

Tumor-Infiltrating T Cells — Friend or Foe of Neoplastic Cells?

Giorgio Parmiani, M.D.

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During the past 20 years, there have been many reports of the presence of inflammatory and immune-cell infiltrates in various neoplasms, including colorectal cancer. Initially, these findings were thought to indicate that although the immune system can recognize autologous tumor cells, it mounts an ineffective response to them. In the 1990s, functional studies demonstrated the presence of HLA-restricted T cells specific for colorectal cancer in populations of tumor-infiltrating lymphocytes and peripheral-blood mononuclear cells from patients with colorectal cancer. Since there were negligible numbers of such T cells in the blood of control subjects, the findings indicated that some of the patients' T cells could target and potentially kill colorectal-cancer cells.

Later, a more detailed analysis of the cells that infiltrate tumors was made possible by the availability of new techniques (e.g., polymerase chain reaction [PCR]) and a large array of monoclonal antibodies useful in immunohistochemical analyses of tumors. These methods showed that the composition of the tumor infiltrate is crucial. For example, early in the evolution of the tumor, an inflammatory response, probably caused by changes in normal cells in the microenvironment of the tumor, mobilizes the innate immune system. This effect can favor the dissemination of tumor cells through a complex interaction in which stromal cells surrounding the tumor release inflammatory cyto-

kines (e.g., tumor necrosis factor α) that enhance tumor growth. During the past three years, new data on the relation between inflammation and cancer suggest that specific genetic alterations of tumor cells — the activation of RAS in colorectal-cancer cells, for example — lead to the release of interleukin-8. This cytokine, in turn, promotes inflammation, angiogenesis, and tumor growth.

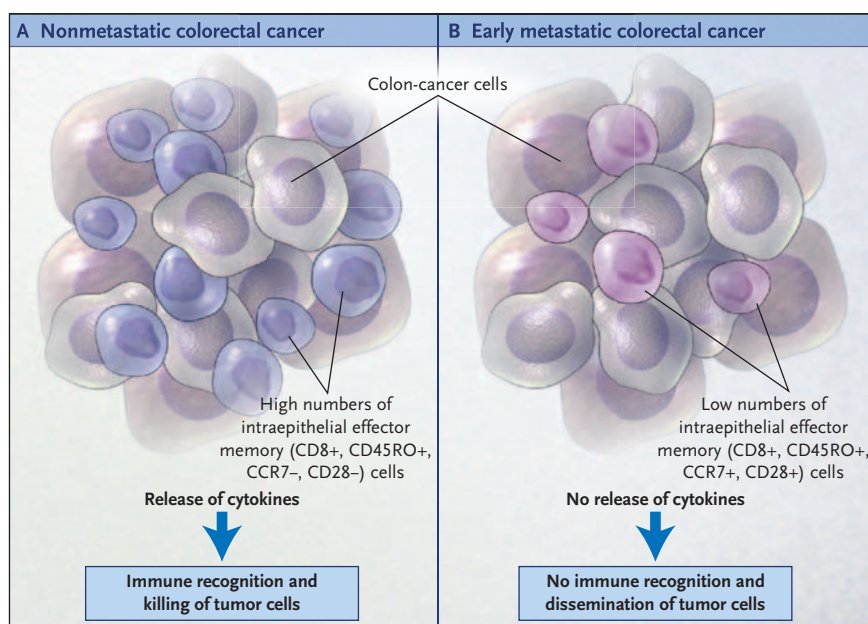
A close look at the literature suggests that tumor-infiltrating lymphocytes in primary melanoma are quite different from those in metastases. Like the primary tumor, the metastases contain tumor-specific cytotoxic T cells, but in metastatic melanoma, the final step of the killing mechanism of these cells is disabled. The inhibition of the cytotoxic machinery of these CD8+ T cells may be due to immunosuppressive cytokines released by tumor cells or to the presence of CD4+CD25+ regulatory T cells, which can suppress the immune response. With respect to colorectal cancer, the presence of CD8+ T cells within nests of tumor cells, and not in the stroma, has been reported to portend a favorable outcome (see diagram). It is likely, however, that what these earlier researchers were seeing in sections of colorectal-cancer tissue did not accurately reflect what was really occurring in vivo.

Only recently — thanks to improved knowledge of the phenotype and function of different subpopulations of CD8+ T cells (central and effector memory CD8+ T cells) and the use of reverse transcrip-

tase-PCR, complementary DNA microarrays, and immunohistochemical techniques — have we begun to understand the immune response to tumors with clarity (see diagram). We now have convincing evidence of an association between a favorable prognosis and the infiltration of melanoma, ovarian cancer, and non-Hodgkin's lymphoma by effector memory CD8+ T cells that can release cytotoxic cytokines.

In this issue of the *Journal*, Pagès et al. (pages 2654–2666) report similar findings for colorectal cancer. In addition, their search for microscopical evidence of early signs of invasiveness and metastasis in tumors led them to conclude that the infiltrate of effector memory CD8+ T cells in the tumor prevents these initial steps in the development of metastatic disease (see diagram). The data provide strong but indirect evidence of immune-mediated control of the growth of colorectal cancer. What we lack are functional studies showing an ongoing, tumor-specific immune response mediated by T cells in untreated patients with colorectal cancer. The presence of antibodies against P53, RAS, or survivin, reported in some patients with colorectal cancer, is usually considered a marker of the presence of tumor cells, rather than of a protective antitumor response.

One of the main drawbacks in the development of effective immunotherapy for colorectal cancer is the lack of an adequate number of molecularly defined tumor



The Immune Response in Colorectal Cancer.

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antigens that could be used in clinical studies. Whereas more than 100 antigenic molecules have been identified in melanoma (though less than 20 have been used as vaccines in clinical trials), far fewer colorectal-cancer antigens have been characterized. They include peptides in normal proteins (e.g., carcinoembryonic antigen) and epitopes in the protein products of mutated oncogenes or tumor-suppressor genes, such as *K-RAS* and *P53*. Pagès et al. could not study the colorectal-cancer antigens that the infiltrating memory T cells recognized to determine whether one or more of these antigens could be the target of tumor-infiltrating lymphocytes.

The findings of Pagès et al. and others working along similar lines can be used in two ways to design clinical studies of immunotherapy: first, tumor antigens

can be used as vaccines in patients with early disease to amplify ongoing recognition and, possibly, killing of cancer cells by the immune system and, second, a variation of adoptive immunotherapy can be used that entails the administration of partially purified populations of anti-colorectal-cancer T cells. In the latter case, tumor-infiltrating lymphocytes could be isolated from early-stage cancers and expanded in vitro. These expanded lines could serve as a source of T cells after in vitro stimulation with autologous tumor cells, lysates of such cells, or molecularly defined colorectal-cancer antigens. The population of tumor-infiltrating lymphocytes from early-stage cancers should be naturally enriched for anti-colorectal-cancer T cells that have greater anti-tumor activity than lymphocytes that infiltrate metastatic lesions.

The use of interleukin-15 and interleukin-21, both recently shown to expand populations of effector memory CD8+ cytotoxic T cells in vitro and in vivo, could augment the effectiveness of vaccination and adoptive immunotherapy.

Up to now, most studies of vaccination or adoptive immunotherapy have been performed in patients with advanced colorectal cancer. The few exceptions include trials involving patients made disease-free by surgery and then vaccinated with a mixture of autologous tumor cells with bacille Calmette-Guérin or heat-shock proteins derived from the autologous tumor. The results of some of these trials suggest a better outcome among vaccinated patients whose primary tumor contained CD8+ tumor-infiltrating lymphocytes and whose peripheral-blood T cells were able to mount a colorectal-cancer-specific response in vitro.

The clinical response to immunotherapy for metastatic colorectal cancer remains unsatisfactory, especially as compared with results that can be obtained with new agents such as bevacizumab, alone or combined with chemotherapy. However, the notion that tumor-specific cytotoxic T cells control tumor growth by clearing early micrometastases, if further substantiated, may help in the design of new trials of immunotherapy in patients with early disease or patients made disease-free by surgery.

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Dr. Parmiani is the chairman of the Unit of Immunotherapy of Human Tumors, Istituto Nazionale Tumori, Milan.