Numerous reports have demonstrated that some conventional chemotherapeutic agents, which have originally been developed to target malignant cells, may enhance the infiltration of neoplastic lesions by immune cells, a process that may have impact on disease outcome. The mechanisms by which chemotherapeutics can influence the host immune system so to contribute to the elicitation of functional antitumor immune responses are subject of intense investigation. Experiments based on transplantation tumor models have revealed that anthracyclines as well as other DNA-damaging agents can trigger the immunogenic demise of malignant cells. This occurs through a series of molecular events that foster the presentation of tumor-associated antigens and hence elicit T-cell-dependent, interferon-γ-based antitumor immune response, thus substantially contributing to the efficacy of chemotherapy. One of the drawbacks of transplantation tumor models for the study of tumor-specific immunity elicited by chemotherapy is their rapid and aggressive growth after transplantation. In this setting, malignant cells bypass the early steps of oncogenesis as well as the progressive establishment of an immunosuppressive microenvironment through long-term tumor-host interactions, as it occurs in the development of the majority of autochtonous neoplasms in humans and mice. A well-studied model for tolerizing spontaneous carcinogenesis is provided by mouse mammary tumor virus (MMTV)/neu transgenic mice. In these animals, the rat variant of v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2 (Erbb2, also known as neu) is specifically overexpressed in mammary epithelial cells, leading to the development of palpable tumors with a latency of about 6 mo. Through their lifespan, MMTV-neu mice become tolerant to Erbb2, as demonstrated by their inability to mount a productive CD8⁺ T-cell response against cancer cells despite the presence of Erbb2-specific CD8⁺ T lymphocytes. CD4⁺CD25⁺ regulatory T cells (Tregs) have been implicated in the suppression of Erbb2-specific T-cell clones. Whether tumor-associated macrophages (TAMs), the major population of immune cells infiltrating spontaneous, fully-established Erbb2-driven tumors under steady-state conditions, are also involved in the maintenance of this state of tolerance/immunosuppression remains to be investigated. Of note, the neoplastic lesions developing in MMTV-neu mice are scarcely infiltrated with CD8⁺ T cells and are devoid of myeloid-derived suppressor cells (MDSCs).

We investigated the impact of two chemotherapeutic agents that are frequently employed for the treatment of breast cancer, namely, the anthracycline doxorubicin and the ERBB1/ERBB2 dual kinase inhibitor lapatinib, on the...
infiltration of Erbb2-driven tumors by immune cells. Both these drugs lead to an increase of the number of activated IFNα-secreting CD4+ and CD8+ T cells within neoplastic lesions (Fig. 1). Moreover, the antineoplastic effects achieved with the combined administration of doxorubicin and lapatinib were significantly impaired upon the antibody-mediated depletion of CD8+ T cells, demonstrating a crucial role for this population in the overall outcome of therapy. By contrast, the depletion of CD4+ T cells augmented the anticancer efficacy of doxorubicin plus lapatinib, via a hitherto unknown mechanism. This finding is consistent with the idea that anthracyclines can induce a therapeutically relevant CD8+ T-cell response in spontaneous tumor models. These findings limit the conclusions of a previous publication based on similar tumor models, in which the doxorubicin response was found to be independent of adaptive immunity. These conflicting results may (at least in part) be explained by the differences in the dose and schedule of chemotherapy. Indeed, Ciampricotti et al. initiated therapy at a more advanced stage of tumor progression than us and, contrarily to what we did, they administered doxorubicin on a repetitive schedule, with possible detrimental effects on T-cell abundance and function.

An important question is how these 2 unrelated chemotherapeutic agents can break immunological tolerance and promote the activation of tumor-specific T cells in the MMTV-neu model. One possibility is that these agents are not only efficient at inducing immunogenic cell death in the neoplastic epithelium, but also deplete or disturb the function of immunosuppressive Tregs and TAMs. This attractive hypothesis remains to be investigated. Further insights into the mechanism whereby T cells are activated and recruited to the tumor and how this contributes to the efficacy of chemotherapy was provided by the study of MMTV-neu mice lacking signal transduction and activator of transcription 1 (STAT1). Strikingly, the growth of Erbb2-driven Stat1−/− tumors was insensitive to doxorubicin and lapatinib. Furthermore, these mice exhibited impaired T-cell activation and a reduced chemotherapeutically-elicited tumor infiltration as compared with their STAT1-proficient counterparts. The inefficient T-cell priming observed in Stat1−/− can be attributed to the cell-intrinsic role of STAT1 in T-cell expansion and maturation. As for the impaired tumor infiltration characterizing these animals, we propose a mechanism relying on the presence of STAT1 in the neoplastic epithelium. Indeed, we demonstrated that
STAT1-deficient tumor cells fail to express chemokine (C-X-C motif) receptor 3 (CXCR3) ligands that operate as T-cell attractants, i.e., chemokine (C-X-C motif) ligand (CXCL)9, CXCL10, and CXCL11, in response to IFN-γ, thus exhibiting a reduced ability to recruit primed CD8+ T cells (Fig. 1).

Taken together, our findings point to the vital contribution of CD8+ T-cell mediated immune response to the efficacy of chemotherapy in spontaneous models of tumorigenesis. As these results were obtained in models that closely mimic tolerizing human malignancies, they may provide further insights into the mechanism of action of conventional chemotherapeutics and open a possibility for combining standard anticancer interventions with immunotherapy.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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