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A novel preclinical murine model of immune-mediated metastatic dormancy

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The mechanisms underlying cancer dormancy are poorly understood. We have developed a preclinical murine model in which immunosurveillance restrains spontaneous metastases in permanent dormancy. The model faithfully recapitulates human metastatic dormancy and may be useful to decipher the immune mechanisms constraining disease progression, thereby facilitating the development of novel immunotherapeutic approaches to control metastatic disease.

Cancer dormancy during tumor progression is a widely described phenomenon. An apparently successful therapeutic intervention can be followed by a prolonged disease-free period of years or even decades before tumor relapse or metastasis, with disseminated metastatic cells remaining in latency.[1] It has been proposed that angiogenesis, cellular quiescence (proliferation counterbalanced by apoptosis), microenvironment interaction, and immunosurveillance may participate in the control of dormant cancer cells.[1] However, the precise mechanisms underlying the maintenance of the dormant state and the subsequent expansion of cancer cells are poorly understood. This is largely due to the difficult clinical detection and isolation of dormant micrometastases and the lack of preclinical animal models faithfully reproducing metastatic dormancy.

The role of immunosurveillance in cancer dormancy is supported by evidence from numerous clinical and preclinical animal studies.[1] Thus, an increased tumor incidence has been observed in transplant hosts undergoing immunosuppressive treatments, and there have been reports of undetected tumors from donors with no history of cancer disease that awake after transplantation in an immunosuppressed recipient. In a mouse model of chemical induced carcinogenesis, depletion of CD4+ and CD8+ T cells was found to be sufficient to disrupt the equilibrium between primary tumor cells and the adaptive immune response.[2] Our group recently developed a murine model of immune-mediated dormant spontaneous metastases.[3] We had previously induced a fibrosarcoma in BALB/c mice with methylcholanthrene, excising the primary tumor followed by disaggregation and tissue culture adaptation to obtain the GR9 tumor cell line. Various fibrosarcoma clonal cell lines were established from GR9 tumor cells with a wide range of MHC class I (MHC-I) phenotypes, ranging from highly positive to completely negative. Clones with greater MHC-I positivity had a reduced local growth rate and increased spontaneous metastatic capacity, whereas those with greater MHC-I negativity had an increased local growth rate and very low or no spontaneous metastatic capacity.[4] Only one clone, GR9-B11, showed no spontaneous metastatic capacity, whereas all other clones producing overt spontaneous lung metastases. The GR9-B11 tumor-bearing mice remained metastasis-free after removal of the primary local tumor (**Fig. 1**). Interestingly, GR9-B11 tumor cells do not express MHC-I surface expression but their primary tumors are MHC-I positive, with the expression of at least two MHC class I molecules (H-2 K and D).[3]

We initially attributed the absence of spontaneous metastases in the mice to an inability of the GR9-B11 tumor cells to migrate to and invade other tissues. However, a new possibility was suggested by observations of the abolition of spontaneous metastatic capacity by immunotherapy in mice injected with GR9-A7, a highly metastatic clone.[5] Given this finding of an important role for the immune response in controlling metastatic dissemination in this model, we postulated that the immune system might control and/or destroy disseminated GR9-B11 tumor cells, preventing metastatic progression. We performed spontaneous metastasis assays to test these possible mechanisms, injecting GR9-B11 tumor cells into immunodeficient mice. In the assays in nude BALB/c mice, 80% of the hosts developed overt spontaneous pulmonary metastases.[3] This striking finding suggested that the injection of GR9-B11 tumor cells in immunocompetent hosts promotes an antitumor immune response responsible for controlling metastatic dissemination. Indeed, analysis of systemic and local immune populations in these mice at 25 or 50 d after primary tumor removal, revealed increases in T lymphocytes, including CD4+ and CD8+ lymphocytes,

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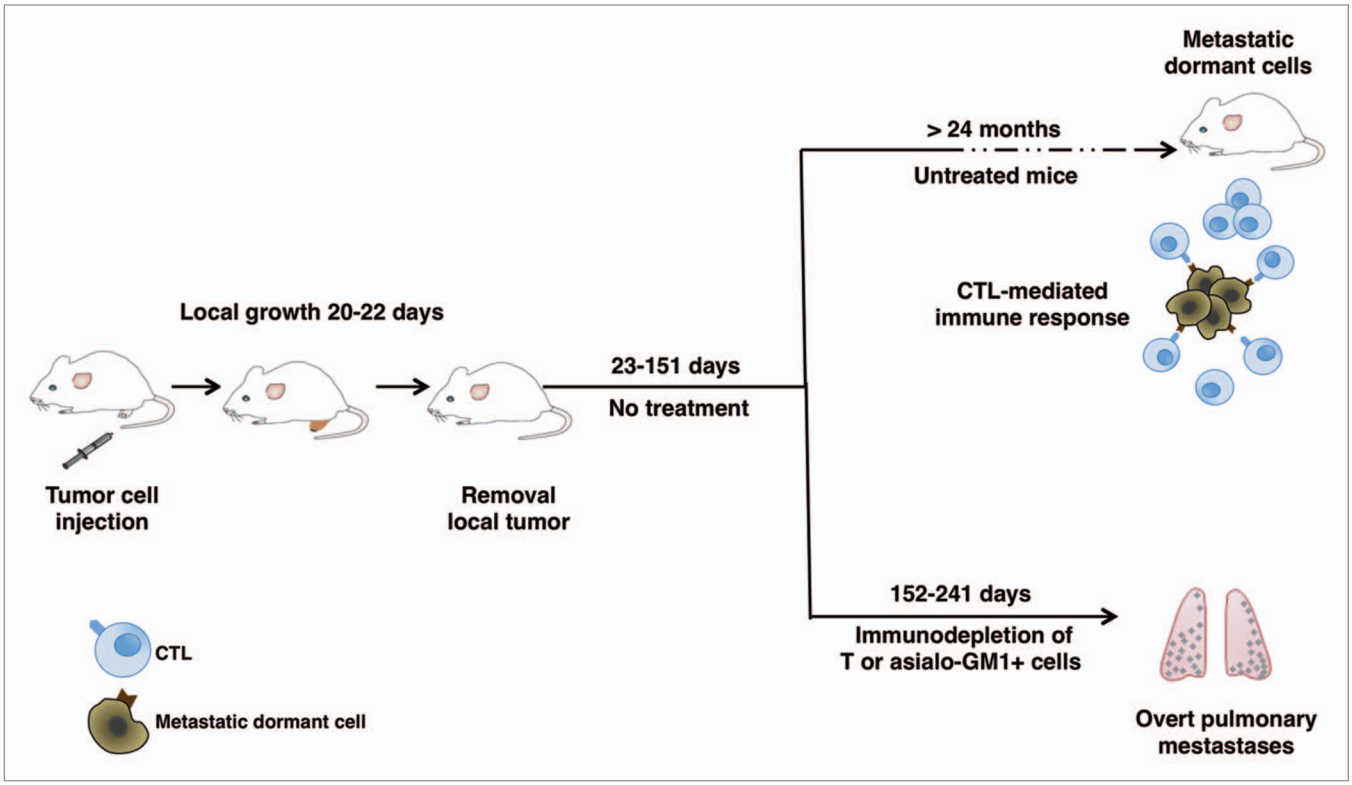

Figure 1. Immunosurveillance stimulated by GR9-B11 fibrosarcoma cells restrains spontaneous metastases in permanent dormancy. Cytotoxic T lymphocytes (CTLs) are directly implicated in this phenomenon. Depletion of T lymphocytes or asialo-GM1+ cells via antibody-based immunodepletion promotes the "awakening" of overt pulmonary metastases from disseminated metastatic cells.

and in dendritic cells (DCs) and macrophages.[3] The next issue to be addressed was whether the disseminated GR9-B11 tumor cells were eliminated or only controlled by the immune system, remaining in a dormant state. For this purpose, GR9-B11 tumor-bearing mice were left for five months after tumor removal with no processing or treatment and were then depleted of different immune cell populations (**Fig. 1**). The mice were euthanized and examined at 3 mo after this depletion, finding overt spontaneous lung metastases in 100% of mice depleted of CD8+T lymphocytes, 87% of those depleted of asialo-GM1+ cells, and 23% of those depleted of CD4+T lymphocytes.[3] These results demonstrated that T lymphocytes and asialo-GM1+ cells restrained GR9-B11 disseminated metastatic cells in a dormant state. It was previously reported that NK (asialo-GM1+) cells can modulate an antitumor protective cytotoxic T lymphocyte response.[6] It can be hypothesized that MHC-I negative GR9-B11 cells rapidly activate NK cells in vivo, priming local DCs toward an IL-12-producing DC phenotype, which stimulates a strong protective CD8-T cell response against MHC-I-positive metastatic cells. The DC population was increased in GR9-B11-injected mice, and the 'awakened' metastatic colonies showed MHC-I positive surface expression. Hence, the microenvironment enhanced MHC-I expression on GR9-B11 metastatic cells. In this context, the study of chemoresistance in patients with hormone-refractory prostate cancer (HRPC) showed that MHC-I-negative and -positive cells may coexist in a tumor in dynamic equilibrium in response to signals from the microenvironment.[7] The MHC-I-negative cells exhibited resistance to chemotherapy, and their number correlated with the stage of the disease and its recurrence. In the same line, relapse in patients after adoptive cell transfer therapies (ACTs) has been related to the reversible downregulation of antigen expression on tumor cells.[8] Our group previously reported that MHC-I molecules may act directly as tumor suppressor genes, exercising control over cellular proliferation.[9] Furthermore, the surface expression of MHC-I molecules is positively controlled by the tumor suppressor gene Fhit and directly correlates with the expression of another suppressor gene, p21/WAF1.[9,10] It can therefore be hypothesized that the recovery of MHC-I surface expression may promote the dormant state in disseminated metastatic cells through immune and oncogenic-suppression mechanisms. Additional studies are needed to fully elucidate the role of MHC-I surface expression in the control of cancer dormancy.

We emphasize that non-transgenic immunocompetent mice were used in these assays, and no immunotherapeutic treatment was administered. Furthermore, the behavior of the cancer in our experimental model faithfully reproduces the progression of human cancers in cases of a long dormant period of metastases-free survival after local tumor removal. This model system may contribute to the study of mechanisms underlying immune-mediated metastatic dormancy and may provide useful insights for developing immunotherapeutic strategies to control metastatic disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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