Application of a novel immunohistochemical panel for the differential diagnosis of Canine Sterile Granulomas

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Introduction

Cutaneous Sterile Granuloma/Pyogranuloma Syndrome (SGPS) and **Reactive Histiocytosis (RH)** are two uncommon cutaneous disorders of dogs that may show similar clinical and histopathological features [1]. SGPS is characterized by an inflammatory infiltrate consisting mostly of macrophages intermingled with neutrophils, lymphocytes and plasma cells; RH is characterized by nodular to diffuse infiltration of dermal dendritic cells that accumulate in tumor-like configurations. In the last stage of RH, the increased number of macrophages can make a differential diagnosis with SGPS difficult [1].

Aim

Usually, immunohistochemistry (IHC) can help to differentiate the dendritic cells present in RH from the macrophages present in the SGPS. Unfortunately, for this test, commercially available antibodies require fresh-frozen tissue [2]. **In order to immunophenotype the inflammatory infiltrate characterizing SGPS and RH in Formalin-fixed paraffin-embedded biopsies, we propose the potential diagnostic use of primary antibodies directed against Ionized calcium-binding adapter molecule 1 (Iba1), a marker that has been demonstrated to be expressed by almost all subpopulations of cells of the monocyte/macrophage lineage and MAC387 described as recognizing blood-derived and infiltrating monocytes/macrophages [3] (figure 1).**

Materials and Methods

20 skin biopsies diagnosed as sterile granuloma and 10 canine cutaneous histiocytomas (CCH) were retrospectively collected. In order to further differentiate SGPS and RH, immunohistochemistry was performed using antibodies against **Iba1 (code 19-19741, WAKO), MAC387 (cod. M0747, DAKO),** proliferating histiocytic marker **CD45 (ab10558 ABCAM),** T-cell marker **CD3 (cod. A0452, DAKO)**, **B-cell marker CD20 (cod. ACR300D, BioOptica)** and **E-cadherin (clone NCH-38, DAKO)** for neoplastic histiocytic proliferation in CCH. A semi-quantitative score ranging from 1 to 4 was applied according to the percentage of positive cells.

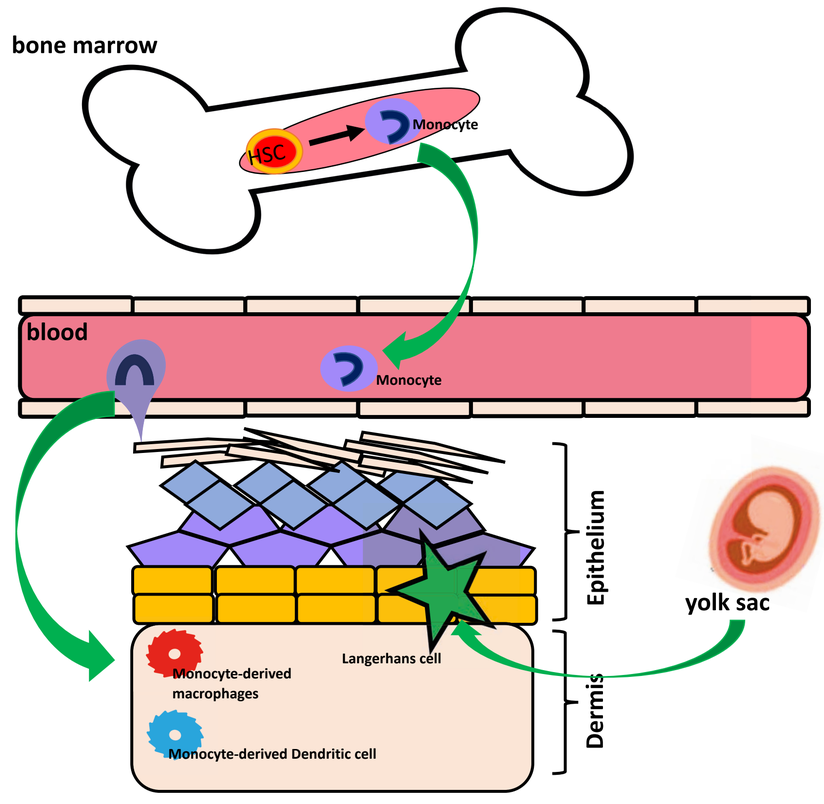

Fig. 1. Monocyte Differentiation into Dendritic Cells (DCs) and Tissue Macrophages. Monocytes are continuously generated in the bone marrow from haematopoietic stem cells (HSCs). Monocytes can give rise to monocyte-derived DCs (Iba1 +, CD45 +) or they can rapidly be recruited to sites of inflammation and sites of tissue remodelling, where they extravasate and give rise to monocyte-derived macrophages (Iba1+, MAC387 +). Langerhans cells (Iba1 +, MAC387-) reside in the epidermis and originate from yolk sac-derived progenitors.

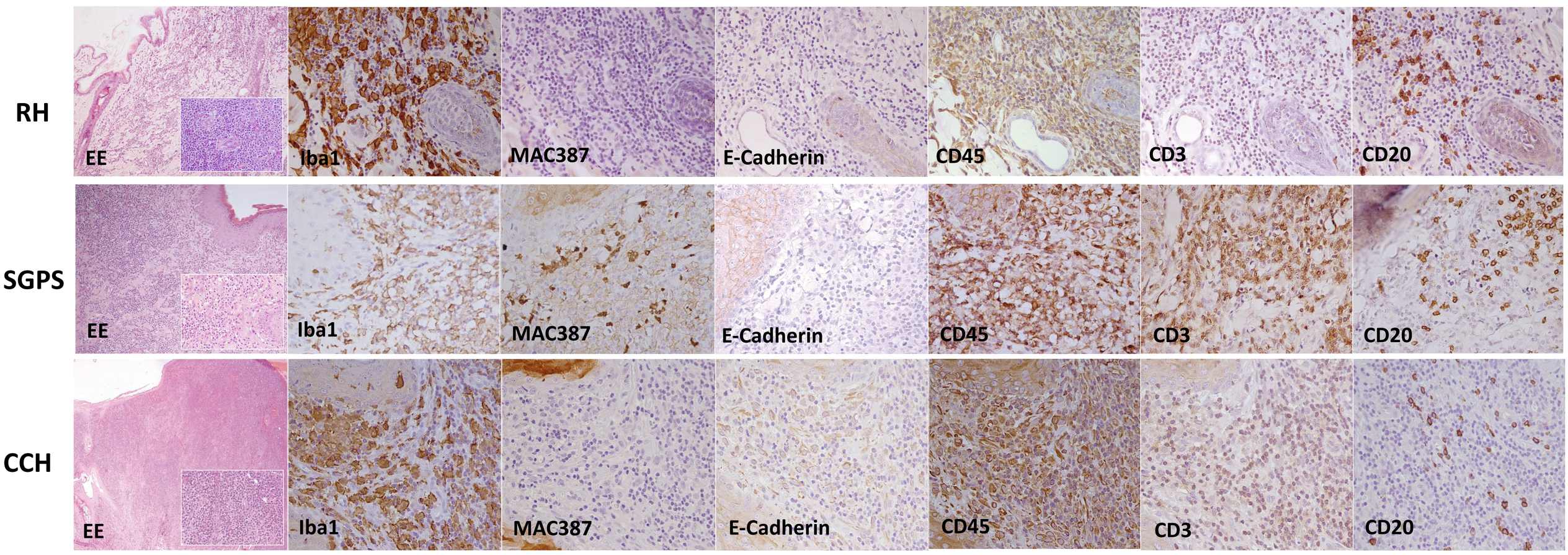

Fig. 2 Immunohistochemical panel for RH, SGPS and CCH, dog, haired skin. Iba1 + cells were consistently found in all cases. MAC387 + cells, consisting in macrophages and neutrophils, were non observed in RH and CCH, but they were consistently found in SGPS. E-cadherin was only expressed by neoplastic cells in CCH. The proliferating histiocytic cells expressing CD45 were found in all cases. CD3 and CD20 immunolabelling were moderate to strong in all cases.

Results and Conclusions

By immunohistochemistry, a final diagnosis of SGPS and RH was made for, respectively, 12 and 8 cases. Our preliminary results (figure 2) reveal that an immunohistochemical panel including Iba1 and MAC387 has the potential to be used in the immune-phenotyping of SGPS and RH allowing the differentiation between these conditions also for therapeutic purposes. A panel including Iba1 and MAC387 may confirm the origin of the inflammatory cells of interest, perchance followed by assessment of the expression of other specific histiocytic markers, such as E-cadherin and CD45 that can provide a more precise classification of histiocytic proliferative and neoplastic disorders of dogs.

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