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Procedia in Vaccinology 8 (2014) 43 – 49

Procedia in Vaccinology

www.elsevier.com/locate/ procedia

7th Vaccine & ISV Congress, Spain, 2013

Assessment of goat tuberculosis model for use in vaccine trials

Bernat Pérez de Val[a,*], Enric Vidal[a], Miquel Nofrarías[a], Sergio López-Soria[a], Pere-Joan Cardona[b], Mariano Domingo[a,c]

[a]*Centre de Recerca en Sanitat Animal (CReSA), UAB-IRTA, Campus de la Universitat Autònoma de Barcelona 08193 Bellaterra (Cerdanyola del Vallès)08193 Bellaterra, Catalonia, Spain.*

[b]*Fundació Institut per a la Investigació en Ciències de la Salut Germans Trias i Pujol, 08916 Badalona, Catalonia, Spain.*

[c]*Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, 08193 Bellaterra(Cerdanyola del Vallès), Catalonia, Spain.*

Abstract

Progressin development of tuberculosis (TB) vaccines largely depends on the availability of animal models to test their safety and efficacy before starting with expensive clinical trials. The present study provides a comprehensive evaluation of bacillus Calmette-Guerin (BCG) effects on clinical, immunological, pathological and bacteriological parameters in goats after an experimental challenge with *Mycobacterium caprae*.Vaccination of goats with BCG reduced the volume of lung gross lesions, the bacterial load in pulmonary lymph nodes and increased the weight gain when compared to unvaccinated animals. Differences in post-challenge IFN-γ responses to ESAT-6/CFP-10 were found to be a useful follow-up biomarker of disease progression and vaccine efficacy. Our results endorse this animal model for further TB vaccine trials.

Selection and peer-review under responsibility of the 7th Vaccine Conference Organizing Committee.

*Keywords:*Tuberculosis; Vaccines; BCG; Goats; Animal model; Computed Tomography; Interferon gamma.

Introduction

The domestic goat is a natural host of tuberculosis (TB). Caprine TB is an emerging zoonotic disease in many European countries[1,2]. Vaccination could be taken into consideration as an alternative to test-and-slaughter strategy for long-term TB control in livestock [3]. Moreover, the use in vaccine trials of experimental animals that

* Corresponding author. Tel.: +34935813284; fax:+34935814490.
*E-mail address:*bernat.perez@cresa.uab.cat

Selection and peer-review under responsibility of the 7th Vaccine Conference Organizing Committee.
doi:10.1016/j.provac.2014.07.008

are as well natural hosts of TB, such as goats, may be essential for a better understanding of mechanisms involved in protection and the development of new vaccines and therapies [4–6].

Currently, bacillus Calmette-Guérin (BCG) is the only licensed vaccine against human TB and is also being included in TB vaccine clinical trials. The majority of new prophylactic vaccine candidates in clinical trials are designed either as recombinant BCG vaccines oras subunit vaccines for use after a previous immunization with BCG[7–9].

Small laboratory animal models are usually employed in preliminary screeningsfor new vaccine candidates. However, larger animals, which efficiently reproduce the main features of human TB, are required in subsequent steps for developing TB vaccines.Domestic goats are easy to house in BSL-3 conditions, involve relatively low maintenance costs compared to other large mammals and, importantly, develop similar TB lesions and immunological responses than active diseased human patients[4].

The aim of this study was to assess the potential use of theexperimental goat model inTB vaccine trials. Comparison of ante-mortem and post-mortem parameters was carried out in BCG vaccinated and unvaccinated goats after beingchallenged with *M. caprae,*which is the main causative agent of caprine TB in Spain [2]

Materials and methods

Experimental schedule

Eleven goat kids were vaccinated with 5×10^5 CFU of BCG Danish strain, and other 11 goat kids remained as non-vaccinated control group. Fifteen weeks later, all goats were anesthetized as previously described [4]and challenged with approximately 1.5×10^3 CFU of *M. caprae* through the endobronchial route.After challenge, goats were weighed weekly throughout the experiment. Blood samples were taken at weeks 0, 5, 11 and 13 post-challenge. All goats were euthanized and subsequently necropsied at 13 weeks post-challenge. All animal experimental procedures were undertaken in accordance with the European Union Laws for protection of experimental animals (86/609), and ethical approval was obtained from the Animal Welfare Committee of the Government of Catalonia (Permit Number: 6332).

Antigen-specificIFN-γ responses

One ml of whole blood from each animal was stimulated in 96-deepwell cell culture plates (Eppendorf Ibérica, Madrid, Spain) withand ESAT-6/CFP-10 peptide cocktail at a final concentration of 5 µg/ml.Phosphate buffered saline was added to another ml of whole blood, which was used as unstimulated control.Blood cultures were incubated overnight at 37°C and 5% CO_2. Afterwards, plasma supernatants were collectedand tested by using a commercial IFN-γ *sandwich*enzyme-linked immunosorbent assay(Bovigam®, Prionics, Switzerland). Results were obtained as Optical Density determined at 450 nm (OD_{450}). Specific reaction was expressed as ΔOD_{450} (OD_{450} of ESAT-6/CFP-10-stimulated sample minus OD_{450} of non-stimulated sample).

Post-mortem examination and bacteriological culture

After necropsy, lungs were removed and fixed through intra-tracheal perfusion and immersion in 10%-buffered formalin and, thereafter, were scanned with a multi-detector computed tomography (CT) scanner (Brillance CT 64-channel, Philips Medical Systems, Cleveland, Ohio, USA). TB lesions were analyzed on a work station (Aquarius Station, TaraRecon, Foster City, California, USA) and the total volume of lung gross lesions was calculated. Whole pulmonary lymph nodes (cranial and caudal mediastinal, and tracheobronchial) were collected, homogenized and cultured for bacterial count as previously described [4].

Data analysis