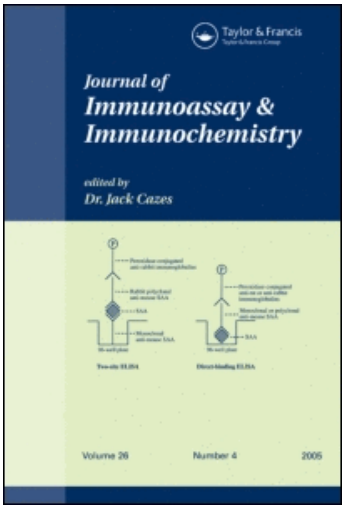

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KINETICS OF INTERFERON-γ (IFN-γ) RELEASE IN THE PERIPHERAL BLOOD OF CALVES VACCINATED WITH BCG

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ABSTRACT

A study was conducted on 13 Friesian-Zebu crossbred calves, the ages of which lie between 6 and 18 months, to investigate the kinetics of IFN-γ release in the peripheral blood following Bacille Calmete Guerine (BCG) vaccination. After being screened for bovine tuberculosis (BTB), the calves were vaccinated with 1 mL inoculums containing 6×10^6 CFU of BCG. The level of IFN-γ in the peripheral blood was measured two times before vaccination and seven times after vaccination, using a sandwich ELISA. The kinetics of IFN-γ post vaccination presented itself in three phases: rising, falling, and steady phases. The concentration of IFN-γ, before and after vaccination, both in stimulated and non-stimulated samples, was statistically significant ($P < 0.01$). Strong positive

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correlation ($r = 0.86$) was recorded between the levels of IFN-γ release in avian PPD- and bovine PPD-stimulated samples. Of the total 13 calves, 11 (84.6%) reacted positively to tuberculin inoculation 15 weeks post vaccination. It is concluded that the IFN-γ rises immediately after BCG vaccination, reaching its peak two weeks post vaccination, and then declines gradually in the following weeks. The strong positive reaction of calves to tuberculin inoculation 15 weeks post vaccination showed the capability of BCG in causing the release of IFN-γ in the peripheral blood, indicating its role in protection against infection with *Mycobacterium bovis* in calves.

Key Words: BCG; IFN-γ; Calves; Vaccination; Bovine tuberculosis

INTRODUCTION

Mycobacterium bovis, one of the species of *Mycobacterium tuberculosis* complex, is the causative agent of BTB, a zoonotic disease which is responsible for increasing animal and human health problems in several countries.(1) Development of an effective vaccine and/or improved diagnostic tests would greatly assist the control of BTB. T-helper 1 (Th 1) cell cytokines (IFN-γ) and interleukin-2 (IL-2) have been associated with protection from *M. tuberculosis* in mice.(2) Similarly, an assay of IFN-γ release from whole blood cultures stimulated with bovine purified protein derivative (PPD) has shown to have a high sensitivity for detecting BTB in cattle.(3,4) It has been accepted that immunity to mycobacterial infections depends on the interaction between T cells and macrophages. T cells, like CD4, CD8, and γδ, have been shown to produce IFN-γ in response to mycobacterial infection, suggesting macrophage-activating capabilities.(5) This, again, underlines the significance of IFN-γ in the protection against mycobacterial infection. Recent studies (6) on the efficacy of BCG have shown that a low dose of BCG was found to protect calves from experimental infection with *M. bovis*. One of the mechanisms by which BCG protected calves from experimental infection is by inducing the release of IFN-γ. In this line, investigation into the kinetics of IFN-γ release, post vaccination, seems to be of paramount importance, partly for the determination of the efficacy of BCG. Therefore, this study was undertaken to determine the kinetics of IFN-γ release in peripheral blood of calves vaccinated with BCG.

EXPERIMENTAL

Animals

Thirteen Friesian-Zebu crossbred calves whose ages lie between 6 and 18 months were screened for BTB twice using IFN-γ testing and recruited to the experimental site from the Debre Zeit Agricultural Research Center. They were kept in an isolated place during the experimental period (15 weeks).

Blood Collection and Processing

About 5 mL of blood was collected (two times before, and seven times after vaccination) every week during the first few weeks and every two weeks in the later weeks, for 10 weeks, into heparinized vacutainers from the jugular vein of each of the study calves. One and one-half mL of whole blood was added into three wells of a 24-well tissue culture plate for each of the study calves within 8 h of collection. The blood in the first two wells was stimulated, either by 100 μL (200 μg/mL) avian PPD (Commonwealth Serum Laboratories (CSL), Victoria, Australia) or by 100 μL (200 μg/mL) bovine PPD (CSL, Victoria, Australia). Nothing was added into the 3rd well so that it could serve as a negative sample control. The blood cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 for 24 h, after which plasma supernatants were collected and stored at −20°C until the assay was initiated.

Vaccination

Each of the 13 calves was vaccinated subcutaneously in the right side of the neck with 1 mL inoculums containing 6×10^6 CFU of BCG (Staten Serum Institute, Denmark).

IFN-γ Assay

The level of IFN-γ in the culture supernatants was measured two times before and seven times after vaccination using sandwich ELISA for bovine IFN-γ as described earlier (7) and supplied by CSL (Victoria, Australia) to determine the level of IFN-γ released. Results were expressed as optical density (OD) measured at 450 nm.

Comparative Intra-Dermal Tuberculin Test (CIT)

This test was conducted on each of the study calves at the 15th week post vaccination. Two sites on the mid-neck of a calf, 10 cm apart, were shaved and the skin thickness was measured in millimeters with a caliper before the injection of tuberculin. An aliquot of 0.1 mL of 20 000 IU/mL bovine PPD (Bovituber, Rhône Mérieux, France) and 0.1 mL of 25 000 IU/mL avian PPD (Avituber, Rhône Mérieux, France) were injected into the dermis of these sites. After 72 h, the thickness of the skin at the sites was measured again. When the change in skin thickness was greater at the avian PPD injection site, the calf was considered negative for BTB. When change in skin thickness increased at both sites, the difference between the two changes was considered. Thus, if the increase in the skin thickness at the injection site for the bovine PPD (*B*) was greater than the increase in the skin thickness at the injection site at the avian PPD (*A*), and B–A, was less than 1 mm, between 1 and 4 mm, or 4 mm and above, the calves were classified as negative, doubtful, or positive for BTB, respectively.

Statistical Analysis

The dependent variable analysed was IFN-γ concentration (OD) with the fixed effect of vaccination (before and after vaccination) and state of stimulation (stimulated with avian PPD or bovine PPD or non-stimulated control). The least squares means (LSM) of concentration of IFN-γ released in the peripheral blood before and after vaccination were compared using General Linear Model (GLM) procedures of the Statistical Analysis System.(8) LSM concentrations of IFN-γ were used to draw the kinetics of IFN-γ release. Pearson's Correlation Coefficient (*r*) was used to indicate the IFN-γ relationship between avian PPD- and bovine PPD-stimulated samples.

RESULTS

Kinetics of IFN-γ

The dynamics of IFN-γ in the peripheral circulation of calves vaccinated with BCG is presented in Figure 1. Accordingly, it was classified into the following three phases:

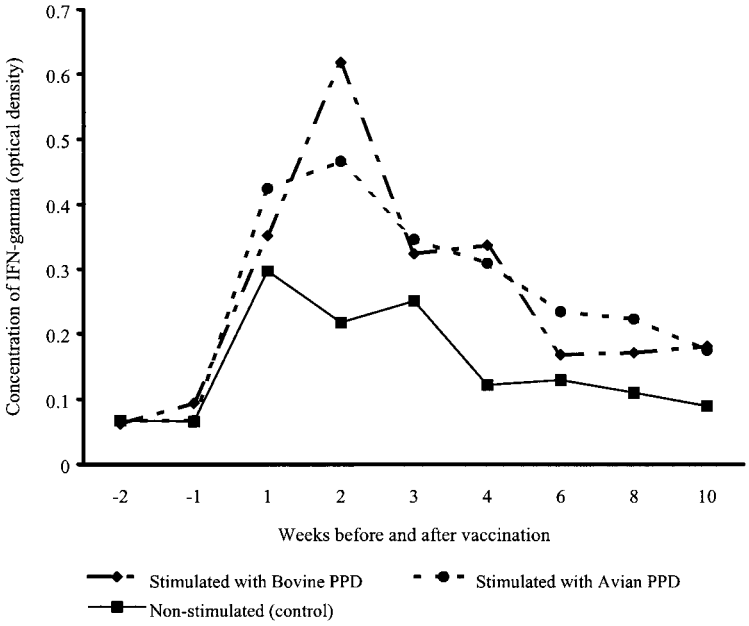

Figure 1. Dynamics of IFN-γ release in PPD stimulated and non-stimulated peripheral blood of calves vaccinated with BCG.

a) Rising Phase

This first phase has occurred following vaccination, and was characterized by a sharp increase in the level of IFN-γ in the peripheral blood. This sharp rise was observed within a week after vaccination, attaining its peak at the 2nd week of vaccination. Although there was a significant difference ($P < 0.01$) in the level of IFN-γ release in stimulated and control, a sharp rise has also been observed in the negative control samples, post vaccination. The highest increase was observed in bovine PPD stimulated samples followed by avian PPD stimulated samples.

b) Falling

This phase was observed between weeks 2 and 6 post vaccination, and was classified into two sub-phases: the rapid and slow falling sub-phases. The former was observed for one week (between weeks 2 and 3) while the

latter occurred between the 3rd and 6th weeks post vaccination. Sharp falling in the level of IFN-γ release in the peripheral blood was observed both in avian PPD and bovine PPD stimulated samples. In the slow falling, the level of IFN-γ has been observed to decrease slowly. Similar to the previous phase, there was still a statistically significant ($P < 0.01$) difference in the level of IFN-γ concentration between stimulated and non-stimulated samples.

c) Steady Phase

This phase was extended for four weeks (between weeks 6 and 10) post vaccination. Although the level of IFN-γ was higher in stimulated samples than in the control, unlike the previous phases, there was no significant difference in the level of IFN-γ between the stimulated and control samples. However, level of IFN-γ during this phase was still higher than its level before vaccination.

Comparison Between the Concentrations of the IFN-γ Before and After Vaccination

Least squares means (LSM) concentrations of IFN-γ in peripheral blood of calves, before and after vaccination, are presented in Table 1. The difference in concentration of IFN-γ before and after vaccination, both in stimulated and non-stimulated samples, was statistically significant ($P < 0.01$). Strong positive correlation ($r = 0.86$) was found between the levels of IFN-γ release in avian PPD- and bovine PPD-stimulated samples.

Table 1. Least Squares Means (LSM) Concentrations (Optical Density) of IFN-γ in Peripheral Blood of Calves Before and After Vaccination

	Vaccination	
State of Stimulation	Before	After
Avian PPD stimulated	0.0672 ± 0.0046^{b}	0.3110 ± 0.0348^{a}
Bovine PPD stimulated	0.0779 ± 0.0114^{b}	0.3071 ± 0.0388^{a}
Non-stimulated control	0.0667 ± 0.0028^{b}	0.1737 ± 0.0284^{a}

Row means with different superscript (^{a,b})differ significantly ($P < 0.01$).

Comparative Intradermal Tuberculin Test Result

The result of CIT indicated that 11 out of 13 (84.6%) calves positively reacted to tuberculin 15 weeks post vaccination.

DISCUSSION

In the present study, the dynamics of IFN-γ in the peripheral blood was determined for 10 weeks following vaccination of calves with BCG. Avian PPD and bovine PPD were used for the stimulation of whole blood. In general, the stimulation led to the formation of three phases: rising, falling, and steady phases. Immediately after vaccination, the IFN-γ concentration in the peripheral blood started to rise, attaining its peak at two weeks post vaccination. A similar finding was reported earlier,(6) indicating that two weeks after vaccination with BCG, they observed a strong IFN-γ response of lymphocyte cultured in vitro with bovine PPD, but the response was markedly reduced five weeks post vaccination. In their study, four weeks after challenge with *M. bovis*, calves were observed to produce strong IFN-γ response, but non-vaccinated *M. bovis* culture negative calves produced low IFN-γ responses. A similar pattern was observed in our study, and this increased level of IFN-γ in the peripheral blood may, in part, reflect clonal expansion of IFN-γ—secreting T cells.(9)

In the falling phase, the level of IFN-γ was observed to decrease, first rapidly, but later slowly. The slow falling in the IFN-γ response might have resulted from the partial control of BCG organisms during the first few weeks following vaccination or from sequestration of BCG organisms.(6) This phase was followed by the steady phase, in which the BCG and the immune component have achieved the equilibrium level.

Related to the present study, previous works have recorded three phases in the dynamics of T cell subpopulations, post infection with *M. bovis*.(10) The first phase was characterized by an increase in γδ T cells while, in the second phase, the number of $CD4^+$ T cells was found to increase. The third phase was characterized by an increase in the number of $CD8^+$ T cells. As all the three T cells have demonstrated the capability of secreting IFN-γ,(11,12) in the present study, it can be suggested that the γδ T cells produced IFN-γ in early stages, while $CD4^+$ and $CD8^+$ T cells produced it in the later stages.

This BCG-induced production of IFN-γ in the peripheral blood of the study calves was not specific, as it was observed to be produced both in avian PPD- and bovine PPD-stimulated samples. This might be due to the common antigenic structure that exists between *M. bovis* and *M. avium*.

The fact that the level of IFN-γ remained higher after vaccination, even in the steady phase, than the level before vaccination, implies that, once the BCG is injected into the calf's body, it is being recognized by memory cells and, when avian or bovine PPD is injected into vaccinated calves, the memory cells recognize it and combat against it by producing IFN-γ. Similarly, the reaction of vaccinated calves to tuberculin inoculation after 15 weeks of vaccination indicates the presence of memory cells that have been induced by vaccination. On the other hand, as indicated earlier,(13) it shows the interference of BCG vaccination with diagnostic value of skin test as both infected and vaccinated animals reacted to the test.

It can be concluded that the concentration of IFN-γ rises immediately after BCG vaccination, reaching its peak 2 weeks post vaccination, and then declines gradually during the consecutive weeks. The fact that the concentration of IFN-γ remained higher after vaccination than the concentration before vaccination, and the positive reaction of calves to tuberculin injection at the 15th week post vaccination, shows the capability of BCG in agitating memory cells, thereby causing the release of IFN-γ in the peripheral blood which, in turn, indicates its efficacy in the protection against infection from *M. bovis* in calves.

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REFERENCES

1. Neill, S.D.; Pollock, J.M.; Byson, D.B.; Hanna, J. Pathogenesis of *Mycobacterium bovis* Infection in Cattle. Vet. Microbiol. **1994**, *40*, 41.
2. Kawamura, I.; Tsukada, H.; Yashikawa, H.; Fujita, M.; Nomoto, K.; Mitsuyama, M. IFN-γ Producing Ability as a Possible Marker of Protective T Cell Against *Mycobacterium bovis* BCG in Mice. Cell Biol. **1992**, *73*, 362–368.
3. Wood, P.R.; Corner, L.A.; Rothel, J.S.; Ripper, J.L.; Fifis, T.; McCormick, B.C.; Francis, B.; Melville, L.; Small, K.; De Wittee, K.; Tolson, J.; Ryan, R.J.; de Lisle, G.W.; Cox, J.C.; Jones, S.L. A Field Evaluation of Serological and Cellular Diagnostic Tests for Bovine Tuberculosis. Vet. Microbiol. **1992**, *31*, 7, 1–79.

4. Ameni, G.; Miorner, K.; Roger, F.; Tibbo, M. Comparison Between Comparative Tuberculin and Gamma Interferon Tests for the Diagnosis of Bovine Tuberculosis in Ethiopia. Trop. Anim. Hlth. Prod. **2000**, *32*, 267–276.
5. Flesh, I.E.A.; Kaufmann, S.H.E. Role of Cytokines in Tuberculosis. Immunology **1993**, *189*, 316.
6. Buddle, B.M.; de Lisle, G.W.; Pfeffer, A.; Aldwell, F.E. Immunological Responses and Protection Against *Mycobacterium bovis* Vaccinated Calves with a Low Dose of BCG. Vaccine **1995**, *13*(12), 1123–1130.
7. Rothel, J.S.; Jones, S.L.; Corner, L.A.; Cox, J.C.; Wood, P.R. A Sandwich Enzyme Immunoassay for Bovine Interferon Gamma and Its Use for the Detection of Tuberculosis in Cattle. Austral. Vet. J. **1990**, *67*, 134–137.
8. *SAS® Procedures Guide for Personal Computers*, *Version 8 Edition*; SAS (Statistical Analysis System) Institute Inc.: Cary, NC, 1994.
9. Kee, H.N.; Aldwell, F.E.; Wedlock, D.N.; Watson, J.D.; Buddle, B.M. Antigen-Induced Interferon-γ and Interleukin-2 Responses in Cattle Innoculated with *Mycobacterium bovis*. Vet. Immunol. Imunopathol. **1997**, *57*, 59–68.
10. Pollock, J.M.; Pollock, D.A.; Campbell, D.G.; Girvin, R.M.; Crockard, A.D.; Neill, S.D.; Mackie, D.P. Dynamic Changes in Circulating and Antigen-Responsive T-Cell Subpopulations Post *Mycobacterium bovis* Infection in Cattle. Immunology **1996**, *87*, 236–247.
11. De Libero, G.; Flesh, I.; Kaufmann, S.H. Mycobacteria Reactive Lyt-2^+ T Cell Lines. Europ. J. Immunol. **1998**, *18*, 59–66.
12. Kaufmann, S.H. Immunity to Intracellular Bacteria. Ann. Rev. Immunol. **1993**, *11*, 129–163.
13. OIE. *Report of the meeting of the Office International des Epizooties* (*OIE*) *ad hoc group on bovine tuberculosis control in developing countries with the participation of World Health Organisation* (*WHO*); Paris, 20–21 February 1996.

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