TCD4+ tissue-specific lymphocyte profile in homeostasis and during immune response in an experimental model of metastatic breast cancer.

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Introduction

Tumor metastasis is the leading cause of death in breast cancer patients. Each organ has its specific parenchyma, stroma and vasculature as potential targets for metastatic colonization, but they must undergo changes in order to be a favorable environment for tumor growth. It has already been shown that fibroblasts from the metastatic microenvironment are able to contribute to the establishment of circulating tumor cells in this new tissue. The modification of future colonization tissue seems to be so important that tumor microvesicles can instruct bone marrow cells to assist the tumor vessel formation process, promoting the establishment of pulmonary metastasis. In this sense, we believe that immune system cells that infiltrate the new metastatic niche can help or prevent tumor colonization. Thus, the aim of this work is to assess whether different profiles of CD4$^+$ T lymphocytes are related to site-specific metastasis during tumor progression at the target sites of metastasis in for 4T1 murine breast tumor model.

Casuistry and Methods

In this work we used the syngeneic murine breast tumor model, 4T1. By injecting the 4T1 tumor cell line into the mouse breast, we can follow the development of the primary tumor as well as the establishment of metastases that spread spontaneously to distant sites, such as lymph nodes, spleen, lung, liver and bone, as well as in women with more aggressive breast cancer. In addition to being similar to what happens physiologically in more aggressive breast cancer cases, we have an immunocompetent animal that allows us to evaluate the immune system, which is our interest.

Using this model, we analyzed the cytokine profile of resident and circulating CD4$^+$ T lymphocytes from each metastasis target organ by flow cytometry. To observe if there are changes in the production of cytokines in each organ by CD4 T cells during tumor progression, we used the control group (no tumor), the D17 group (animals with 17 days of tumor inoculum, with metastasis in the draining lymph node of the tumor, spleen and lung) and group D23 (animals with 23 days of tumor inoculum, with metastasis to all organs evaluated) (Figure 1).

Results

Using the 4T1 highly metastatic breast tumor model, we evaluated the profile of cytokines produced by CD4$^+$ T cells at two distinct times in tumor progression, distinguishing lymphocytes that reside in the tissue from those that only circulate. The heatmap shows that we were able to identify the organs along the tumor progression, suggesting that the resident lymphocyte profile of each organ is a good predictor of organ-specific metastasis, since the change in the cytokine profile occurred prior to tumor colonization (Figure 2).

However, the CD4$^+$ T lymphocytes circulating in each organ also formed an organ-specific signature, which is different from the peripheral blood signature, denoting a possible modification of cellular phenotype as it enters the tissue. After 17 days of tumor inoculum, when there is still no metastasis in all organs, we observed that there is still an organ-specific signature, but all organs have a similar cytokine profile, including peripheral blood, suggesting that at this time of tumor progression, changes occur in a systemic way (Figure 3).

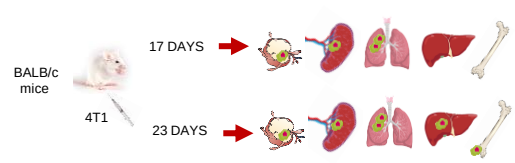

Figure 1: Mouse 4T1 breast tumor model and dissemination to target organs of metastasis.

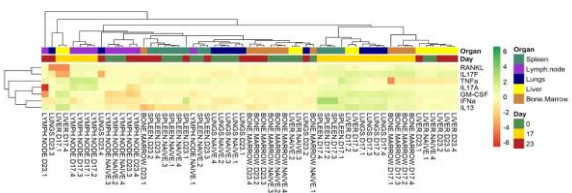

Figure 2: Cytokine profile of **resident** CD4 T cells. Cluster analysis demonstrates difference in CD4$^+$ T cell profiles according to microenvironment in control animals and at 17 and 23 days of tumor inoculum.

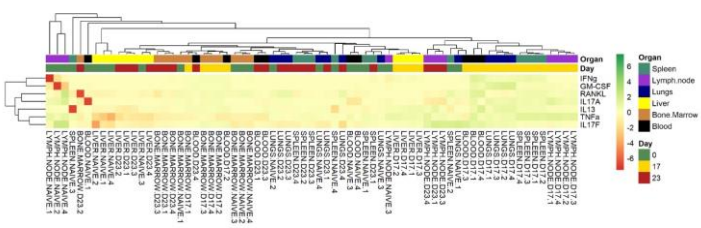

Figure 3: Cytokine profile of **circulant** CD4 T cells. Cluster analysis demonstrates differences in CD4$^+$ T cell profiles according to the microenvironment in control animals and at 17 and 23 days of tumor inoculum.

Conclusions

Our data show that from the cytokines produced by CD4$^+$ T cells can define a specific signature to predict the establishment of metastasis organ specific. Furthermore, even if there are changes in each organ, apparently there is a moment of tumor progression when CD4$^+$ T lymphocytes change their phenotype in a systemic way, suggesting that the control of the antitumor or pro-tumor response occurs in the spleen.

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