OncoImmunology 1:1, 97-99; January/February 2012; © 2012 Landes Bioscience

Immunochemotherapy against colon cancer by gene transfer of interleukin-12 in combination with oxaliplatin

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Key words: interleukin-12, oxaliplatin, colorectal cancer, liver, adenovirus

Abbreviations: 5-FU, 5-fluorouracil; Gem, gemcitabine; HC-Ad, high-capacity adenovirus; IL-12, interleukin-12; Iri, irinotecan; MDSC, myeloid-derived suppressor cells; Mif, mifepristone; NK, natural killer; OXP, oxaliplatin; Treg, regulatory T cells

Using a murine model of liver metastases, we found that oxaliplatin can enhance the immunostimulatory effect of interleukin-12 delivered by an adenoviral vector. A shift toward a favorable immune microenvironment was observed in tumors, with a relative increase in $CD8^+$ T cells vs. T regulatory and myeloid-derived suppressor cells.

The presence of liver metastases, before or after removal of primary lesions, compromises the long-term survival of colorectal cancer patients. Therefore, effective treatments against this disease should avoid liver colonization and dissemination of cancer cells in this organ. A clinical setting presenting diffuse deposits but relatively low tumor burden is, in principle, a favorable scenario for immunotherapy. However, tumors thrive in an environment hostile for the establishment of efficient immune responses, in part due to the accumulation of T regulatory cells (Tregs) and myeloid-derived suppressor cells (MDSC).[1]

The ability of certain chemotherapeutic agents to stimulate the immune system is emerging as a key determinant of their antitumor properties.[2] Among them, oxaliplatin (OXP) has been recently described to increase the immunogenicity of cancer cells.[3] However, standard chemotherapy protocols designed to maximize the cytotoxic effect have shown limited efficacy against metastatic colorectal cancer. Hopefully, strategies that favor the immunostimulatory properties of these drugs might change this situation. In a recent report in reference 4, we describe that OXP used in combination with interleukin-12 (IL-12) is able to eradicate pre-existing liver metastases of colorectal cancer and to protect from relapse in a murine model.

IL-12 is a potent immunostimulatory cytokine that activates the proliferation and function of key mediators of the innate and adaptive systems such as T lymphocytes and natural killer (NK) cells. The ability of IL-12 to stimulate the immune response against tumors has been extensively demonstrated.[5] The systemic toxicity of this cytokine is a serious obstacle for its use as a recombinant protein in humans, but genetic transfer to specific locations is an attractive alternative. Adenoviral vectors carry out efficient gene transfer to the liver in humans. However, transgene expression with early versions is transient, and the efficacy of re-administration is severely impaired by the rapid appearance of neutralizing antibodies. The use of high-capacity (also called helper-dependent or "gutless") adenoviral vectors (HC-Ads) allows long-term expression in animal models after a single administration, and their extended cloning capacity facilitates the incorporation of inducible expression systems.[6] These features have been introduced in the HC-Ad/RUmIL-12 vector designed to fight against primary or metastatic liver cancer.[7] It contains a liver-specific, mifepristone-inducible system for the expression of murine IL-12 that allows a tight control on the intensity and duration of cytokine expression. Following vector administration, the induction regime is adjusted based on the response to a low dose of mifepristone, to compensate for differences in viral transduction. In addition, a step-wise increase of inducer is needed to cope with the transient silencing of liver-specific promoters caused by gamma-interferon.[8] We found that this individualized protocol allowed several cycles of IL-12 expression in the therapeutic range.[4,7] Using a syngeneic model of liver metastases in mice, we observed that a single cycle consisting on 10 daily inductions significantly extended the survival of animals and achieved eradication of hepatic tumors in 50% of them (**Fig. 1B**). Importantly, a single dose of OXP administered 3 d before the initiation of IL-12 induction increased the rate of tumor eradication above 80%, whereas the same dose of drug had no significant antitumor effect by its own. In principle, this improvement in the therapeutic effect is not surprising, because it may reflect collaboration with the cytotoxic effect on cancer cells. In fact, the same phenomenon

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Submitted: 08/29/11; Accepted: 08/31/11
http://dx.doi.org/10.4161/onci.1.1.17930

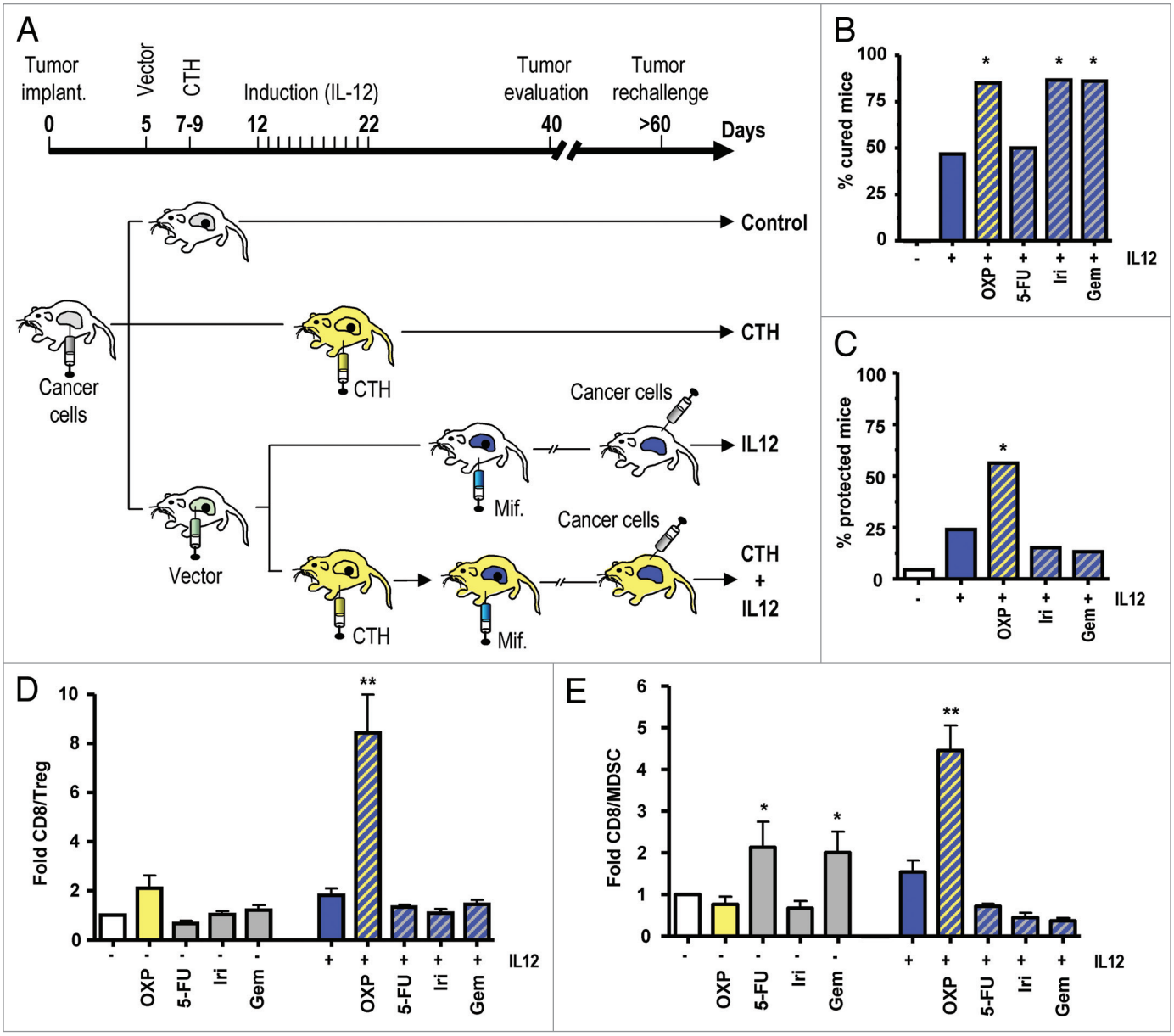

Figure 1. IL-12 and oxaliplatin cooperate to induce an efficient immune response against tumors. Schematic representation of experiments, including schedule and description of treatment groups (A). Hepatic tumors were established by intrahepatic inoculation of murine colon cancer cells in syngeneic C57BL/6 mice. The HC-Ad/RUmIL-12 vector was administered by direct hepatic injection in the liver surrounding the tumors, and expression of IL-12 was activated by daily injections of mifepristone (Mif). Some mice were treated with intraperitoneal chemotherapy using single agents (OXP, 5-FU, Iri or Gem, named collectively CTH). Other groups received the same drugs preceding IL-12 expression (CTH+IL12). The presence of tumors was evaluated by direct inspection through laparotomy one month after initiation of treatments. (B) Percentage of tumor-free animals. No tumor regressions were observed in control animals or those treated with chemotherapy alone, and are not represented in the graphic. (C) Cured animals received a subcutaneous inoculation of the same cancer cells, and the graphic shows the proportion of mice that were completely protected (absence of tumor growth). (D and E) In a separate set of experiments, tumor samples were obtained in the course of the treatments. Leucocytes were isolated and $CD8^+$ T cells, Tregs ($CD25^+$ $FoxP3^+$) and monocytic MDSC ($CD11b^+$ $Ly6C^+$ $Ly6G^-$) were identified by flow cytometry. The fold increase in the ratio of CD8/Treg and CD8/MDSC for each group is represented in (D and E), respectively, considering the control group as a reference. *$p < 0.05$; $p < 0.005$ (analysis of variance).

occurred when IL-12 was combined with other chemotherapeutics frequently used in the treatment of colorectal cancer, such as irinotecan (Iri) and gemcitabine (Gem), but not 5-Fluorouracil (5-FU) (**Fig. 1B**). However, we found that the effect of OXP had something special, because animals cured from their hepatic tumors showed an improved immunological protection from a subcutaneous tumor rechallenge (**Fig. 1C**), whereas this did not occur when IL-12 was combined with Iri or Gem. When we studied the potential mechanisms behind this fact, we found that OXP was the only drug that promoted a favorable immune microenvironment in the tumors (**Fig. 1D and E**). In accordance with previous reports, 5-FU and

Gem were able to reduce the abundance of MDSCs in the tumors,[9,10] but this effect was lost when they were combined with IL-12 in our experimental conditions (**Fig. 1E**). In contrast, OXP decreased both Treg and MDSCs, especially in the context of IL-12 overexpression. As a consequence, a dramatic increase in the ratio of cytotoxic $CD8^+$ T lymphocytes vs. immunosuppressive cell populations was detected in the tumor microenvironment. These changes in the infiltrate of tumors are compatible with a stronger antitumor immune response, although alternative or complementary mechanisms of action cannot be ruled out at this point.

Further work using different tumor models will determine if the cooperation between IL-12 and OXP can be generalized to other situations, and will help to predict the potential applicability of this protocol in the clinical practice. This approach could be applied with a curative intention against pre-existing liver metastasis, or as prophylaxis after removal of the primary tumor in colorectal cancer patients at high risk. In principle, once the liver has been transduced with the HC-Ad vector, the mifepristone-dependent activation of IL-12 expression can be scheduled as needed according to the evolution of the patient. We believe this opens new possibilities in the field of immunochemotherapy. Nevertheless, the function of all the components integrating the vector system should be carefully evaluated in pilot clinical trials before we can safely proceed to the implementation of this strategy in cancer patients.

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