culture from lung-associated lymph nodes from three of five animals and from the medial retropharyngeal lymph nodes from one of five cattle. Analysis of the spoligotype patterns confirmed that the mycobacterial strains isolated from tissues matched the respective challenge strains (i.e., H$_{37}$R$_v$ for *M. tuberculosis* and AF2122/97 for *M. bovis*; data not shown). *Mycobacterium* spp. were not detected in *M. kansasii*-inoculated animals. The mycobacterial burden differed ($P < 0.05$) between challenge species (Table 1), as follows: *M. bovis* > *M. tuberculosis* > *M. kansasii*.

Cell-mediated responses. Injection of *M. bovis* PPD for the skin test (at ~16 weeks postinoculation) resulted in palpable reactions in all calves, regardless of the mycobacterial treatment; however, differences were detected, with the responses (means ± SEMs) of the *M. tuberculosis*-inoculated cattle (22.6 ± 5.1) and *M. bovis*-inoculated cattle (12.8 ± 1.9) exceeding ($P < 0.05$) the responses of the *M. kansasii*-inoculated cattle (4.5 ± 0.3). Similarly, IFN-γ responses to *M. bovis* PPD were elicited in all calves regardless of the mycobacterial treatment. Two weeks after inoculation, the mean IFN-γ responses did not differ ($P > 0.05$) between treatment groups. From 4 weeks to the end of the study, the mean IFN-γ responses to *M. bovis* PPD by *M. tuberculosis*- and *M. bovis*-inoculated cattle exceeded ($P < 0.05$) the mean response of the *M. kansasii*-inoculated cattle (Fig. 1). Throughout the study, the IFN-γ responses to *M. bovis* PPD did not differ ($P > 0.05$) between the *M. tuberculosis*- and the *M. bovis*-inoculated cattle. The patterns of the IFN-γ responses to the ESAT-6 and CFP10 antigens were similar to the pattern of responses to *M. bovis* PPD (data not shown) (30). Thus, the delayed-type hypersensitivity and IFN-γ responses did not correlate with the disease progression differences detected between *M. tuberculosis*- and *M. bovis*-inoculated cattle. Likewise, transient IFN-γ responses may be elicited to mycobacterial species (e.g., *M. kansasii*) that are efficiently cleared by cattle. It is interesting to note that the IFN-γ response kinetics toward challenge with all three pathogens developed very similarly up to 2 weeks postinfection, after which time the responses in the *M. kansasii*-infected calves were curtailed and remained at a significantly lower level throughout the rest of the observation period. One caveat concerning this observation is that *M. bovis* antigens were used for the assay; thus, the responses may be indicative of early cross-reactive responses. Two weeks postinfection is most likely the time point when a developing cellular immune response becomes effective, and this response appears to be capable of eliminating *M. kansasii*, while it is unable to fully control *M. bovis* infection and, to a lesser extent, *M. tuberculosis* infection. This response kinetic is very similar to that observed after mouse aerosol infection, in which *M. tuberculosis* is exported to the regional lymph nodes for the development of the initial primary response (7, 21, 36). This delayed
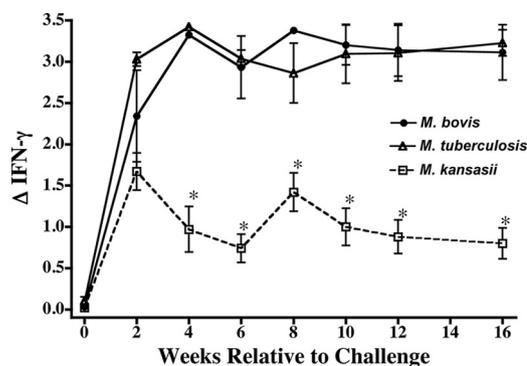

FIG. 1. IFN-γ responses upon experimental inoculation of calves with *M. bovis* (n = 5), *M. tuberculosis* (n = 5), or *M. kansasii* (n = 4). Data are presented as optical densities (means ± SEMs) at 450 nm of the response to *M. bovis* PPD minus the response to no antigen (ΔIFN-γ). *, the response was significantly different ($P < 0.5$) from the responses by the *M. bovis*- and *M. tuberculosis*-inoculated animals at the same time point.

TABLE 2. Kinetics of serum antibody responses to MPB83, ESAT-6, and CFP-10 in cattle during experimental mycobacterial infection detected by VetTB Stat-Pak lateral-flow assay

Group	No. of animals positive/no. of animals tested at the following wk after inoculation:					
	0	3	6	9	12	18[a]
M. bovis[b]	0/4	4/4	4/4	3/4	3/4	NA[c]
M. tuberculosis	0/5	5/5	4/5	2/5	0/5	NA
M. kansasii	0/4	0/4	0/4	2/4	1/4	4/4

[a] For the *M. kansasii*-inoculated cattle, the responses at 18 weeks were 2 weeks after the injection of PPD for the skin test.

[b] One of the *M. bovis*-infected cattle was euthanized at week 6, and therefore, kinetics data were available for only four of the five original study animals.

[c] NA, animals in the *M. tuberculosis*- and *M. bovis*-inoculated groups were euthanized 16 weeks after inoculation; thus, samples were not available for analysis at that time point.

immune kinetic in mice is associated with the slow and delayed migration of parenchymal dendritic cells to pulmonary lymph nodes (26). It would be of interest to conduct similar studies with *M. kansasii* to see if the migration kinetics are identical to those for its more virulent cousins, *M. tuberculosis* and *M. bovis*.

Antibody responses. Specific antibody responses were detected in all *M. tuberculosis*- and *M. bovis*-inoculated cattle 3 weeks after inoculation (Table 2). At 12 and 16 weeks after inoculation, the IgG responses to MPB83 and *M. bovis* culture filtrate by *M. bovis*-inoculated cattle exceeded ($P < 0.05$) the respective responses by the *M. tuberculosis*-inoculated cattle (Fig. 2). Antibody levels to MPB83 persisted from 3 to 16 weeks after challenge with the *M. bovis* group, whereas the responses to MPB83 by the *M. tuberculosis*-inoculated cattle waned from 6 to 16 weeks after challenge (Fig. 2). Using a nonquantitative assay (i.e., MAPIA), three of four *M. bovis*-inoculated cattle responded to MPB83 by 3 weeks after challenge and four of four cattle responded at all other subsequent time points, whereas three of five, four of five, four of five, and three of five *M. tuberculosis*-inoculated cattle responded to MPB83 at 3, 6, 9, and 12 weeks after challenge, respectively. *Mycobacterium kansasii* inoculation elicited LAM-specific IgG: the preinoculation response (given as the mean ± SEM S/P ratio) was 0.12 ± 0.13, whereas the response was 1.01 ± 0.28 at 7 weeks after inoculation ($P < 0.05$). The initial early responses waned by 10 weeks after challenge to 0.50 ± 0.20 and then increased ($P < 0.05$) 2 weeks after injection of PPD for the skin test at 18 weeks after challenge to 1.47 ± 0.26. Prior to the skin test, only two of four *M. kansasii*-inoculated calves had antibody specific to the MPB83, ESAT-6, and CFP10 antigen cocktail (i.e., as determined by the VetTB Stat-Pak lateral-flow assay), whereas after the skin test, all four animals had responses to this antigen cocktail (Table 2).

The strong and persistent generation of MPB83-specific IgG following infection with *M. tuberculosis* is an interesting observation, given that *in vitro M. tuberculosis* expresses very small amounts of MPB83 compared with the amount expressed by *M. bovis* (35). Potent immune responses to MPB83 have previously been observed in *M. tuberculosis*-infected mice (13), and the response is also upregulated in *M. tuberculosis*-infected macrophages (i.e., *in vitro*) (22, 23). Together with the present findings of MPB83-specific responses by *M. tuberculosis*-inoc-

ulated cattle, these data support the notion that MPB83 expression is upregulated by *M. tuberculosis in vivo*.

Immune response-disease progression associations. As indicated in the early 1900s (25), the administration of culture-derived *M. tuberculosis* (in this case, $H_{37}R_v$) to healthy cattle elicits an immune response without the expression of disease (i.e., pathology), thus providing the rationale for early 20th century studies evaluating live *M. tuberculosis* as a vaccine for bovine tuberculosis. In the present study, *M. tuberculosis* was detected in three of five inoculated animals upon necropsy, which was at ~16 weeks after inoculation. MPB83-specific antibody was detectable early after challenge in all five *M. tuberculosis*-inoculated animals, yet these responses waned to preinoculation levels by the end of the study (Fig. 2A). Thus, with experimental *M. tuberculosis* $H_{37}R_v$ infection of cattle, antibody responses are associated with mycobacterial burden rather than pathology. A wane in the early MPB83-specific antibody response likely followed the clearance of *M. tuberculosis* from the host. In contrast, persistent *M. bovis* infection resulted in lesions and sustainable specific antibody responses (Fig. 2). Together, these findings indicate that antibody responses upon mycobacterial infection are indicative of the antigen load rather than the mycobacterium-induced pathology.

Inoculation of *M. kansasii* elicited LAM-specific responses in four of four cattle and MPB83-specific responses in two of four cattle, as detected by MAPIA. Injection of PPD for the skin test boosted both the LAM- and the MPB83-specific re-

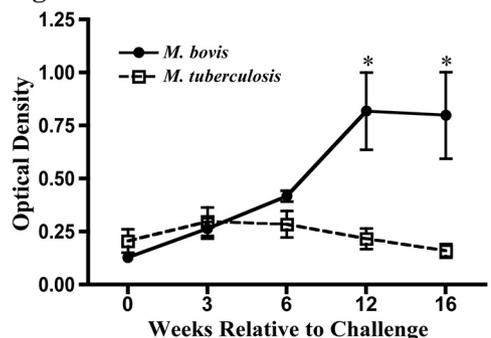**A. IgG to MPB83**
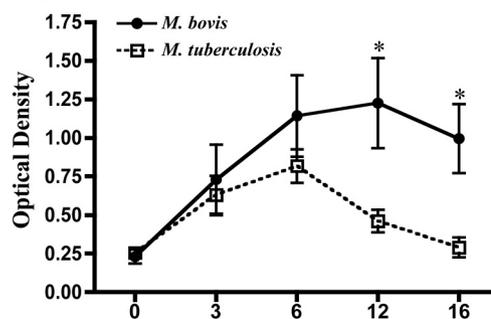
B. IgG to *M. bovis* Culture Filtrate

FIG. 2. Antibody responses upon experimental inoculation of calves with *M. bovis* ($n = 5$) or *M. tuberculosis* ($n = 5$). Data are presented as the optical densities (means ± SEMs) at 450 nm of the response to MPB83 (A) or *M. bovis* culture filtrate (B). ∗, the response was significantly different ($P < 0.5$) from the response by *M. tuberculosis*-inoculated animals at the same time point.

sponses in all four animals, as previously detected with *M. bovis* infection of cattle (33). Mycobacteria were not isolated upon necropsy, and lesions were not detected. These findings demonstrate the potential for a correlation of the specific antibody responses to a boosting effect resulting from exposure to cross-reactive antigens, even when the mycobacteria have likely been cleared weeks prior to subsequent exposure.

Conclusions. In this study, cellular immune responses correlated with mycobacterial infection but not necessarily with the pathology or bacterial burden. The findings of the present study highlight several scenarios for the interpretation of antibody responses by cattle to mycobacterial exposure. (i) The first scenario is the classic model, in which infection (e.g., *M. bovis* infection of cattle) is not controlled by the host, resulting in significant pathology and mycobacterial burden. With this scenario, the association of the antibody responses to pathology (15) is likely coincident with the antigen burden. (ii) The second scenario is an antigen load model in which the antibody responses are positively correlated to the antigen burden. With *M. tuberculosis* $H_{37}R_v$ infection of cattle, as the mycobacterium is cleared, the antibody responses wane. With *M. bovis* infection of cattle, as the infection progresses and the mycobacteria persist, the antibody responses also persist or increase. (iii) The final scenario is the sensitization model, in which exposure to a mycobacterium (e.g., *M. kansasii* inoculation) induces an antibody response which wanes over time, yet the response may be boosted significantly by reexposure to mycobacterial antigens (e.g., PPD) or other live mycobacteria.

Good and reliable models of human latent tuberculosis are not readily available for support of both applied tuberculosis research (e.g., vaccinology and diagnostic studies) and basic tuberculosis research (e.g., immunopathogenesis and bacterial pathogenesis). An animal model in which different infection outcomes can be modeled with organisms with overlapping antigen repertoires, such as the three organisms studied here, would be of advantage. *Mycobacterium bovis* infection of cattle could be considered a model of clinical tuberculosis, while *M. tuberculosis* $H_{37}R_v$ infection could be akin to latent, subclinical tuberculosis. Infection with *M. kansasii*, on the other hand, might be a model for a human tuberculous infection that has successfully been cleared from its human host. Therefore, we propose that these three infection systems be developed into useful models mimicking different stages of human *M. tuberculosis* infection.

ACKNOWLEDGMENTS

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