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Does a polarization state exist for mast cells in cancer?

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ABSTRACT

The data of literature are discordant about the role of mast cells in different types of neoplasms. In this paper the authors propose the hypothesis that tumor-associated mast cells may switch to different polarization states, conditioning the immunogenic capacities of the different neoplasms. Anti-inflammatory polarized mast cells should express cytokines such as interleukin-10 (IL-10) and then mast cells number should be inversely related to the intensity of inflammatory infiltrate. On the contrary, when mast cells do not express anti-inflammatory cytokines their number should be directly related to the intensity of the inflammatory infiltrate. In this paper we briefly argue around feasible approaches, based on the retrospective studies of tumor tissue samples from neoplasms considered "immunologically hot" and neoplasms considered "immunologically cold", through immunohistochemistry and immunofluorescence techniques (confocal microscopy). The establishment of the actual existence of a polarization interchange of mast cells, could lead to a new vision in prognostic terms, useful to contrive new approaches in immunotherapy of tumors.

Introduction

Mast Cells (MCs) are long-living immune cells, associated with chronic inflammation and allergic reactions, which contain large basophilic cytoplasmic granules, filled of pro-inflammatory substances. Mature MCs no longer circulate into the bloodstream. Nevertheless, mast cell progenitors migrate into tissues and differentiate into mature elements under the influence of cytokines and stem cell factor. MCs are present throughout the body and play important roles in the maintenance of some physiological functions as well as in the pathophysiology of many diseases, such as allergy, parasitic infection, and neoplastic disorders. Tissue MCs are classified into distinct subtypes based on their tissue of residence (mucosal, serosal, or brain subtype). Invasion or injury of tissue may cause activation of MCs leading to their degranulation and cytokines release. Many other molecules could be released, triggering and boosting inflammatory response or regulating other processes such as angiogenesis [1]. Although there is extensive knowledge about MCs physiology, there are conflicting data regarding their role in cancer [2,3]. In several malignancies MCs would seem to have a permissive role on tumor growth; in some others they seem to adopt a host protective behavior [4]. However, it has never been described, for MCs, any phenotypic polarization process analogous to that well known for macrophages.

Depending on the extent of the inflammatory infiltrate, the tumors have been defined as "immunologically cold" or "immunologically hot" [5] even if it must be kept in mind that also neoplasms of the same type can have different degrees of inflammation.

In recent years, many studies have been devoted to characterize the role of MCs in tumors. In some cases, it has been observed a tendency to a reduced number of MCs in malignant neoplasms, compared to benign counterparts, like in malignant melanoma (MM) and nevi respectively [6]. In MM the number of tumor associated macrophages (TAMs) appears to be associated with a worse prognosis, but TAMs are in a certain percentage polarized towards the M1 state [7]. Interestingly, the abundance of lymphocyte infiltrate is considered an important prognostic parameter in MM and the neoplasm is considered a typical "immunologically hot" tumor. This kind of reversal behavior between MCs and macrophages can also be observed in other neoplasms, in which it can denote a greater aggressiveness and propensity to relapse [8].

A major function attributed to MCs in malignant neoplasms along with macrophages is the pro-angiogenetic support to cancer [9]. However, this commission is probably carried out in non-biologically aggressive neoplasms, especially if they have a possible endothelial

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nature [10–12].

The role of MCs in tumor of the Central Nervous System such as gliomas is not well understood and both positive and negative relationships between MCs action and tumor progression have been reported [13]. MCs are enrolled in gliomas by the action of chemokines produced by cancer cells or macrophages [14,15]. However, while the number of macrophages can reach up to 70% of the total glioblastoma cell population [16], MCs can be identified only as scattered cells within the tumor tissue. Interestingly, the extent of lymphocyte infiltrate is a prognostic factor also in glioblastomas even if, most often, it is quite modest [17] and it is not regularly mentioned in histopathological diagnostic reports.

In summary, the relationship between the number of mast cells, macrophages and the extent of the inflammatory infiltrate in tumors is not well estimated so far. If mast cells are unable to adopt alternative polarization states in which they have a pro-inflammatory or a predominantly anti-inflammatory activity, the mast cells abundance should likely be always proportional to the extent of the inflammatory infiltrate present in the neoplasm. On the other hand, if MCs can turn in different polarization phenotypes the state of polarization could be regulated by both macrophages and tumor cells. and they could probably be recognized by the different type of cytokines released in the tumor tissue.

The hypothesis

In this paper it is hypothesized that two different and alternative polarization states are adoptable by MCs, similarly to what happens for macrophages. These two different polarized phenotypes will be called MC1 and MC2 and correspond respectively to an anti-inflammatory setting, in malignant “immunologically cold” neoplasms and to a pro-inflammatory setting, in “immunologically hot” neoplasms. It is acceptable that switching between the two alternative phenotypes is depending on a functional and numerical balance with tumor cells and with TAMs.

Evaluation of the hypothesis

Proposed methodologies

A reliable way to test this hypothesis, could be carrying out a series of immunohistochemical analyses on archival paraffin embedded tissues. Thus, it should be possible to characterize lymphocytes, macrophages and mast cells, according to the principles and methods previously described for light and fluorescence microscopy techniques [7,8,10,12,18].

Patients and specimens

A series of patients that underwent to surgical intervention for melanoma, colon carcinoma and glioblastoma, should be retrospectively evaluated; specimens from tumor resection should be histologically assessed and graded, according to the World Health Organization criteria, on formalin-fixed and paraffin-embedded tissue sections by three experienced pathologists. Deparaffinized tissue sections (4 μm thick) should be used for all different staining procedures employed in our study: hematoxylin and eosin, immunohistochemistry and immunofluorescence.

Immunohistochemistry

Tissue samples obtained from all cases should be immunohistochemically assayed for CD68 and CD163. The CD68 positive cells ($CD68^{+)}$ must be considered as M0, M1, and M2 macrophages and $CD163^{+}$ cells are recognized as M2 macrophages. MCs will be recognized as cells immunohistochemically stained for c-kit ($CD117^{+}$).

A CD3 and CD8 staining for T-lymphocyte can provide a method for evaluation of the inflammatory infiltrate, counting number of cells for mm^2.

A direct cell count will be carried out by using the cell count function in Image J 1.42 [19] software, on five fields per area, for all the cases involved in this study. Each field consisted of a photo obtained at 400× magnification.

Immunofluorescence and confocal analysis

Confocal microscopy can allow to study the distribution pattern of MC1/MC2 mast cells evaluating, by the double staining methods, both $CD117^{+}$/IL-10^{+} and $CD117^{+}$/IL-10^{-} combined phenotypes.

Statistical analysis

The Spearman's rank correlation analysis will be employed in order to test the strength and direction of a relationship between: (i) the numerosity of MCs and T lymphocytes; (ii) between MC1 (IL-10^{+}) or MC2 (IL-10^{-}) and T lymphocytes; (iii) between MCs and macrophages; (iv) between MC1 (IL-10^{+}) or MC2 (IL-10^{-}) and $CD68^{+}$ or $CD163^{+}$ macrophages. These analyses will be performed in all selected neoplasms.

A p value lower than 0.05 will be chosen for statistical significance. Experimental data will be analyzed by SPSS Version 20.0 software (IBM Italia).

Data coming from confocal microscopy analyses should be compared to those obtained by immunohistochemistry, in light microscopy. In particular, in the same cases, the number of CD 68^{+}, $CD163^{+}$ and $CD117^{+}$ cells should be analyzed.

Possible results

Here we hypothesize some probable scenario rising from immunohistochemical analysis carried out by using the specific antibodies against staining with CD117 and CD3:

1. A direct correlation, intended as concordance of cell numerosity, between T lymphocytes ($CD3^{+}$) and MCs ($CD117^{+}$) could be found (Fig. 1). This can occur in two different modalities: a relative high number of both T lymphocytes and MCs cells is highlighted (Fig. 1A) or a relative low number for both populations is found (Fig. 1B).
2. An inverse correlation between infiltrated MCs and T lymphocytes could be found; as the population of T lymphocytes increases in neoplastic tissue, the MCs presence declines and *vice versa*.
3. Any correlation could be found between lymphocytes and MCs numerosity with the amplitude of each population being independent from the other one (Fig. 2).

Double staining immunofluorescence combined with confocal microscopy analysis on neoplasm tissue sections, stratified for “dense” or “poor” lymphocyte infiltrate, may reveal a significant difference in $CD117^{+}$/IL-10^{-} (MC1) and $CD117^{+}$/IL-10^{+} (MC2) distribution (Fig. 2) with a greater presence of $CD117^{+}$/IL-10^{-} (MC1) elements in lymphocytes-rich neoplasms (Fig. 2A) or an increased presence of $CD117^{+}$/IL-10^{+} (MC2) elements in neoplasms with poor lymphocyte infiltrate (Fig. 2B).

To highlight the relationship between infiltrated macrophages and MCs, by using specific antibodies directed against CD68 and CD163 and again anti-CD117, similarly to the first analysis, one can expect to see:

1. a direct correlation between the MC1 (IL-10^{+}) or MC2 (IL-10^{-}) number and the number of $CD68^{+}$ or $CD163^{+}$ macrophages;
2. that any correlation can be recognized between the number of MC1 (IL-10^{+}) or MC2 (IL-10^{-}) and the $CD68^{+}$ or $CD163^{+}$ macrophages numerosity.

It should be borne in mind that all the hypothetical correlations described above, could be observed in all selected neoplasms or could specifically be tied to a specific form of cancer.

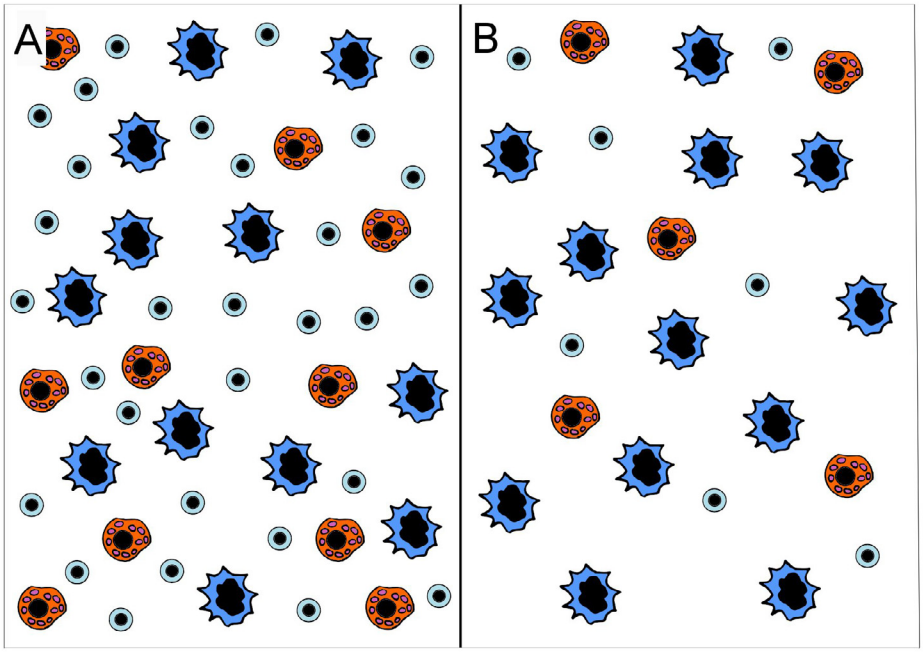

Fig. 1. In immunohistochemistry for optical microscopy the analysis can show a direct, inverse or no correlation between the number of MCs and inflammatory infiltrate. In case of direct correlation, the number of MCs is directly proportional to the extent of inflammatory infiltrate. MCs: Orange Cytoplasm; Tumor Cells: irregular nuclei and blue cytoplasm; Lymphocytes: little round cells. Left panel (A): a high number of MCs corresponds to many lymphocytes. Right panel (B): a low number of MCs corresponds to few Lymphocytes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Consequences of the hypothesis

MCs can have a pro-inflammatory or an anti-inflammatory activity [20,21], but a polarization state, defined as a featured and alternative phenotype, is not currently recognized. A hypothetical anti-inflammatory phenotype for mast cell, indicated by "MC2" state, should be characterized by the production of IL-10; interleukin-10 notably inhibits Th1 cells activation and contribute to push on macrophages towards an M2 polarization state. Interleukin-37 is known to inhibit the production of inflammatory mediators from M1 macrophages.

Findings coming from the proposed analyses may give us indications about the behavior of infiltrating tumors mast cells; for example, one of this could be the evidence that is only one homogeneous population of MCs does exist and infiltrates a given type of neoplasm. All the $CD117^+$ cells could be immunoreactive for IL-10 contributing to an anti-inflammatory atmosphere. Alternatively, no mast-cell could be immunostained for IL-10 and their number would be related to inflammatory infiltrate or associated to a particular type of macrophage polarization.

On the other hands, in "immunologically cold" tumors, we could find $CD117^+/IL\text{-}10^+$MCs that would outnumber $CD117^+/IL\text{-}10^-$. In turn, in "immunologically hot" tumors $CD117^+/IL\text{-}10^-$ MCs would outnumber $CD117^+/IL\text{-}10^+$. Such findings might be related to inflammatory infiltrate or to a specific type of macrophage polarization.

In conclusion throughout these experiments we would be able to collect evidences that:

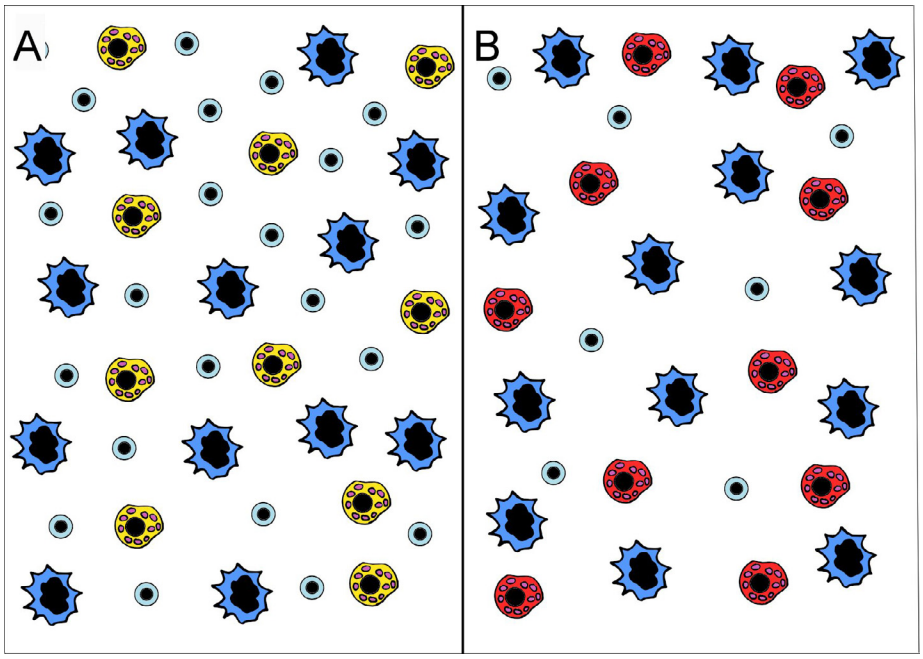

Fig. 2. If a polarization state of MCs does exist, immunofluorescence combined with confocal microscopy will demonstrate that density of inflammatory infiltrate correlate with the polarization state of MCs rather than their number. Pro-inflammatory ($CD117^+/IL\text{-}10^-$) polarized MCs (yellow cytoplasm) and their hypothetical effect is shown on the left panel (A) and anti-inflammatory ($CD117^+/IL\text{-}10^+$) polarized MCs (red cytoplasm) and their hypothetical effect is shown on the right side (B). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

1. a polarization state for MCs does exist and is in correlation with the polarization state of macrophages. It is also able to influence the grade of inflammatory infiltrate of tumors;
2. Alternatively, the inflammatory infiltration of tumors and the macrophage polarization state is only correlated to the MCs number.

This study could have a translational fallout, because it may lay the foundation for recognizing mast cells in their setting of "tumor helpers". This concept could lead to develop new tailored therapies able to inflect inflammatory components of neoplasms, mainly in "immunologically cold" neoplasms such as glioblastoma, whose therapy is largely unsatisfactory [22,23].

Declaration of Competing Interest

All the authors, Ivan Presta, Annalidia Donato, Paolo Zaffino, Maria Francesca Spadea, Teresa Mancuso, Natalia Malara, Eusebio Chiefari and Giuseppe Donato, declare that they do not entertain no relationships with financial entities or people that could inappropriately influence the develop of the present work.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2019.109281.

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