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NEUTROPHILS AND MACROPHAGES IN CANINE MELANOCYTIC TUMORS

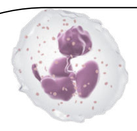
Ilaria Porcellato[1], Monica Sforna[1], Alice Musi[1], Ilaria Bossi[1], Serenella Silvestri[2], Luca Mechelli[1], Chiara Brachelente[1]

[1]*Dipartimento di Medicina Veterinaria, Università degli studi di Perugia.*
[2]*Dipartimento di Medicina e Chirurgia, Sezione di Ematologia clinica ed Immunologia, Università di Perugia*

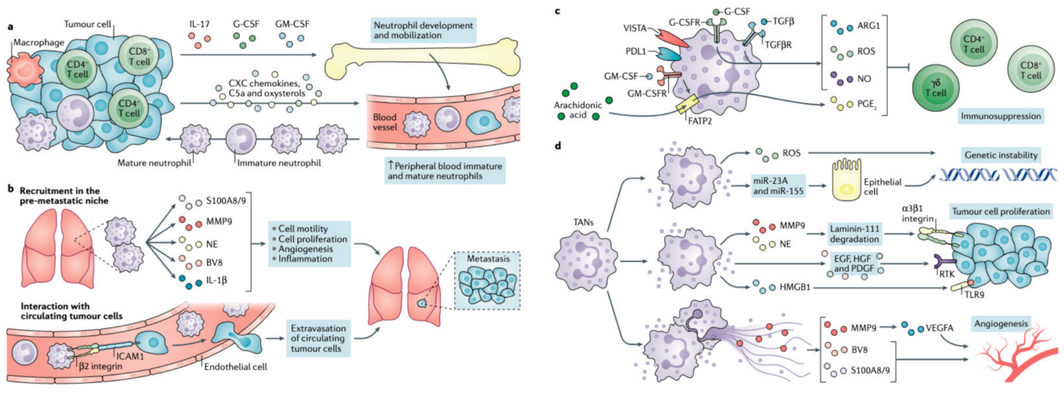
[+]ilariaporcellatodvm@gmail.com

Despite promising immunotherapy strategies in human melanoma, there are few studies on the **immune environment** of canine melanocytic tumors.[1] Many studies conducted on humans highlight the key role of **innate immunity,** in particular of **neutrophils and macrophages**, in the development, growth and prognosis of human malignant melanoma, through the release of pro- and/or anti-inflammatory cytokines, and tumour growth factors.[2,3] Numerous studies focused on the evaluation of the infiltration of lymphocytes and T cells,[4] also in dogs,[5] but few did focus on the role of other immune cells.

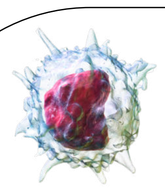
This study **aims** at the characterization of the **innate immune system** in canine melanocytic tumors, by the investigation, by means of immunohistochemistry, of tumor-associated macrophages (TAMs) and tumor-associated neutrophils (TANs); moreover, the role of these cellular populations in predicting the prognosis has been assessed.



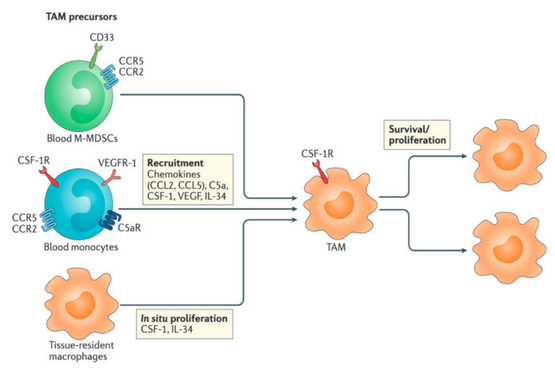
TANs (Tumor-associated neutrophils)

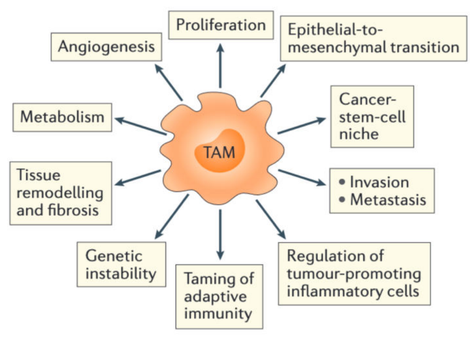


from Jaillon et al., 2020



TAMs (Tumor-associated macrophages)

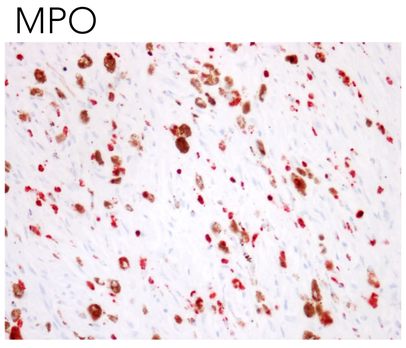




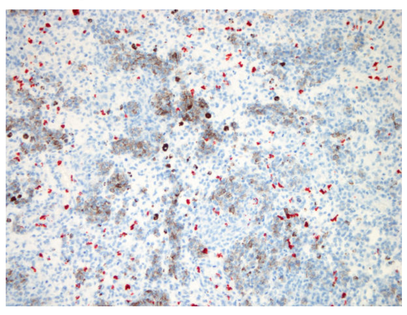
from Mantovani et al., 2017

Immunohistochemistry was performed on 65 cases of melanocytic tumors (24 oral melanomas, 26 cutaneous melanomas, 15 cutaneous melanocytomas), with antibodies for MPO, MAC387 (that recognizes both neutrophils and macrophages) and IBA1. Cells were counted by two operators in 5 randomly selected fields, avoiding areas of necrosis and near ulceration.

MPO

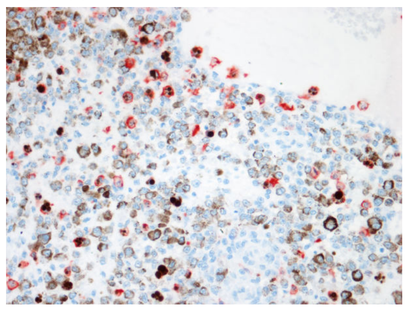


MAC387

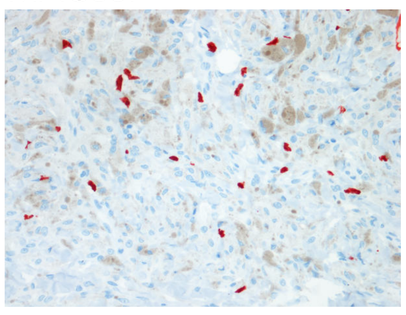


The results did not show correlation between the number of MPO+ and MAC387+ cells and death from melanoma, or with the death risk. However, a statistically significant correlation between the MPO+ cells and diagnosis (P<0,001), mitotic count (P<0,001), pigmentation (P=0,002), nuclear atypia (P=0,00245), and, though weakly, tumor thickness (P=0,044) and major diameter (P=0,042). Additionally, a statistically significant association was observed between the presence of neutrophils-MAC387+ and diagnosis (P=0,011) and pigmentation (P=0,003).

IBA1



MAC387



Results showed that both IBA1+ and MAC387+ macrophages were more numerous in oral melanomas, when compared to cutaneous melanomas and melanocytomas (P<0.001). Moreover, an increased number of IBA1^{+} cells was associated with macroscopic and histological negative prognostic factors, such as the major diameter (P<0.05), the tumor thickness (P<0.05), the mitotic count (P=0.001), and the nuclear atypia (P<0.05). These associations were not observed instead with the number of MAC387^{+}cells.

Conclusions

None of the markers tested did show significant association with the death for melanoma or with the risk of death for melanoma. However, the associations observed with different **histological features**, such as mitotic count and cellular atypia, commonly associated with a worse prognosis, seem to indicate a possible role for both TANs and TAMs. In particular, they could be involved in providing the tumor with an **immunosoppressive immune microenvironment**, therefore contributing to its progression, local invasion and metastasis. Further studies involving *in vitro* characterization of these cells and their products are currently running.

REFERENCES [1] Tarone L et al. Naturally occurring cancers in pet dogs as pre-clinical models for cancer immunotherapy. Cancer Immunol Immunother. 2019 Nov;68(11):1839-1853. [2] Mignogna C et al. Innate immunity in cutaneous melanoma. Clin Exp Dermatol. 2017 Apr;42(3):243-250. [3] Tucci M et al. Immune System Evasion as Hallmark of Melanoma Progression: The Role of Dendritic Cells. Front Oncol. 2019;9:1148. [4] Nguyen T et al., Multiplex Immunohistochemistry Analysis of Melanoma Tumor-Infiltrating Lymphocytes. Methods Mol Biol. 2021;2265:557-572. [5] Porcellato I et al. Tumour-infiltrating lymphocytes in canine melanocytic tumours: An investigation on the prognostic role of CD3+ and CD20+ lymphocytic populations. Vet Comp Oncol. 2020 Sep;18(3):370-380.