Veterinary Immunology and Immunopathology 167 (2015) 185–189

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Short communication

Goats challenged with different members of the *Mycobacterium tuberculosis* complex display different clinical pictures

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ARTICLE INFO

Article history:
Received 20 May 2015
Received in revised form 7 July 2015
Accepted 20 July 2015

Keywords:
Animal tuberculosis
Domestic goat
Lesion score
Shedding
Strain genotype

ABSTRACT

Tuberculosis (TB) in goats (*Capra hircus*) is due to infection with members of the *Mycobacterium tuberculosis* complex (MTC), mainly *Mycobacterium bovis* and *Mycobacterium caprae*. We report a comparative experimental infection of goats with *M. bovis*, *M. caprae* and *M. tuberculosis* strains. We hypothesized that goats experimentally infected with different members of the MTC would display different clinical pictures. Three groups of goats were challenged with either *M. bovis* SB0134 (group 1, $n = 5$), *M. caprae* SB0157 (group 2, $n = 5$) and *M. tuberculosis* SIT58 (group 3, $n = 4$). The highest mean total lesion score was observed in *M. bovis* challenged goats (mean 15.2, range 9–19), followed by those challenged with *M. caprae* (10.8, 2–23). The lowest score was recorded in goats challenged with *M. tuberculosis* (3, 1–6). Culture results coincided with the lesion scores in yielding more positive pools (7/15) in *M. bovis* challenged goats. By contrast, only three pools were positive from goats challenged *M. tuberculosis* (3/12) and with *M. caprae* (3/15), respectively. Differences in the performance of the intradermal and gamma-interferon (IFN-γ) tests depending of the group were observed since all goats from group 1 were diagnosed using intradermal test and these goats reacted earlier to the IFN-γ assay in comparison to the other groups. This study confirmed that goats experimentally infected with different members of the MTC display different clinical pictures and this fact may have implications for MTC maintenance and bacterial shedding.

1. Introduction

Like many other mammals, domestic goats (*Capra hircus*) are susceptible to infection with members of the *Mycobacterium tuberculosis* complex (MTC), the causal agents of animal and human tuberculosis (TB). Caprine TB has a broad distribution and is often recorded in Mediterranean Europe and in Africa. Most infections are due to *Mycobacterium bovis* and *Mycobacterium caprae*, while infections with *M. tuberculosis* are rare (Pesciaroli et al., 2014). The lesion characteristics, often involving cavernous lung TB, along with the frequent direct and indirect contact with cattle suggests that goats can act as a domestic reservoir for bovine TB (Napp et al., 2013).

In animal models such as rabbits and mice, different MTC strains cause diverse disease outcomes. For instance, *M. tuberculosis* infection in rabbits is cleared over time, whereas infection with *M. bovis* results in chronic disease leading to death (Manabe et al., 2003). Similarly, mice infected with a diversity of *M. bovis* strains isolated from different host species had different survival probabilities and clinical pictures (Aguilar et al., 2009). In natural infections it has been observed that the genotype of the species included in the MTC may affect disease outcome in human beings (Malik and Godfrey-Faussett, 2005), and in animal hosts such as the Eurasian wild boar (*Sus scrofa*) (Garcia-Jimenez et al., 2013). However, experimental MTC infection in goats has only been reported using either *M. bovis* or *M. caprae* (Perez et al., 2012; Gonzalez-Juarrero et al., 2013). Such experiments have never been carried out in goats comparing several MTC species. Hence, we run a comparative experimental infection with *M. bovis*, *M. caprae* and *M. tuberculosis* strains. We hypothesized that goats experimentally infected with different members of the MTC would display different clinical pictures.

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http://dx.doi.org/10.1016/j.vetimm.2015.07.009
0165-2427/

2. Materials and methods

The study was performed in 14 adult goats from a TB-free dairy flock of Spanish Guadarrama breed. This flock was negative to the previous single intradermal tuberculin (SIT) test and interferon-gamma (IFN-γ) detection assay (Bovigam, ThermoFisher, USA) performed two months before the challenge. Procedures were previously accepted by the Ethical Committee (CEA-UCM 37-2013) and performed by veterinarians according to Spanish Legislation (R.D. 1201/2005). Animals were located in a level 3 biosafety facility and randomly assigned to group 1 (*M. bovis*; $n=5$), group 2 (*M. caprae*, $n=5$) or group 3 (*M. tuberculosis*, $n=4$) and challenged by the transthoracic route as previously described (Bezos et al., 2010). The immune response was evaluated for 5 weeks using the single and comparative intradermal tuberculin (SIT and SCIT respectively) tests and IFN-γ assay. At the end of the study animals were subjected to necropsy and the true infection status was determined by bacteriology.

The inocula consisted of field isolates of *M. bovis* SB0134 (group 1) and *M. caprae* SB0157 (group 2) both isolated from TB infected wild boar in Spain. Group 3 was challenged with the strain *M. tuberculosis* SIT58, isolated from cattle in Spain. Each goat received a final volume of 0.5 mL containing 10^4 UFC.

Blood samples were collected before inoculation, and then weekly for the 5 weeks after the challenge. Whole blood samples were stimulated by *M. bovis* (bovine) and *M. avium* (avian) purified protein derivatives (PPDs; CZ Veterinaria, Spain) at 20 μg/mL sample and PBS as non-stimulated controls. Additionally, an aliquot was stimulated with human PPD (2 UT/0.1 mL of Evans PPD, gently supplied by Hospital Ramón y Cajal, Madrid, Spain) at the last week of the study to evaluate its accuracy for detection of *M. tuberculosis* infected goats. The supernatant was assayed for the presence of IFN-γ using the Bovigam ELISA according to the manufacturer's instructions and interpretation was performed according to the Spanish legislation: a positive reactor was considered when the absorbance (optical density at 450 nm, OD_{450}) in the aliquot stimulated with bovine PPD minus the OD_{450} of the nil was ≥0.05 and also this value was higher than that observed in the aliquot stimulated with avian PPD minus the nil.

The SIT was performed on the left side of the neck before challenge, and then after blood sampling for IFN-γ assay at the end of the study (week 5 post-inoculation).

SIT and SCIT tests were carried out according to Council Directive 64/432/EEC using bovine PPD and avian PPDs. Additionally, a SIT test using human PPD was also performed. Using the standard interpretation of the SIT test, positive reactors were those showing a skin fold thickness increase of 4 or more mm or the presence of clinical signs such as oedema, exudation, necrosis, pain or inflammation at the injection site. Using the SCIT test, a bovine reaction greater than avian reaction more than 4 mm was considered as positive result. A more stringent interpretation considering inconclusive reactors to SIT (3 mm) or SCIT test (>1 and ≤4 mm) as positive reactors recommended in infected settings was also applied as described elsewhere (Bezos et al., 2014b).

At the end of the study the goats were sedated by injection of intravenous Xylazine (Xilagesic 2%, Calier S.A., Spain) at 10 mg/50 kg and euthanized by intravenous injection of T-61 (Intervet, Spain). Organs were examined and sliced into thin sections for the identification of TB-compatible lesions. The severity of the gross pathological changes was scored according to a semi-quantitative system (Vordermeier et al., 2002). Tissue samples were pooled for culture into three anatomical regions: one pool contained head lymph nodes (LNs) and the tonsils of the soft palate; a second one contained the thoracic LNs and lung tissue; and a third one containing samples of spleen, liver, hepatic LN and mesenteric LNs. Pooled tissue samples were decontaminated with a final concentration of 0.37% hexadecylpyridinium chloride, cultured onto Coletsos medium and identified by PCR as described (Bezos et al., 2010).

3. Results and discussion

Table 1 summarizes the lesion scores and culture results by challenge group. The highest mean total lesion score was observed in *M. bovis* challenged goats, followed by those challenged with *M. caprae*. The lowest score was recorded in goats challenged with *M. tuberculosis*. This difference was significant (Median Test, Chi-Square = 9.2, 2 d.f., $p=0.0101$). In fact, TB-compatible lung lesions were recorded in 4 out of 5 goats from group 1 (mean 10.4, range 5–13), 3 out of 5 from group 2 (mean 9.6, range 0–18) and only 1 out of 4 from group 3 (mean 0.25, range 0–1). Culture results coincided with the lesion scores in yielding more positive pools (7/15; 46.7%) in *M. bovis* challenged goats. By contrast, only three pools were positive from goats challenged with *M. caprae* (3/15; 20%) and *M. tuberculosis* (3/12; 25%), respectively (Table 1). Only 4 out of 14 goats confirmed by bacteriology (26.7%) had positive cultures outside the thorax, indicating generalized infection. All four goats had been infected with *M. bovis* SB0134.

Overall results obtained using the IFN-γ and SIT and SCIT tests are summarized in Table 2. The highest number of positive reactors to the IFN-γ test was observed in the *M. bovis* infected group. Moreover, in this group, the first positive reactors were detected at week 2 post-infection whereas in the *M. caprae* and *M. tuberculosis*-infected groups was later. Accordingly, significant differences (Mann-Whitney's test, $p=0.032$) were found between the ODs measured at week 2 in the *M. bovis* infected group and the other groups. Excluding the *M. tuberculosis* infected group, all infected goats were positive reactors at week 4. In all the infected groups there was a significant increase (Friedman's test, $p=0.022$, $p=0.001$ and $p=0.022$ for groups 1, 2 and 3, respectively) of the overall ODs from week 2 to the end of the study (Fig. 1). Nevertheless, using human PPD for blood stimulation, no reactors were detected regardless the MTC species used for infection and the ODs measured were significantly lower (Kruskal-Wallis test, $p<0.05$).

Regarding the intradermal tests, no significant differences (Kruskal-Wallis test, $p>0.05$) were found regarding the skinfold thickness measured at the bovine, avian and human PPD injection sites between the three infected groups. Using the stringent interpretation of the bovine PPD-based SIT test, all the infected animals were detected at 5 week after challenge. Using the stringent interpretation of the SCIT test, all infected animals were detected excluding one goat from *M. caprae* infected group. Using standard interpretation of the SCIT test, two and one positive reactors were missed in groups 2 and 3, respectively (Table 1). Human PPD used for intradermal testing showed higher sensitivity than that reported for the IFN-γ test excluding the *M. tuberculosis* infected group where no reactors were also detected both using human-PPD based intradermal and IFN-γ tests. Nevertheless, the number of infected goats detected using human PPD-based SIT test was lower than those detected using bovine PPD, particularly in group 3 where no reactors were detected.

This experimental infection confirmed the hypothesis that goats challenged with different members of the MTC display different clinical pictures. Goats challenged with *M. bovis* SB0134 had the highest lesion scores and culture results, while goats challenged with *M. tuberculosis* displayed clearly lower lesion scores, probably due to a lower rate of growth throughout the study in comparison to the other MTC members. These differences were particularly evident regarding the proportion of animals with lung lesions, and the lung lesion scores. This might imply MTC species-related differences in the likelihood of bacterial shedding, as suggested for naturally infected wild boar (Garcia-Jimenez et al., 2013). However,