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Gamma interferon-producing CD4 T-cells correlate with resistance to *Mycoplasma mycoides* subsp. *mycoides* S.C. infection in cattle

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Abstract

Contagious bovine pleuropneumonia (CBPP), caused by *Mycoplasma mycoides* subsp. *mycoides* SC (*Mmm*SC), is one of the most significant cattle disease in Africa. The control measures, which led to eradication from numerous countries are not feasible in Africa where the only prophylaxis relies on vaccination. However, the attenuated vaccines, used up to now in Africa, are of low efficiency. The development of an improved vaccine is, therefore, a necessity.

The purpose of this study was to compare some immunological parameters in *Mmm*SC-infected cattle (endobronchial versus natural in-contact infection) and assess the response in correlation with the clinical outcome (death versus recovery). Characterization of the immune parameters elicited in recovered animals, known to be refractory to new infection, will be an important step towards development of new vaccines against CBPP.

A significant outcome of this study was the demonstration that all *Mmm*SC-infected cattle developed a *Mmm*SC-specific cell-mediated immune response.

A kinetic analysis of the *Mmm*SC responsiveness showed that the main difference between endobronchially- and in-contact infected animals was the delay before the onset of the *Mmm*SC-specific immune response. The first *Mmm*SC-responding PBMC sample was selected from each animal for cell phenotyping. The phenotypic analysis of this early *Mmm*SC-induced response revealed the predominant contribution of the CD4 T-cells in all animals whereas IFNγ was only constantly produced in recovered animals.

Evolution of this early *Mmm*SC-specific immune response was then followed by a kinetic analysis of the *Mmm*SC-induced CD4 T-cell response and IFNγ released. The results demonstrated that in recovered animals, the *Mmm*SC-specific CD4 Th1-like T-cell response was maintained until slaughtering whereas in animals with acute disease, progression of CBPP was associated with a decreased ability of the PBMC to produce IFNγ.

Abbreviations: CBPP, contagious bovine pleuropneumonia; *Mmm*SC, *Mycoplasma mycoides* subsp. mycoides Small Colony; MdFI, median of fluorescence intensity

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The results led to the identification of immune parameters, which correlate with protection against CBPP and to a relevant strategy for the development of improved vaccines against this disease.

Keywords: Contagious bovine pleuropneumonia; *Mycoplasma mycoides* subsp. *mycoides* S.C.; Vaccine; Cell-mediated immunity; Gamma interferon

1. Introduction

Contagious bovine pleuropneumonia (CBPP), caused by *Mycoplasma mycoides* subsp. *mycoides* biotype Small Colony (*Mmm*SC), is one of the most serious cattle diseases in Africa. The principal pathological mechanism is a strong inflammatory reaction, usually restricted to the lungs, leading to death by respiratory distress due to lung consolidation in 15–30% of cases. In most cases, however, CBPP evolves into a chronic form, followed by recovery (Provost et al., 1987; F.A.O., 2003).

In recent years, CBPP has emerged from areas where it has been persisting in endemic form to reinvade zones from which it had previously been eradicated (Botswana, Tanzania, Rwanda...). In addition to these newly-infected areas, even the endemic zones are experiencing an upsurge in incidence. The disease is responsible for heavy economic losses due to mortality, loss of weight, reduced working ability or fertility. Furthermore, CBPP is the only bacterial disease included in List A of the Office International des Epizooties (OIE), and infected countries are excluded from international trade. It is therefore considered as the most important threat to the cattle industry in Africa.

Although CBPP was widespread until the middle of the 19th century, its distribution has been considerably reduced through the successful application of control measures. It has been completely eradicated from numerous countries such as Australia, the USA and many European countries through a combination of a stamping-out policy, control of cattle movement and quarantine. However, such measures are impracticable in Africa, where the only realistic prophylaxis is through vaccination.

The history of CBPP vaccination dates back to 1852, when Willems established that subcutaneous inoculation of CBPP-infective lymph in the thick connective tissue protected cattle against contact challenge (Willems, 1852). This immunisation procedure was widely used in Europe and South Africa until the policy of eradication by stamping-out was adopted (Turner, 1959). Since then, various types of vaccines have been tested (Provost, 1974; Provost et al., 1987; Turner, 1959). Nowadays CBPP prophylaxis in Africa relies on the use of live vaccines based on the attenuated T1 strain. However, the efficacy of the T1 vaccines is low and they induce only short-term protection, making annual vaccination necessary to achieve a sufficient level of protection (Thiaucourt et al., 2000). As a consequence, the development of an improved vaccine is a prerequisite for the eradication of CBPP in Africa. This objective is based on Willems's experiments and on the long-term immunity to reinfection observed in CBPP-recovered cattle, demonstrating their ability to mount a protective immune response against *Mmm*SC (Provost et al., 1987).

The basis of immune protection against *Mmm*SC infection has so far remained unknown. Previous immunological studies have primarily examined serological responses, but with contradictory results concerning their role in protection (Lloyd, 1967; Masiga et al., 1975). However, while a role for antibody-dependent mechanisms cannot be ruled out, cell-mediated immunity is likely to be involved in protection (Roberts et al., 1973; Roberts and Windsor, 1974; Tulasne et al., 1996).

The role of the cellular immune response in the pathogenesis and control of *Mmm*SC infection has never been analysed, nor have the T-cell subsets involved been identified. However, an understanding of the protective and immunopathological mechanisms will determine the most appropriate and efficient vaccine strategy against CBPP.

This study explores a number of immunological parameters in *Mmm*SC-infected cattle and assesses the immune response in correlation with the clinical outcome of the infection (fatal acute infection versus recovery). Characterisation of the immune parameters