Distribution of lymphocytes, immunoglobulin-containing cells, macrophages, and dendritic cells in the accessory sex glands of rams experimentally infected with *Actinobacillus seminis*1

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ABSTRACT.- Acosta-Dibarrat J., Tenorio-Gutiérrez V., Soriano-Vargas E., Talavera-Rojas M., Cal-Pereyra L., Montes de Oca-Jiménez R., Velázquez-Ordoñez V. & Tórtora-Pérez J. 2016. Distribution of lymphocytes, immunoglobulin-containing cells, macrophages, and dendritic cells in the accessory sex glands of rams experimentally infected with *Actinobacillus seminis*. Pesquisa Veterinária Brasileira 36(5):363-372. Centro de Investigación y Estudios Avanzados en Salud Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Km 15.5 Carretera Toluca-Atlacomulco, Toluca, ME 50100, México. E-mail: jpacosta00@hotmail.com

The distribution of cells involved in the immune response in accessory sex glands of rams experimentally infected with *Actinobacillus seminis* was studied. Twelve one-year old rams were experimentally infected by intraurethral (IU) (n=4) and intraepididymal (IE) (n=4) route, and four control (CON) animals were used. The animals were slaughtered 35 days post-inoculation, samples were taken from accessory sex glands, and bacteriology and histopathology tests were performed. The presence of CD4, CD8 and TCRγδ (WC1) lymphocytes, CD45RO cells, macrophages (CD14), dendritic cells (CD1b), IgA-, IgG- and IgM-containing cells (IgCC) was determined. Animals of the IE group developed clinical epididymitis. No lesions were seen in rams of the IU group; two of the intraepididymal inoculated CON developed small lesions in the epididymis. *A. seminis* isolates were achieved from 6:16 (37.5%) accessory sex glands in the IE group, but not in the IU and CON groups. In the CON group, IgA- and IgM-containing cells predominated in the bulbourethral glands and the disseminated prostate, and they were scarce or null in the vesicles and ampullae. A significant increase of IgA-, IgG- and IgM-containing cells was confirmed in the seminal vesicles, the ampullae and the bulbourethral glands in the IE group. In the IE and IU groups, an increase in CD4, CD8, WC1, CD45RO and CD14 was evidenced in the vesicles and ampullae. CD1b dendritic cells were present in the ampullae and vesicles with inflammatory processes. *A. seminis* triggered a local immune response in the IE and IU groups. These results indicate a different pattern of infiltrating immune cells in the accessory sex glands of infected *A. seminis* rams.

INDEX TERMS: Accessory sex glands, rams, *Actinobacillus seminis*, lymphocytes, immunoglobulin-containing cells, macrophages.

RESUMO.- [Distribuição de linfócitos, células carreadoras de imunoglobulinas, macrófagos e células dendríticas em glândulas sexuais acessórias de carneiros infectados experimentalmente com *Actinobacillus seminis*.] A distribuição das células envolvidas na resposta imune em glândulas sexuais acessórias de carneiros experi-
rimentally infected with *Actinobacillus seminis* was studied. Doze carneiros de um ano de idade foram experimentally infected with *Actinobacillus seminis* via intrauretral (IU) and via intraepididimal (IE) inoculation, and quatro animais controies (CON) were utilized. Os animais foram abatidos 35 dias após a inoculação, amostras foram retiradas das glândulas sexuais acessórias e testes bacteriológicos e histopatológicos foram realizados. A presença de linfócitos CD4, CD8 e TCRyβ (WC1), células CD45RO, macrófagos (CD14), células dendriticas (CD1b) e células contendo IgA, IgG and IgM (IgCC) foi determinada. Os animais do grupo IE desenvolveram epididimite clínica. Não foram visualizadas lesões nos carnieiros do grupo IU, dois dos CON inoculados intraepididimalmente desenvolveram pequenas lesões no epidídimo.

Isolados de *A. seminis* foram obtidos de 6:16 (37,5%) nas glândulas sexuais acessórias no grupo IE mas não nos grupos IU e CON. No grupo CON células contendo IgA and IgM predominaram nas glândulas bulbouretrais e na próstata e foram escassas ou ausentes nas vesículas e na ampolla. Um incremento significativo de células contendo IgA e IgM foi confirmado nas vesículas seminais, nas ampolla e nas glândulas bulbouretrais no grupo IE. Nos grupos IE e IU foi evidenciado um aumento em CD4, CD8, WC1, CD45RO e IgM nas vesículas seminais, na ampolla e nas glândulas bulbouretrais. No grupo IU, a presença de linfócitos CD1b e IgM foi confirmada nas vesículas seminais. As células dendriticas CD1b e IgM foram detectadas nos grupos IE e IU, destacando-se a presença de linfócitos CD4, CD8 e IgM nas vesículas seminais. Isto indica um padrão diferente de células imunológicas que infiltrantram nas glândulas sexuais acessórias de carnieiros infectados por *A. seminis*.

**INTRODUCTION**

Sheep epididymitis has been mainly associated with *Brucella ovis* and *Actinobacillus seminis* infections (Burgess 1982, Moustacas et al. 2013). The pathogenesis of the disease caused by *A. seminis* has been explored to a small extent; the presence of pathogenicity factors in *A. seminis*, such as adhesins (Healey et al. 1991) or RTX toxins (Schaller et al. 2000), is likely to contribute to the development of the pathology. However, the susceptibility to infection of the accessory sex glands, especially of the seminal vesicles in sheep (Foster et al. 1987, Al-Katib & Dennis 2005, Acosta-Dibarrat et al. 2006), bulls (Bagshaw & Ladds 1974, Cavalieri & Van Camp 1997), and humans (Furuya et al. 2004) suggests that the presence of other factors, especially immunological factors, would be implicated in such susceptibility.

The male genital tract has to accomplish functions of defense against infections, and it must also tolerate germ cells that present differential antigens. Recently, the study of the immune response mechanisms in the reproductice system has been intensified, focused on the fight against sexually transmitted diseases (Anderson & Pudney 1999).

The information on the immune response mechanisms in the male reproductive tract in different species, and particularly, the information on the presence and distribution of immune response cells in the reproductice tract of she-
Immunohistochemistry. Slices were hydrated with PBS for 10 minutes and endogenous peroxidase was blocked with Perox-o-Block (Lab. Zymed, San Francisco, USA) for 45 seconds. An unspecific blockade was carried out with 10% caprine serum overnight. Then, primary monoclonal antibodies for the different receptors were applied (Table 1), which were incubated for 2 hours at room temperature. The biotin-goat anti mouse IgG complex was subsequently applied (Lab. Zymed, San Francisco, USA) for 30 minutes; then, the Streptavidin-Peroxidase complex (Lab. Zymed, San Francisco, USA) was administered for 15 minutes; the DAB substrate (Lab. Zymed, San Francisco, USA) was applied until a reaction was observed (2 to 5 minutes) and hematoxylin contrast staining was carried out. Three washings with PBS were performed between each step. Finally, the slices were dehydrated, cleared and mounted.

In the control slice, the primary antibody was replaced with PBS. Additionally, plates with prepulse sections were used as positive control in all runs, since it had been previously confirmed that cells positive to all monoclonal antibodies used in this work were present in this organ.

Immunofluorescence. For the determination of immunoglobulin-containing cells (IgCC), tissue Igs were eliminated first by placing the plates in PBS all night; an unspecific blockade with 10% caprine serum was subsequently performed for 1 hour at room temperature, and three 5-minute washings with PBS were carried out. Anti-ovine IgA primary antibody (Bethyl Lab, Montgomery, USA) was applied in a 1:40 dilution, as well as anti-ovine IgG primary antibody (Bethyl Lab, Montgomery, USA) in a 1:200 dilution, and anti-ovine IgM primary antibody (Bethyl Lab, Montgomery, USA) in 1:60 in PBS with 1% ASB. Incubation was performed for 1 hour at 37°C. Three washings were performed and the secondary antibody, TRICT-conjugated anti-rabbit IgG antibody (Sigma-Aldrich, St Louis, USA), was applied in a 1:30 dilution (in 10% goat serum and 1% ASB) for one hour at 37°C. Finally, it was washed again 3 times for 5 minutes per washing, and was mounted in glycerin and observed under an epifluorescence microscope. The primary antibody was replaced with PBS in the control slice, and the technique described above was then followed. This control was included due to the possibility of unspecific labeling by the anti-rabbit IgG conjugate. In this case, a prepuce section was also used as positive control, on which positive labeling had been previously demonstrated for the three immunoglobulins.

Cell count and statistical analysis. Cell count was carried out in ten 40 X 10x fields with the Image Pro Plus 4.5 software. The cells present in the projected image were counted and the resulting average counts were expressed as cells per mm². The possible differences between treatments (groups) for each gland and each monoclonal antibody used were determined by the Kruskal-Wallis test, followed by the Mann-Whitney test. The difference was considered significant with p<0.05.

**RESULTS**

The results of the histopathology and bacteriology studies are shown in Table 2. At the moment of slaughter, only the challenge bacterium could be re-isolated in the IE group in 3 of 4 sheep from the epididymal tail, in 3 of 4 sheep from the ampullae, and in 1 of 4 sheep from the seminal vesicle, the prostate and the bulbourethral gland. Inflammatory lymphoplasmocytary infiltrates, with different intensity degrees, occurred in the ampullae and seminal vesicles, and were concurrent with the presence of lymphocytes, immunoglobulin-containing cells, macrophages, and dendritic cells.
Table 3. CD4, CD8, CD45RO, WC1, CD14 AND CD1b cells per (mm\(^2\)) in the accessory sex glands of rams inoculated with *Actinobacillus seminis*

| Gland                | Group | CD4  | CD8  | CD45RO | WC1  | CD14 | CD1b  |
|----------------------|-------|------|------|--------|------|------|-------|
| Seminal Vesicle      | IU    | 78.0±60.2\(^a\) | 93.0±75.7\(^b\) | 274.3±125.5\(^h\) | 19.4±7.1\(^h\) | 52.7±27.7\(^n\) | 0.0±0.0 |
|                      | IE    | 334.7±144.2\(^a\) | 316.6±24.6\(^a\) | 864.6±466.2\(^a\) | 75.0±43.7\(^a\) | 142.7±37.1\(^a\) | 2.8±5.6 |
|                      | CON   | 11.1±22.3       | 38.9±31.1       | 67.9±39.7         | 1.4±28       | 41.6±132  | 0.0±0.0 |
| Ampulla              | IU    | 124.1±173.1\(^e\) | 180.4±1320.4\(^e\) | 220.0±178.9\(^e\) | 31.9±25.4\(^e\) | 166.5±59.9\(^h\) | 0.0±0.0 |
|                      | IE    | 76.3±18.9\(^e\) | 205.4±141.3\(^e\) | 295.2±730.0\(^e\) | 33.3±19.8\(^e\) | 263.4±129.4\(^e\) | 5.5±6.4 |
|                      | CON   | 16.7±4.5        | 33.3±15.7       | 40.3±15.9         | 1.4±28       | 33.3±19.5  | 0.0±0.0 |
| Disseminated Prostate| IU    | 52.7±28.2\(^a\) | 41.5±34.7       | 140.3±121.8       | 0.0±0.0      | 86.1±35.3  | 0.0±0.0 |
|                      | IE    | 13.9±11.5\(^a\) | 30.5±14.7       | 51.4±38.9         | 1.4±32       | 97.2±29.4  | 0.0±0.0 |
|                      | CON   | 14.8±11.5       | 7.4±8.5         | 27.8±11.1         | 3.7±6.4      | 42.6±36.1  | 0.0±0.0 |
| Bulbourethral Gland  | IU    | 23.6±15.9\(^e\) | 19.5±19.2       | 8.3±5.5\(^b\)    | 0.0±0.0      | 22.2±12.0  | 0.0±0.0 |
|                      | IE    | 12.5±5.3        | 18.0±18.3       | 36.0±45.3\(^a\)  | 5.5±0.0      | 27.8±23.5  | 0.0±0.0 |
|                      | CON   | 9.7±11.5        | 11.1±18.7       | 4.2±8.4           | 0.0±0.0      | 28.2±3.2   | 0.0±0.0 |

IE = intraepididymal, IU = intraurethral, CON = control. \(^a\) Significance compared with the CON group p<0.05, \(^b\) significance compared with the IE group p<0.05, \(^e\) compared with the CON group p<0.1, \(^f\) compared with the IU group p<0.1. Median ±SD.

Fig.1. Number of CD4, CD8, WC1, CD45RO, CD14 and CD1b lymphocytes per mm\(^2\) in the accessory sex glands of rams inoculated with *A. seminis*. IU Intraurethral group, IE Intraepididymal group, CON Control group. a significance compared with the CON group p<0.05, b significance compared with the IE group p<0.05, e compared in the CON group p<0.1, f compared in the IU group p<0.1.
**Actinobacillus seminis** isolate in 3:4 animals in the IE group.

The average counts obtained for the evaluated cells in each group are summarized in Table 3 and Figure 1. A greater overall cellularity can be observed in the seminal vesicles and the ampullae from the IU and IE groups, compared with the control group. The most important differences occurred in the ampullae and the seminal vesicles from the animals with concurrent isolation of *A. seminis* from these structures.

In the ampullae of the deferent duct in the IE group, there were differences from the CON group in CD4 (p=0.003), CD8 (p=0.028), CD45RO (p=0.005), WC1 (p=0.007) and CD14 (p=0.028) counts, but no differences were noted in CD1b, although the presence of labeled cells was demonstrated (5.5±6.4 mm²). Differences were also found between the IU and CON groups in CD8 (p=0.028), WC1 (p=0.002) and CD14 (p=0.003), with a trend in CD45RO (p=0.083). No significant differences were found between cell populations from the ampullae in the IU and IE groups.

In the seminal vesicles, differences were present between the IE and CON groups in CD4, CD8, WC1 and CD45RO (p=0.029), as well as a tendency in CD14 (p=0.057); these differences were also maintained between the IE and IU treatments, and were even significant for CD14 (p=0.029). There was a significant difference in the vesicle counts in WC1 between the CON and IU groups (p=0.029), with a tendency in CD45RO (p=0.057). A scarce number of CD1b (2.8±5.6 mm²) was found in the IE group.

No differences were found in the disseminated prostate; a trend was demonstrated only for CD4 between IU, CON and IU, IE (p=0.057).

In the bulbourethral glands, differences were demonstrated between the IE and CON groups, and between the IE and IU groups in WC1 (p=0.006 and p=0.029, respectively) and in CD14 cells between the IU and CON groups (p=0.042).

In the seminal vesicles, lymphoid accumulations were found in the periphery of the acini with presence of CD8 and CD4. CD4 lymphocytes were frequently present in the conjunctival stroma of the glands, and CD8 were present with a closer relationship with the basal membrane of acini (Fig.2). CD45RO were present in both positions and were frequently located within the acini (Fig.3). CD14 labeling occurred in the stroma, on the macrophages and in the endothelia.

Regarding immunoglobulin-containing cells (IgCC), the most frequent labeling in the accessory sex glands corresponded to IgA, followed by IgM and IgG. In the CON group, the bulbourethral gland and the disseminated prostate presented IgA- and IgM-containing cells, which were not seen in the ampullae and the vesicle (Table 4, Fig.4)

IgG-containing cells were seen in the IE and IU groups, in a greater proportion in the seminal vesicle and in the deferent duct ampullae. Coincidentally, it was from these glands that *A. seminis* isolates were obtained more frequently. Statistical differences were found in the ampullae for IgM- and IgG-containing cells between the CON and IE groups (p=0.029), and between the IE and IU groups (p=0.029).

Significant differences were found between IgA-containing cells (p=0.029) in the bulbourethral glands in the CON and IE groups, while IgM-containing cells showed a trend.
Trends were present in the vesicles in the Kruskal Wallis test \((p<0.1\)) but the great variability in the cell counts from these samples prevents these differences from being evident between the groups (data not shown).

IgG-containing cells were only seen in the IU and IE groups, mainly in the latter, with percentages of 11, 9 and 2\% in the ampulla, the seminal vesicle and the bulbourethral gland, respectively. Conversely, no IgG labeling was seen in the CON group in any gland.

The greater cellularity observed in the bulbourethral glands and the disseminated prostate stands out in all groups, mainly due to the presence of IgA- and IgM-containing cells (Figure 5). In the bulbourethral gland, IgA-containing cells ranged between 73 and 79\%, and IgM-containing cells ranged between 21 and 25\%, while in the disseminated prostate, 82 to 90\% were IgA-containing cells and between 10 and 18\% were IgM-containing cells, and the...
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positive IgG-containing cells counts were almost null. The histological location of IgCC also showed differences between the glands studied. In the disseminated prostate and the bulbourethral gland, IgA- and IgM-containing cells were observed mainly in the scarce connective tissue surrounding the acini. A clearly positive IgA labeling was observed in the inside of some acini in association with glandular secretion. IgG- containing cells from the seminal vesicles were located in the connective tissue that separates the glandular lobules, while IgA- containing cells were observed in the acini.

DISCUSSION

In this experiment, several facts are confirmed that may contribute to demonstrating the different susceptibility of the accessory glands to *Actiobacillus seminis* infection (Al-Katib & Dennis 2005, Acosta-Dibarrat et al. 2006). As previously mentioned regarding *A. seminis*, the bacterium was isolated more frequently from the ampullae and seminal vesicles, suggesting that, in these structures, bacterial establishment and permanence is somehow facilitated, although these were the glands that showed the most notable inflammatory changes behind the epididymides, which were directly inoculated. In the vesicles and ampullae of the CON group, no positive IgA-, IgM- or IgG-containing cells were found, or they were found in a lower number than in the inoculated groups, a condition that may facilitate bacterial establishment. This finding differentiates the seminal vesicles and the ampullae from the bulbourethral gland and the disseminated prostate, which, conversely, demonstrated an important number of these cells in the aforementioned group.

The distribution of IgCC in control and infected sheep glands observed in this work is consistent with the findings demonstrated in bulls, where IgCC are also not found or are present in a very limited number in the seminal vesicles of normal animals (Campero et al. 1989, 1990), while in the presence of an inflammatory response in the genital tract, IgCC appear in the ampullae and the seminal vesicles (Campero et al. 1989). It has been noted that the production of IgA and IgG in the seminal vesicles in the bull is an essential component of the local immune response (Bier et al. 1977). However, in other studies, no IgCC are found, or these are found in a very limited number in the seminal vesicles of normal bulls; therefore, it is unlikely that this is the origin of the immunoglobulins present in the semen (Campero et al. 1989).

| Table 4. IgA, IgG- and IgM-containing cells in the accessory sex glands per (mm²) |
|-----------------------------------------------|
| Gland                                     | Group | IgA-containing cells | IgG-containing cells | IgM-containing cells |
|-----------------------------------------------|
| Seminal Vesicle                             | IU    | 97.4±194.9*          | 1.4±2.8*             | 7.0±14.0* |
|                                             | IE    | 319.9±233.9*         | 52.8±78.5*           | 110.6±75.2* |
|                                             | CON   | 0.0±0.0*             | 0.0±0.0*             | 0.0±0.0* |
| Ampulla                                     | IU    | 12.4±24.9            | 0.0±0.0              | 1.4±2.8 |
|                                             | IE    | 49.1±76.4            | 16.7±15.8            | 123.5±128.1 |
|                                             | CON   | 11.2±15.9            | 0.0±0.0              | 0.0±0.0 |
| Disseminated Prostate                       | IU    | 280.3±106.1          | 1.4±2.8              | 32.5±64.9 |
|                                             | IE    | 243.7±949            | 0.0±0.0              | 48.1±21.4 |
|                                             | CON   | 143.6±167.2          | 0.0±0.0              | 31.8±25.3 |
| Bulbourethral                               | IU    | 84.5±44.8            | 0.0±0.0              | 22.2±7.8 |
|                                             | IE    | 237.4±40.6           | 5.5±6.4              | 81.2±53.1 |
|                                             | CON   | 58.3±58.5            | 0.0±0.0              | 15.3±17.8 |

*IE = intraepididymal, IU = intraurethral, CON = control. *Significance compared with the CON group p<0.05, *Significance compared with the IU group p<0.05, *Significance compared with the IU group p<0.1, *p<0.1 (Kruskal Wallis test) very heterogeneous reactions within the group.

Fig. 5. IgA and IgM positive cells in the accessory sex glands of the reproductive system. A) IgM positive plasma cells present in disseminated prostate. IF, obj. 40x. B) Appearance of the bulbourethral gland with abundant IgA positive cells, located in the interstitial tissue. IF, obj. 10x.
Foster et al. (1988), using 5 one-year-old sheep and 12 sheep older than 4 years, reported the presence of IgA- and IgG-containing cells, mainly in the bulbourethral gland and the prostate, and only occasionally in the seminal vesicles and the ampullae; these results are consistent with the results of this work. However, in contrast with the results of this work, these authors found high percentages of IgG-containing-cells, a condition that only occurred in a small number in the IE group in the seminal vesicles and ampullae with active inflammatory processes. This difference may be attributed to the fact that the animals used by Foster et al. (1988), had subclinical alterations of the reproductive system with local infection, since no bacteriological examinations of the semen were carried out and they only verified that the animals were free of B. ovis by serology; they also do not distinguish between young animals and adult animals, and variations are likely to occur in the amount and distribution of IgG due to age, as has been reported in bulls (Campero et al. 1989).

In humans, positive IgA- and IgM-containing cells are described in the prostate, the bulbourethral gland and the penile urethra, where only a limited number of IgG-containing cells are found. However, little is known about the immunobiology of seminal vesicles, where no plasma cells are seen, but an occasional expression of plgR is found (Anderson & Punney 1999).

The information available on the presence and distribution of the cell types studied in the male reproductive apparatus, especially in the accessory sex glands, is limited. In this study, no differences were found in the distribution of CD4, CD8, WC1 and macrophages in the disseminated prostate, the bulbourethral gland, the seminal vesicle and the ampulla of the deferent duct in sheep of the CON group, in contrast to the observations for IgCC. The disseminated prostate is closely related to the prostatic urethra and its submucosa, strongly infiltrated by CD4 and CD8 lymphocytes and macrophages, a condition that may favor antigenic recognition and presentation and may promote the proliferation and differentiation of B lymphocytes at this zone. In the case of bulbourethral glands, this relationship is not clear; therefore, it may be possible that this gland behaves as an effector site for the immune response, to which cells activated in other parts of the reproductive apparatus or other organs may arrive (Russell & Mestecky 2002). There is no information available on the presence of endothelial VCAM-1, ICAM-1 or MadCAM-1 receptors in the bulbourethral glands.

A. seminis triggered an inflammatory response in the accessory glands, mainly in the seminal vesicles and the ampullae, with a significant increase in CD8, CD4, TCRγδ and CD14 lymphocytes and CD45RO cells in both the IU and IE groups at 35 days post-inoculation, although in the IU group no A. seminis isolations were achieved, and only two animals demonstrated a serologic response (Acosta-Dibarrat et al. 2007). These facts suggest an immune response for controlling the establishment and development of the infection induced by the inoculation of the bacterium by IU route, which is considered as the route that more closely resembles the natural acquisition of A. seminis infection (Jansen 1980, 1983). In the case of IE-challenged animals, the damage caused to the epididymis and the constant presence of bacteria at this site may have prevented the local response of the ampullae and vesicles from eradicating the infection. These facts support the possibility that A. seminis behaves as an opportunistic bacterium, that is only capable of causing disease in the presence of predisposing factors (Jansen 1983, Walker & Leamaster 1986).

No conclusions can be drawn from this experiment regarding the type of response induced by the experimental infection with A. seminis. However, in the inflammatory foci of both the vesicles and the ampullae, there was a significant increase of labeled cells for TCR γ/δ (WC1), CD4 and CD8 lymphocytes, macrophages, and even dendritic cells, particularly in IE animals. The changes in IgCC in these same glands suggest an interaction of type Th1 and Th2 responses in the response to the bacterium and its potential eradication, considering the results for the IU group. It has been reported that lymphocytes from the spleen of mice previously inoculated with A. seminis and challenged in vitro with the whole bacterium, produced IL4, IL-2 and IFN-γ, suggesting that A. seminis induces both responses (Patlani et al. 2006). The changes observed in cell populations in this work may have been influenced by the development of autoimmun responses (Paolicchi et al. 2000), particularly in the case of IE inoculated animals. However, controls intraepididymally inoculated with S. S. demonstrated a reduced number of immune response cells compared to treated animals.

A certain proportion of TCR γδ lymphocytes express the WC1 marker on their surface. These cells are abundant in the peripheral blood and jejunal mucosa of ruminants, especially in young animals, and may provide an immediate mechanism of Th1 cytokine production (Pollock and Welsh, 2002, McClure 2009). The increase in WC1 in the seminal vesicles and the ampullae was significant relative to the IN group, but its absolute number was lower than that of CD4 and CD8 lymphocytes. The increase in TCR γδ lymphocytes has been reported in various inflammatory or infectious diseases (Baldwin et al. 2000). These cells produce keratinocyte growth factor, and may be implicated in epithelial repairing processes (Van der Broek et al. 2005).

The common leukocytic antigen CD45 is a modulator of T-lymphocyte activation signal transduction; the CD45RO isoform is present in memory CD4 and CD8 T-lymphocyte subclasses (Bembridge et al., 1993). This isoform also is expressed in monocytes, granulocytes and mononuclear cells presenting WC1, TCRγδ, CD4 and CD8, but is not expressed by B lymphocytes (Bembridge et al. 1995). This isoform is particularly abundant in mucosa-resident lymphocytes, especially in the intestinal lamina propria, where it may represent 93%, in contrast with 30% in peripheral blood (Stephen & Hiroshi 1999). Its increase in the vesicle and ampulla of the infected animals appears to be an additional indicator of immune response induction by the presence of the bacterium.

The CD1b marker is a surface glycoprotein present in...
dendritic cells, through which it is capable of presenting lipid and glycolipid antigens to T cells (Porcelli et al. 1998, Rhind 2001, McClure 2009). Positive cells are considered excellent antigen presenters for TCR γδ lymphocytes, and are the main cells responsible for the production of an effective response against these intracellular pathogens (Rhind 2001). In this work, only a limited number of positive cells were present in the ampullae and the seminal vesicles, mainly in the IE group, where they appeared concurrently with a significant increase in TCR γδ lymphocytes, suggesting that dendritic cells would not be relevant in the antigenic presentation in the accessory sex glands of rams. In contrast, macrophage increase in the ampulla and the vesicle in the IU and IE groups would facilitate antigen presentation in the glands in A. seminis infection. CD14, that has been reported to be capable of recognizing LPS present in Gram- bacteria, is found in the macrophages' surface, alone or associated with proteins (Wright et al. 1990). The observation of endothelial labeling with CD14 in the ampullae and seminal vesicles with inflammatory processes may be explained, considering that the LPS of Gram-negative bacteria has been reported to stimulate the expression of this marker in the endothelia, with TNF-α and IL-6 production (Dai et al. 2002).

CONCLUSIONS

Inoculation with Actinobacillus seminis caused pathologic alterations, not only at the site of inoculation in the epididymal tails, but also in the accessory glands of the male reproductive system, mainly in the ampullae of the deferent duct and the seminal vesicles.

Differences could be found in the distribution and number of IgCC among glands in the CON group. The ampullae and vesicles from this group presented a limited number of cells or no cells at all, while these were abundant in the bulbourethral gland and the prostate, mainly of the IgA- and IgM-containing cells. In IU- and IE-treated groups, positive IgG-containing cells were present in those organs with a marked inflammatory response, in the ampullae and vesicles and in the inoculated epididymal tails.

The CD4/CD8 ratio did not show a predominance of any of these subtypes in the tissues where A. seminis was isolated.

CD1b dendritic cells were scarce in the accessory glands of the reproductive system, where the antigenic presentation functions in A. seminis infection seem to be performed by CD14 macrophages.

The immunobiology of the accessory glands may be the reason why there are differences in their susceptibility to A. seminis infection, and why they eventually act as a site (refuge) of bacterial permanence.

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