LYMPHOCYTE MARKERS CORRELATION IN TISSUE AND BLOOD IN VISNA/MAEDI EXPERIMENTALLY-INFECTED SHEEP

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Introduction

Visna/maedi (VM) provokes an inflammatory response lead by lymphocytes. The expression of lymphocyte markers is useful to understand the pathogenesis of the disease. The aim of this work was to study the expression of lymphocyte markers involved in the evolution of VM in an experimental infection by using flow cytometry (FC) and immunohistochemistry (IHQ).

Material and Methods

Twenty Raza Aragonesa lambs, negative by ELISA and PCR for VM, were distributed into two infected groups (8 animals each) and one control group (4 animals). Infected lambs were inoculated intratracheally with $10^6 TCID_{50}$ either with an articular (496) or nervous strain (697). Blood was tested periodically by FC for the expression of CD4, CD8 and FoxP3 on all animals. At 210 dpi two animals from each infected group (A1, A2, N1, N2), selected by high ELISA values and one control (C) were killed and studied by pathological means. The inflammatory pattern was characterized by IHQ using CD3, CD4, CD8 and FoxP3.

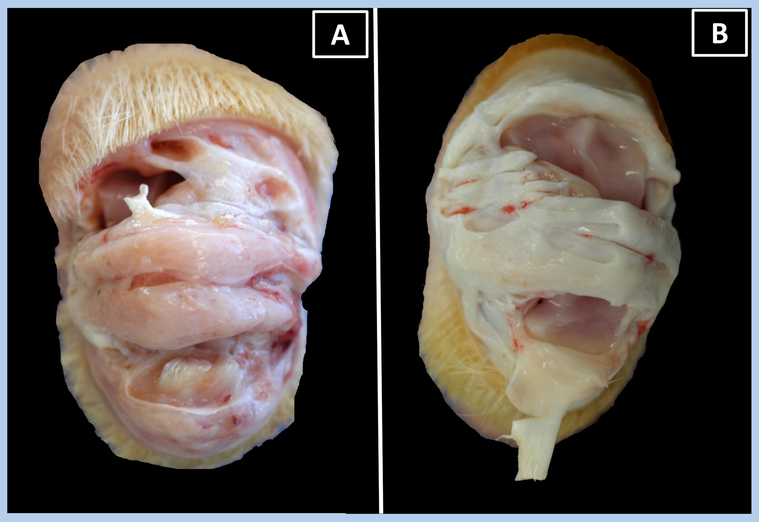

Figure 1. (A) A1 sheep, left carpal joint. Articular capsule showing severe thickness, edema and proliferation of connective tissue. (B) A2 sheep, right carpal joint. Articular capsule showing moderated thickness and proliferation of connective tissue. Note the difference between the two joints (A) Edematous and proliferative (B) Markedly fibrotic.

Results

- Pathological examination demonstrated carpal proliferative arthritis on the two animals from the articular group (A1, A2; Figs 1 and 2). The animals from the nervous group (N1, N2) did not present lesions at the SNC. Microscopically all infected animals presented different degrees of interstitial pneumonia associated with lymphoid follicles. No lesions were found on the control animal.
- Tissue expression of CD4, CD8, FOXP3 and CD3 are presented on Figure 3.
- Blood expression of CD4 and CD8, on each group is shown on the Figure 4.
- Expression of FOXP3 was always low and did not differ between groups.

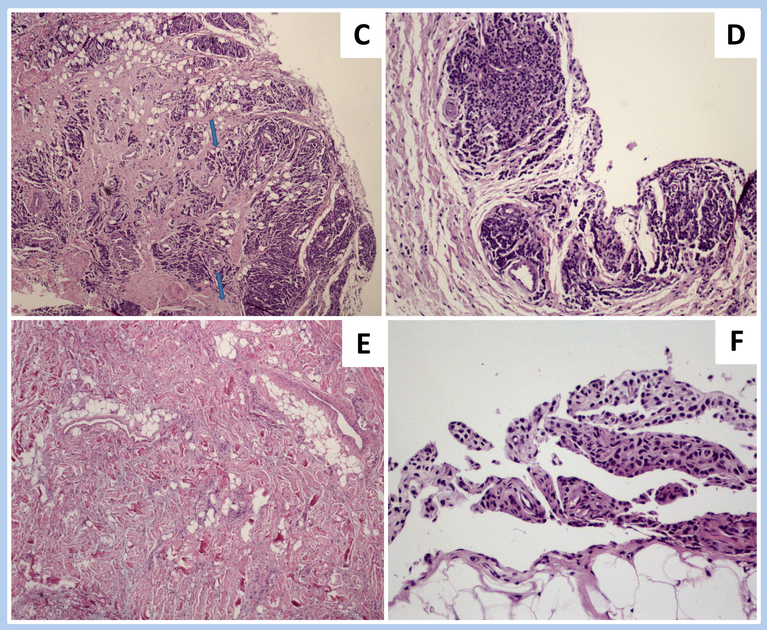

Figure 2. C, D: A1 left carpal joint, H&E. (C) Severe multifocal well demarcated lymphocytic inflammatory infiltrate at the subsynovial area associated with fibroplasia and neovascularization. Note the predominant perivascular pattern (arrows) (D) Synovial membrane showing an intense lymphocytic inflammatory infiltrate with follicular pattern. E, F: A2 sheep, right carpal joint, H&E. (E) Articular tissue showing severe fibroplasia, (F) Moderate digitiform synoviocyte proliferation.

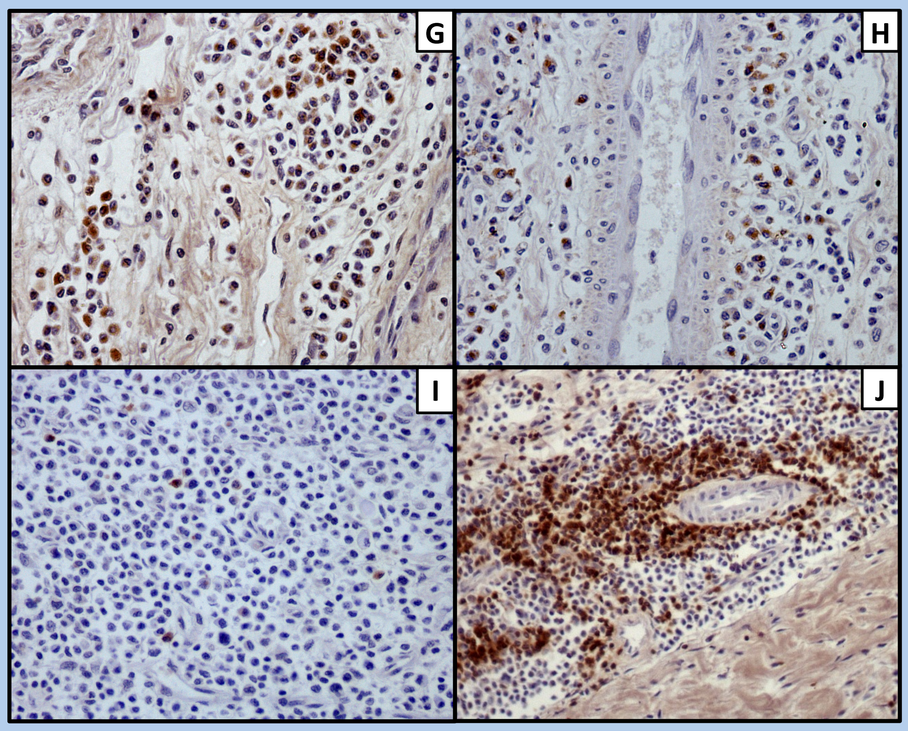

Figure 3. A1 sheep, left carpal joint, IHQ. (G) CD4 positive cells, cytoplasmic staining, 30-40% lymphocytes show positivity; (H) CD8 positive cells, cytoplasmic staining, 15-20% lymphocytes show positivity; (I) FOXP3 positive cells, cytoplasmic and nuclear staining, 1-2% lymphocytes shows positivity; (J) CD3 positive cells, cytoplasmic staining, 20-30% lymphocytes shows intense positivity, mainly perivascular (Percentages based on a single animal , A1)

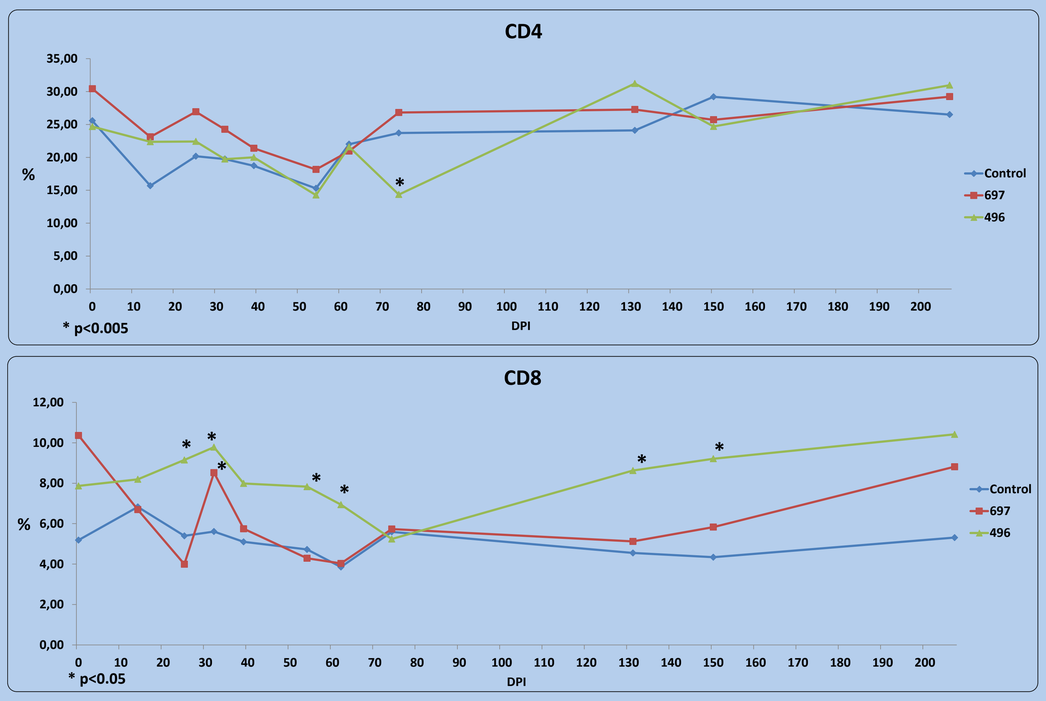

Figure 4. FC blood expression of CD4 and CD8 in infected and control groups. Results expressed as mean of percentages by groups. Significant results are mostly found on CD8 at the 496 group.

Conclusions:

- At 210 dpi, 496 strain is able to reproduce the VM induced arthritis whereas 697 strain does not reproduce VM SNC lesions. However, all infected animals presented typical VM lesions in the lung. VM arthritis may show fibrosis as the main pathological change.
- In tissue, CD4 were the predominant lymphocyte subset in arthritic lesions at 210 dpi. In blood, CD8 were significantly increased only in 496 strain infected animals.
- More data will be achieved by the end of the experiment that is programed to be concluded by the end of 2013 .