Is Interleukin-483 Splice Variant Expression in Bovine Tuberculosis a Marker of Protective Immunity?

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Splice variants of the interleukin-4 (IL-4) cytokine gene have been described for humans, mice, and cattle. IL-4 splice variants have been shown to inhibit IL-4-mediated cellular responses and thus act as IL-4 antagonists. Recent work has highlighted the possibility of a correlation between IL-4 splice variants and protection against clinical tuberculosis. In this study we investigated the potential role of IL-4 splice variants IL-4-422 and IL-4-463 in cattle with bovine tuberculosis, using quantitative real-time reverse transcription-PCR. For this analysis we used naturally exposed tuberculin skin test-positive field reactor cattle, uninfected control cattle, and cattle from two experimental models of protective immunity against Mycobacterium bovis: (i) vaccination with M. bovis BCG and challenge with virulent M. bovis and (ii) infection with M. bovis and treatment with isoniazid (INH) prior to rechallenge. The cytokine levels of field reactor cattle were compared to the levels of uninfected controls, while in kinetic studies of BCG vaccination and INH treatment we compared pre-experimental values with sequential samples for each individual animal. The data revealed a significant increase in IL-4-483 mRNA expression in field reactor cattle, which had no visible pathology compared to cattle with gross pathology typical of bovine tuberculosis. Increased IL-4-483 expression in both cattle models of protective immunity (BCG vaccination and INH treatment) was transient over time, reaching significance in the INH treatment model. Our results support the hypothesis that IL-4-483 is involved in protective immunity against M. bovis infection in cattle and are in accordance with clinical studies that have suggested a role for IL-4 splice variants in protective immunity in tuberculosis.

Tuberculosis (TB) is a disease characterized in the first instance by proinflammatory Th1 cytokines, such as gamma interferon (IFN-γ) and interleukin-12 (IL-12), and also tumor necrosis factor alpha, which are required for protection against and containment of tubercle bacilli via the activation of specific CD4+ T cells and the formation of tissue granulomas, respectively (9, 18, 19). In contrast, IL-4 is a classic Th2 cytokine which down-regulates Th1-mediated responses (12, 16, 17), leading to the suggestion that a successful vaccine must not only stimulate an appropriate, protective Th1 response but also control the level of IL-4 production (27). This framework has scientific merit but could neglect the positive impact of IL-4 as an anti-inflammatory agent which balances and controls tissue-damaging proinflammatory Th1 responses and promotes tissue repair (15, 24, 31), as well as the potential role of IL-4 in cellular recruitment (4, 30, 38). More recently, IL-4 has also been associated with the generation of regulatory T cells (25, 28), which further suggests that any modulation of IL-4 could have profound consequences for host immunity.

Alternative splicing of pre-mRNAs is a powerful and versatile regulatory mechanism that can exert quantitative control of gene expression and result in functional diversification of proteins (23). An IL-4 splice variant, IL-4-482, was first described in 1996 by Atamas et al. (2) as an IL-4 antagonist that could inhibit IL-4-mediated T-cell proliferation. However, IL-4 agonist activities are now also known, such as the positive stimulation of collagen synthesis by fibroblasts (3). Pertinently, increased levels of IL-4-482 mRNA were recently found in people latently infected with Mycobacterium tuberculosis compared with uninfected healthy controls or patients with active pulmonary TB (10). Increased IL-4-482 mRNA levels have also been found in TB contacts compared with noncontacts, and the authors hypothesized that the ratio of IL-4 to IL-4-482 might be important in the progression to active disease (11, 14). Lastly, chemotherapy of TB patients resulted in increased expression of IL-4-482 in both pulmonary TB and in human immunodeficiency virus-TB coexistence (12). Taken together, this information tentatively suggests that the IL-4 splice variant is a potential marker of protective immunity in TB.

Two splice variants of the bovine IL-4 gene, IL-4-482 and IL-4-483, have recently been described in cattle experimentally infected with the parasitic trematode Fasciola hepatica (35). Although no function was assigned to the splice variants in this model, a positive correlation was found between the expression of IL-4-482 and the expression of IL-4-482 and IL-4-483 in antigen-stimulated cells. In this study we used the same tools to analyze the expression of IL-4-482 splice variants in cattle with bovine TB. Specifically, we investigated the expression of IL-4-482, IL-4-483, IL-4, and IFN-γ in unstimulated, ex vivo peripheral blood mononuclear cells (PBMC) from individual cattle belonging to four distinct groups: tuberculin skin test-positive reactors from confirmed herd breakdowns, uninfected controls, and two bo-

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vine experimental models of protective immunity against *M. bovis* in which pathology is much reduced. Comparisons were made between field reactors and uninfected controls, while the values for sequential samples from the experimental models were compared to pre-experimental values for each animal.

**MATERIALS AND METHODS**

**Animals.** (i) Uninfected controls. Thirty-eight Friesian-Holstein heifers and steers, typically ~6 months old, were recruited from herds without a history of bovine TB and with a history of negative tuberculin skin test responses (13). (ii) **Naturally infected field reactors.** Thirty-three individual field reactors were identified in herds with confirmed TB as determined by positive comparative tuberculin skin tests (13). This test compares the responses of individual cattle to both bovine and avian tuberculin (purified protein derivative B [PPDB] and PPDA) in order to discount reactivity to cross-reactive antigens from environmental mycobacteria. A positive result requires the response to PPDB to be at least 5 mm greater than the response to PPDA using the standard interpretation (http://defraweb/animalh/tb/isg/report/diagnosis.htm). Blood samples were collected approximately 2 weeks after the disclosing skin test. All animals also produced increased PPDB-specific IFN-γ levels relative to the PPDA-specific IFN-γ levels (data not shown). (iii) **BCG vaccination model.** Sixteen Friesian-Holstein heifers and steers approximately 6 months old were recruited from herds with a history of negative tuberculin skin test responses (13). Twelve calves were vaccinated by the subcutaneous route with approximately 5 × 10^6 CFU of the BCG Pasteur parent strain (procured by M. B. McGeachie, Wall University, Canada). Twenty-one days postvaccination, vaccinated calves were challenged with 6 × 10^5 CFU virulent *M. bovis* (AF2122/97) by the intratracheal route (6) at 10 weeks postvaccination. A challenge control group of four calves was also infected with the same dose of *M. bovis* at this time. (our unpublished data). (iv) **INH-assisted immunity model.** Twelve Friesian-Holstein steers approximately 6 months old were recruited from herds with a history of negative tuberculin skin test responses (13). The calves were divided into three groups of four animals. The first group received 350 CFU virulent *M. bovis* (AF2122/97) by the intratracheal route as described above plus isoniazid (INH) (25 mg/kg/day, as described by Leite et al. [21]) from 3 weeks postinfection until 17 weeks post-infection. This group was then rested until the postmortem examination at 34 weeks postinfection. The second group received *M. bovis* and INH as described above but were then rested for 4 weeks after the end of the INH treatment regimen before they were rechallenged at week 21 with 1,000 CFU virulent *M. bovis* (AF61-1307). The third group comprised four challenge controls that were also infected with 1,000 CFU *M. bovis* at week 21. All animals were skin test positive prior to postmortem examination (data not shown) (our unpublished data).

**(v) Cattle postmortem examination.** A detailed postmortem examination was carried out for all infected calves as previously described (6). Lymph nodes were removed aseptically, serially (2-mm) sectioned (5), and examined for the presence and extent of lesions. The lymph nodes recovered were the right and left submandibular, right and left medial retropharyngeal, right and left lateral retropharyngeal, right and left parotideal, cranial mediastinal, caudal mediastinal, right and left bronchial, cranial tracheobronchial, and right and left tonsil. Other lymph nodes were macroscopically examined in situ. The lungs were serially sliced following the bronchial tree, and the slices were palpated and inspected. The nasal passages were also opened and inspected. Individual tissues were assigned a pathology score depending upon the number, size, and character of the lesions observed in accordance with the standard methodology used in this laboratory.

**RNA extraction, reverse transcription, and real-time PCR.** Previous clinical studies have shown that the IL-463 splice variant is found in unstimulated PBMC (10-12, 14). Therefore, we used freshly isolated PBMC from all cattle in this study. RNA was prepared from 10^6 PBMC/sample using the RNeasy mini kit system and following the manufacturer’s instructions (QIAGEN Ltd., West Sussex, United Kingdom). PBMC were isolated by layering over Histopaque 1077 (Sigma) and centrifugation at 800 × g for 40 min at room temperature, washed twice in Hanks balanced salt solution (Life Technologies), and counted. A total of 10^6 PBMC were aliquoted into sterile screw-top vials, the cells were pelleted by centrifugation, and the supernatants were discarded. Lysates were prepared by resuspending the pelleted cells in 350 μl of RLT buffer according to the manufacturer’s instructions (QIAGEN Ltd.), and the lysates were stored at −80°C. Cell lysates were centrifuged through DNA shredder columns (QIAGEN Ltd.) prior to ethanol extraction and RNA isolation using the RNeasy column system. RNA preparations were finally treated with Turbo DNase (Ambion (Europe) Ltd., Huntingdon, United Kingdom) for 30 min at 37°C. Reverse transcription was carried out using a QIAGEN Reverse IT T-Primed First Strand synthesis kit and following the manufacturer’s instructions. Reverse transcription controls (no reverse transcriptase) were included for every sample.

The PCR assay was optimized using plasmids containing the constructs for IL-4, IL-462, and IL-463, as well as bovine PBMC (ex vivo, antigen stimulated, and mitogen stimulated) (data not shown). PCR was carried out in triplicate for each sample using a universal PCR master mixture (Applied Biosystems, United States) plus forward and reverse primers and Taqman probes for IFN-γ, IL-4, IL-462, and IL-463 as described previously (35). For a positive signal, at least two of the three PCRs in any triplicate analysis had to give detectable cycle threshold values. Cytokines were normalized to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene (22). All primers and probes were manufactured by MWG-Biotech, Ebersberg, Germany. Standard curves for each cytokine and GAPDH were prepared from the PBMC of two field reactor animals using serial dilutions of cDNA, extracted and processed as described above. The same set of standard curves was used to obtain measurements of RNA in all experiments described in this report. The amount of GAPDH per sample ranged from 3.5 to 885 arbitrary units, and 50% of the samples contained between 17.9 and 304 arbitrary units of GAPDH. A further DNA contamination control (DNase-treated RNA sample prior to reverse transcription) was included for each sample in each run. Reactions were carried out using a Rotor-Gene RG300 (Corbett Research, Cambridge, United Kingdom). Cycling conditions consisting of an initial activation step of 10 min at 95°C, followed by 45 cycles of 95°C for 1 s and 60°C for 6 s, were used for all primers and probes. A calibration control plasmid was included in each PCR run in duplicate so that any minor differences (virtually none) between runs could be normalized and therefore all PCR runs were comparable.

**Statistical analysis.** Data were analyzed using the Mann-Whitney nonparametric test (two-tailed and incorporating 95% confidence intervals), analysis of variance, or an unpaired t test as indicated below and using the GraphPad Prism software package. A P value of <0.05 was considered statistically significant.

**RESULTS**

**Field reactor cytokine expression.** Figure 1 shows the levels of cytokine mRNA for IFN-γ (Fig. 1a), IL-4 (Fig. 1b), and IL-463 (Fig. 1c) for 33 individual skin test-positive animals with confirmed TB plus 38 matched uninfected controls. Interestingly, the levels of both IFN-γ and IL-4 were significantly reduced in the ex vivo, unstimulated PBMC in field reactor cattle compared to the levels in the uninfected controls (*P* = 0.0201 and *P* = 0.0164, respectively). IL-463 mRNA was detected in only a portion of the animals in both groups (15/38 [39.5%] of controls and 16/33 [48.5%] of field reactors). The level of IL-463 appeared to be slightly increased in field reactors compared to the uninfected controls, but the difference was not statistically significant. Dividing the IL-463-positive field reactors into animals with visible gross pathology and animals without visible gross pathology (visible lesions [VL] and no visible lesions [NVL], respectively) (Fig. 1d), however, showed that the highest scores for IL-463 were found in the NVL group and that the level of IL-463 expression in the NVL group was significantly higher than the level of IL-463 expression in the VL group (*P* = 0.0091). Thus, a high level of IL-463 mRNA expression appeared to correlate with the absence of pathology in cattle naturally infected with *M. bovis*. However, we did note that two of the IL-463-negative field reactor animals (Fig. 1c) also had NVL (no gross visible pathology), and therefore protection in these two individuals may have been the result of mechanisms other than IL-463. There was no discernible correlation between a positive IL-463 response and a positive IFN-γ response to the immunodominant proteins ESAT6 and CFP10 (all field reactors were ESAT6/CFP10-specific IFN-γ positive [data not shown]). This is in contrast to clinical studies in which ESAT6 positivity has been used to...
identify groups of individuals infected with *M. tuberculosis* whose elevated IL-4 levels appeared to correlate with their ability to control infection (10, 11).

**BCG vaccination-induced protection model.** Figure 2 shows the levels of cytokine mRNA expression for IFN-γ (Fig. 2a), IL-4 (Fig. 2b), and IL-483 (Fig. 2c) for 12 calves vaccinated with *M. bovis* BCG at week 0 and then challenged with virulent *M. bovis* at week 10 postvaccination. The levels of both IFN-γ expression and IL-4 expression were significantly reduced at week 4 postvaccination compared to the prevaccination levels (*P* < 0.0001 for both cytokines). Subsequently, this significant decrease in IFN-γ and IL-4 levels in unstimulated ex vivo PBMC following vaccination has since been confirmed for a larger group (*n* = 36) of BCG-vaccinated cattle (data not shown). The levels of both cytokines appeared to recover to prevaccination levels by week 10 but then decreased significantly following challenge with virulent *M. bovis* (*P* < 0.01 for IFN-γ at weeks 12 and 14 and *P* < 0.001 for IL-4 at weeks 12 and 14).

![Cytokine mRNA levels in 12 calves vaccinated with *M. bovis* BCG (week 0) and challenged with 6 × 10⁶ CFU virulent *M. bovis* (Mb) at week 10 postvaccination. Each symbol represents a single animal. The horizontal lines indicate the medians for the different times. The data show that there were significant decreases in the levels of both IFN-γ and IL-4 compared to the prevaccination values at week 4 following vaccination with BCG (*P* < 0.0001 for both cytokines [indicated by asterisks]) and then again at weeks 12 and 14 following challenge with virulent *M. bovis* (*P* < 0.01 and *P* < 0.001 for IFN-γ and IL-4, respectively [indicated by asterisks]).]
IL-483 mRNA expression (Fig. 2c) was detected in just 3/12 calves prior to BCG vaccination (week 0). This proportion increased to 5/12 calves by week 4 postvaccination and to 7/12 calves by week 12, 2 weeks after challenge with M. bovis. All three calves that were positive for IL-483 at week 0 also showed positive responses at various times during the study. Other calves with no detectable IL-483 at week 0 showed positive responses at different times as the experiment progressed. Only one calf never showed a detectable IL-483 response. These changes in IL-483 levels in BCG-vaccinated and M. bovis-infected animals did not reach statistical significance. M. bovis challenge control calves showed significant decreases (P < 0.05 in all cases) in the levels of both IFN-γ and IL-4 following infection (Fig. 3). This was in agreement with the data in Fig. 2a and b which show that there were decreases in the levels of both cytokines in unstimulated ex vivo PBMC following infection with virulent M. bovis. IL-483 expression in the challenge controls was detected in two of four animals at just one time each (Fig. 3c). Overall, therefore, IL-483 was detected in the majority of BCG-vaccinated cattle (11/12 cattle altogether), and all 12 of these cattle were shown to be protected from M. bovis infection. Figure 4a shows the pathology scores for BCG-vaccinated, M. bovis-challenged cattle and for M. bovis challenge controls in order to illustrate the relative decrease in tuberculous pathology (and increased protection) following BCG vaccination.

INH-assisted protection model. Figure 5 shows the levels of cytokine mRNA expression for IFN-γ (Fig. 5a), IL-4 (Fig. 5b), and IL-483 (Fig. 5c) for the four calves that were experimentally infected with M. bovis, treated with INH from week 3 postinfection through week 17, and then rested until the end of the experiment (week 34). The data show that the levels of IFN-γ (Fig. 5a) and IL-4 (Fig. 5b) changed little over the course of this experiment and that the small differences that did occur were inconsistent and not statistically significant compared to preinfection values. Interestingly, there was no decrease in the level of either IFN-γ or IL-4 following infection and prior to INH treatment (i.e., at week 3), as observed in the BCG challenge control animals described above. This may reflect the M. bovis dose (6 × 10^6 CFU for the BCG challenge control animals compared to 350 CFU for this experiment). In contrast to IFN-γ and IL-4, a significant increase in IL-483 was observed following M. bovis infection and INH treatment. This increase was apparent at week 13 of the experiment (during the INH treatment regimen) and reached statistical significance at weeks 21 (P = 0.0159) and 25 (P = 0.0317). Therefore, the levels of IL-483 declined to levels that were not significantly higher than the preinfection values (two calves) or were undetectable (two calves).

Similarly, Fig. 6 shows the levels of cytokine mRNA for IFN-γ (Fig. 6a), IL-4 (Fig. 6b), and IL-483 (Fig. 6c) for the four
Calves that were experimentally infected with 350 CFU *M. bovis*, treated with INH, rested, and then rechallenged with a further 1,000 CFU *M. bovis*. In these calves the levels of IFN-γ and IL-4 changed little over the experimental time except that at week 25, 4 weeks after rechallenge with *M. bovis*, increases in the levels of both cytokines were observed in all four calves, reaching statistical significance in the case of IFN-γ (P = 0.0286 and P = 0.0571 for IFN-γ and IL-4, respectively). Similar to the results for the infected and INH-treated calves described above, the levels of IL-43 in calves that were infected, treated with INH, and then rechallenged (Fig. 6c) were increased in three of four calves from week 13 to week 25; however, this increase did not reach statistical significance because of one nonresponder at each time (a different animal at each time). After week 25 the levels of IL-43 declined to preinfection values or were undetectable.

In the *M. bovis* challenge control group (Fig. 7), no change in IFN-γ was seen following infection; however, a significant increase in the level of IL-4 (P = 0.03) was observed at 4 weeks postinfection, similar to results for the rechallenge group described above (Fig. 6b). The levels of IL-43 declined over time from preinfection levels and were undetectable in three of four calves by 13 weeks postinfection.

These data show that there were transient but significant increases in the levels of IL-43 in calves infected with *M. bovis* and treated with INH, a regimen that we know induces protection (Fig. 4b shows the pathology scores of these groups of cattle). In contrast, the levels of IL-43 declined over time following infection in challenge control cattle that went on to develop significant pathology.

**DISCUSSION**

In this report we describe an investigation of IL-4 splice variants in bovine TB. We assessed the presence of splice variants in cattle naturally infected with *M. bovis* and compared the levels of cytokines in this group of field reactors with the levels in a group of uninfected controls. We found that one of the splice variants, IL-43, was detectable in a portion of field reactors and that when these reactors were divided into NVL and VL groups, the NVL animals had higher levels of IL-43 expression than the VL animals, suggesting that there is a link between IL-43 and protection in cattle naturally exposed to *M. bovis*.

We then investigated the presence of IL-4 splice variants in two cattle models of protective immunity against *M. bovis*, BCG vaccination and INH-assisted protection.

Vaccination with *M. bovis* BCG is known to induce protection against subsequent challenge with virulent *M. bovis* both in cattle and in other animal species (27, 31), so that BCG-

![FIG. 5. Cytokine mRNA levels in four calves experimentally infected with virulent *M. bovis* (arrowheads) and treated with INH from week 3 postinfection to week 17 (gray bars). Each symbol represents a single animal. The horizontal lines indicate the medians for the different times. The data show that there were significant increases in IL-43 levels at weeks 21 (P = 0.0159, as determined by a Mann-Whitney test [indicated by an asterisk]) and 25 (P = 0.0317, as determined by a Mann-Whitney test [indicated by an asterisk]) in all calves compared to the preinfection values.](http://iai.asm.org/)

![FIG. 6. Cytokine mRNA levels in four calves experimentally infected with virulent *M. bovis* (arrows), treated with INH (gray bars) from week 3 postinfection to week 17, rested for 4 weeks, and then rechallenged with virulent *M. bovis*. Each symbol represents a single animal. The horizontal lines indicate the medians for the different times. The data show that there were increases in the levels of both IFN-γ (P = 0.0286, as determined by a Mann-Whitney test [indicated by an asterisk]) and IL-4 (P = 0.0571, as determined by a Mann-Whitney test [indicated by an asterisk]) at week 25, 4 weeks after rechallenge with *M. bovis*, and that there was an increase in the level of IL-43 from weeks 13 to 25, although this increase for the group as a whole did not reach statistical significance.](http://iai.asm.org/)
induced protection is now the "gold standard" by which other novel TB vaccines are assessed (5, 37) and there is much interest in BCG prime-boost vaccination strategies to improve the protection afforded by BCG alone (29, 36). Vaccination of cattle with BCG also has been shown to reduce antigen (ESAT6)-specific IFN-γ responses and the bacterial load following infection with virulent M. bovis compared to M. bovis challenge controls. High ESAT6-specific IFN-γ responses have been positively correlated with pathology (34). In this study we showed that there was IL-463 expression in a portion of our BCG-vaccinated animals and that the number of animals expressing IL-463 increased following BCG vaccination and again following M. bovis challenge. In contrast, IL-463 was largely undetectable in the M. bovis challenge controls, with just two calves showing a positive signal at one time each. Even though the changes observed in IL-463 over time did not reach statistical significance, the overall presence and absence of IL-463 in the vaccinated and challenge control groups, respectively, correlated inversely with the pathology score for each group.

INH is commonly used in the therapy of human TB (39), but it is illegal in some countries, including the United Kingdom, for the treatment of cattle because it can interfere with the tuberculin skin test (20). We used an INH-attenuated experimental M. bovis infection in cattle in order to generate drug-assisted protective immunity similar to that described for drug-induced latency in the mouse (1, 33). In this model we observed increases in IL-463 levels in all animals at various times following INH-attenuated M. bovis infection. These animals showed little pathology compared to the M. bovis challenge calves, which had the greatest pathology and in this experiment actually showed a decrease in IL-463 levels following infection.

Interestingly, it was IL-463 and not IL-462 that appeared to be the dominant splice variant in these investigations. IL-462 was detected in ex vivo PBMC in just 5/33 field reactors and in none of the uninfected controls or experimental animals. We noticed, however, that stimulation of PBMC, particularly stimulation with mitogen, resulted in patterns of cytokine expression different from those observed in ex vivo unstimulated PBMC; often there were strong IL-4 and IL-462 responses but no IL-463 response (data not shown). A difference in the kinetics of bovine IL-462 and IL-463 mRNA expression has been noted previously in mitogen-stimulated lymph node cells (35), and it may be that these two bovine IL-4 splice variants have different functions. The IL-4 gene contains four exons, among which there are two potential IL-4 receptor (IL-4R) binding sites. One of these sites is affected in IL-462 by deletion of exon 2 such that binding of the IL-4R occurs but no signal is transmitted (2). In the case of IL-463 exon 3 is deleted, but nothing is known yet about how this deletion affects IL-4R binding, and there is currently no information regarding the function of this splice variant.

Only investigation and comparison of the biological activities of cloned bovine IL-462 and IL-463 may provide some answers to these questions.

This study also revealed interesting kinetics for IFN-γ and IL-4 following vaccination and infection. Vaccination with M. bovis BCG caused a reduction in both IFN-γ and IL-4 that was apparent 4 weeks after the vaccination, but the levels returned to prevaccination levels by week 10 postvaccination. Similarly, infection with 6 × 10^3 virulent M. bovis CFU (BCG model) caused significant decreases in IFN-γ and IL-4 levels both in the vaccinated and challenged animals and in the M. bovis challenge controls. However, there was no such decrease in the level of IFN-γ or IL-4 in the M. bovis challenge controls that received a lower dose of virulent M. bovis (350 CFU) (INH model). Instead, a significant increase in the IL-4 level was observed at 4 weeks postinfection in this group. These data for cytokine expression in ex vivo unstimulated PBMC provide an interesting comparison to in vitro cultured and stimulated PBMC, which typically show increases in antigen-specific cytokine production following infection or vaccination (5, 8, 26, 29, 34, 36, 37). The interpretation of our IFN-γ and IL-4 data is not certain yet, but the data are interesting because the results suggest that there is an M. bovis dose effect on cytokine responses; however, previous work (8) has shown that just 1 CFU of M. bovis can result in the same level of pathology as 1,000 CFU (over a 20-week period of infection) and should therefore perhaps merit an equivalent host response. Clearly, there are subtle kinetic differences about which we currently know little.

In summary, we showed that the level of the IL-4 splice variant IL-463 is increased in cattle that are protected following exposure to M. bovis. This was demonstrated in naturally exposed skin test-positive field reactors that had NVL on post-
mortality examination, in BCG-vaccinated and experimentally infected calves that had reduced pathology compared to their challenge control counterparts, and finally in an INH-attenuated M. bovis infection and rechallenge model which also resulted in low pathology compared with challenge controls. Our data therefore fully support the clinical studies that suggest that IL-4 splicing variants have a positive role in protective immunity against TB, and they further validate the conclusion that bovine TB in cattle is a relevant model with which to study these enigmatic cytokines.

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