CD4+ T Cells Are Able to Promote Tumor Growth through Inhibition of Tumor-Specific CD8+ T-Cell Responses in Tumor-Bearing Hosts

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Abstract

Modulation of the immune response by established tumors may contribute to the limited success of therapeutic vaccination for the treatment of cancer compared with vaccination in a preventive setting. We analyzed the contribution of the CD4+ T-cell population to the induction or suppression of tumor-specific CD8+ T cells in a tumor model in which eradication of tumors crucially depends on CD8+ T cell–mediated immunity. Vaccine-mediated induction of protective antitumor immunity in the preventive setting (i.e., before tumor challenge) was CD4+ T cell dependent because depletion of this T-cell subset prevented CD8+ T-cell induction. In contrast, depletion of CD4+ cells in mice bearing established E1A+ tumors empowered the mice to raise strong CD8+ T-cell immunity capable of tumor eradication without the need for tumor-specific vaccination. Spontaneous eradication of tumors, which had initially grown out, was similarly observed in MHC class II–deficient mice, supporting the notion that the tumor-bearing mice harbor a class II MHC–restricted CD4+ T-cell subset capable of suppressing a tumor-specific CD8+ T-cell immune response. The deleterious effects of the presence of CD4+ T cells in tumor-bearing hosts could be overcome by CD40-triggering or injection of CpG. Together these results show that CD4+ T cells with a suppressive activity are rapidly induced following tumor development and that their suppressive effect can be overcome by agents that activate professional antigen-presenting cells. These observations are important for the development of immune interventions aiming at treatment of cancer. (Cancer Res 2005; 65(15): 6984-9)

Introduction

In general, protective antitumor immunity in tumor-free hosts can be more easily installed than therapeutic immunity following vaccination of tumor-bearing recipients. One of the possible reasons for this difference is the generation of an immunosuppressive environment by the growing tumor. Overcoming this detrimental influence is a major challenge for successful immunotherapy of established tumors.

Tumor-specific CD8+ T-cell responses are often crucial to tumor eradication (1–4). Although CD4+ T helper cells are usually intimately involved in the induction of CD8+ T cells, relatively little is known about the interplay between CD4+ and CD8+ T cells in tumor-bearing animals. Help provided by CD4+ T cells can be crucial for the induction of effective antitumor immunity (5–8).

However, recent evidence suggests that CD4+ cells can also contribute to tumor growth. For example, CD4+ T cells were shown to enhance tumor incidence in human papillomavirus 16 transgenic mice by increased recruitment of neutrophils, which provide matrix metalloproteinase-9 necessary for angiogenesis and neoplastic cell proliferation (9). CD4+ natural killer (NK) T cells can interfere with immune surveillance resulting in incomplete elimination and recurrence of tumors. Depletion of these cells within the first 10 days after tumor cell inoculation resulted in enhanced survival (10). CD4+CD25+ T cells, which have been implicated in control of autoimmune responses, have also been shown to play a role in suppression of antitumor responses (11–14). These cells most likely suppress responses against self-antigens on the tumor cells, but could potentially also be directed against neoantigens expressed by the tumor. Recently, tumor antigen–specific regulatory T cells were cloned from tumor-infiltrating lymphocytes of a melanoma patient (15). Together these observations indicate that CD4+ T cells can play an important and diverse role in the development of tumors.

To gain more insight in the contribution of CD4+ T cells to the development of tumor-specific CTL immunity in tumor-bearing hosts, we wished to analyze the evolution of the naturally occurring antitumor CTL response. To this end, we made use of a tumor model in which eradication of the tumor crucially depends on CD8+ T cell–mediated immunity against a neoantigen, the adenovirus type 5–derived E1A protein (16). E1A-specific CTLs are crucial for tumor clearance as induction of E1A-specific tolerance by injection of the minimal E1A-derived CTL epitope leads to the inability of mice to control the outgrowth of E1A-expressing tumors. In a prophylactic setting, induction of the E1A-specific CD8 response is dependent on CD4+ cells. We now show that, in tumor-bearing mice, depletion of CD4+ cells results in a remarkable increase in the number and systemic spread of tumor-specific CD8+ T cells as well as in tumor eradication and enhanced survival. Because CD4+ T cells are required for proper CTL induction by vaccination of tumor-free animals before tumor challenge, these findings indicate that tumor growth can modulate the CD4+ T-cell response from one supporting CTL activation to a response that inhibits development of tumor-specific CTL immunity. These observations are important to the design of immunotherapeutic strategies for the treatment of cancer.

Materials and Methods

Cell cultures. All in vitro cultures were done in Iscove’s modified Dulbecco’s medium (Life Technologies, Gaithersburg, MD) supplemented with 8% FCS, 50 µmol/L 2-mercaptoethanol, glutamine, and penicillin.

Mice. C57BL/6 (H-2b) mice were purchased from IFFA Credo (Paris, France). C57BL/6 Ks (H-2b) and class II−/− (H-2b) mice were bred at TNO-PG (Leiden, the Netherlands).

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Research Article

Cancer Res 2005; 65: (15). August 1, 2005

6984 www.aacrjournals.org

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Role of CD4+ T cells in naive and tumor-bearing mice. Ad5E1A-transformed tumor cells express the E1A protein containing highly immunogenic CD4+ and CD8+ epitopes. Despite the presence of this immunogenic protein, Ad5E1A-transformed tumor cells form progressively growing tumors in immunocompetent mice. Eradication of these tumors crucially depends on E1A-specific CD8+ T cells (16). We have previously shown that the E1A peptide, recognized by CD8+ T cells, is presented in the tumor-draining lymph nodes and that E1A-specific CD8+ T cells are detected in tumor-draining lymph nodes in 30% of tumor-bearing mice (17). However, the tumors grow progressively, indicating that the CD8+ T-cell response is incapable of eradicating the tumor. To obtain more mechanistic insight in the ineffectual antitumor immune response, we studied the role of CD4+, CD8+, and NK1.1+ cells by depletion of each specific cell type in mice bearing established tumors 3 weeks after tumor challenge. As described before (18), depletion of CD4+ T cells before vaccination of non-tumor-bearing animals completely abrogates the induction of E1-specific CD8+ T-cell immunity, demonstrating the crucial role of CD4+ T cells in the induction of E1-specific T cell–mediated immune responses (data not shown).

CD8+ T cells suppress expansion of antitumor CD8+ T cells. Because E1A-specific CD8+ T cells are crucially involved in the eradication of E1A-transformed tumors (16), we determined whether depletion of CD4+ cells affects the number of E1A-specific CD8+ T cells. A remarkable increase in the number of E1A-specific CD8+ T cells in peripheral blood was observed after CD4 depletion (Fig. 3). Furthermore, whereas E1A-specific CD8+ T cells in untreated tumor-bearing mice are only detected in tumor-draining lymph nodes in 30% of the animals, the E1A response emerging after CD4 depletion was readily detected in other lymphoid organs and the tumor (Fig. 4). These results indicate that the CD4+ cells suppress the initial induction or expansion of the tumor-specific CD8+ T cells in tumor-bearing mice. When CD4 depletion was combined with CD8+ cell depletion, the beneficial effect of CD4 depletion alone was completely abrogated, confirming that tumor rejection in mice depleted of CD4+ cells requires CD8+ T cells (Fig. 2).

CD4+ T cells are not only expressed on a subset of T cells but also on subsets of NK cells and dendritic cells. Therefore, any of these cell types may theoretically be involved in the observed effects of CD4 depletion. To investigate whether the immunosuppressive CD4+ cells were T cells, class II−/− mice, which lack functional peripheral CD4+ T cells but contain CD4+ NK T cells (19) and...
CD25+ cells were depleted, reduced in numbers by 90% to 95% as determined by flow cytometric analysis (data not shown). CD25+ cell depletion, therefore, did not influence tumor growth (data not shown). The role of CD8+ cells in CD4-depleted mice. C57BL/6 mice were injected s.c. in the flank with 1 × 107 live Ad5E1ras cells and left untreated (●) or injected 22 days later, at a time mice had developed established tumors, with GK1.5 (anti-CD4 antibody; □) or 2.43 (anti-CD8 antibody; ⧫), or both (○). Percent of surviving mice. Untreated versus CD4-depleted, P < 0.01; untreated versus CD8-depleted, P = 0.038 (log-rank test).

Discussion

In this study, we show that CD4+ T cells in tumor-bearing mice suppress antitumor CD8+ T-cell responses as depletion of CD4+ cells in mice bearing established tumors results in the rapid, systemic expansion of effective tumor-specific CD8+ T-cell immunity without the need for tumor-specific vaccination. Remarkably, the suppressive CD4+ cells seem to be rapidly induced after tumor growth because the same CD8+ T-cell response that is suppressed by CD4+ T cells in tumor-bearing mice is helper cell dependent in vaccinated tumor-free mice. Thus, our data indicate that rapidly after tumor outgrowth (i.e., within 3 weeks), CD4+ T cells emerge that, instead of providing help for CD8+ T-cell induction, hamper the spontaneous development of effective tumor-specific CD8+ T-cell responses. Suppression in tumor-bearing animals can be overcome via injection of anti-CD40 antibodies or CpG, indicating that the suppressive effect mediated by CD4+ T cells can be by-passed through the activation of CD40- and Toll-like receptor 9–positive cells, respectively, most likely dendritic cells.

Therapeutic vaccination for cancer treatment has proven more difficult than prophylactic vaccination. Although the reasons for this observation are not known, various aspects are likely to contribute to the ineffectiveness of therapeutic antitumor vaccination. Here we show that one of these aspects relates to the induction of suppressive CD4+ T cells rapidly after tumor cell...
CD4+ T cells often leads to the abrogation of tumor-specific CD8+ responses after vaccination, as immunization in the absence of several effector pathways will be affected by the generation of the or PC61 (anti-CD25 antibody; P versus CD4-depleted, 5). Activation, B-cell maturation, and isotype switching. Likewise, CD4+ orchestration of various immune functions such as macrophage infiltration, tissue homeostasis in case the immune system is confronted with new proteins during, for example, pregnancy, lactation, or inhalation. However, in tumor-bearing hosts, such T cells may be detrimental to the emergence of effective antitumor immunity and may hamper effective antitumor immunotherapy.

It is conceivable that inflammatory mediators are produced by immune cells present in a tumor that undergoes rejection. This is likely to lead to influx of more immune cells, including CD8+ T cells. Indeed, the total number of CD8+ T cells in tumor masses from undepleted mice is lower compared with the number of CD8+ T cells in tumors undergoing rejection (Fig. 4).

Recently, we have shown that the spontaneously arising E1A-specific CD8+ T cells in the draining lymph nodes of mice bearing E1A+ tumors produce IFN-γ after in vitro restimulation (25). These data indicate that in CD4-intact mice, tumor-specific CTLs present in tumor-draining lymph nodes are not only present but also functional. However, these CTLs do not systemically appear and, therefore, will not eradicate the s.c. growing tumor (17, 26). In contrast, systemic spread of the tumor-specific CD8+ T cells resulting in tumor eradication is readily induced after treatment with APC-activating agents such as anti-CD40 signaling or CpG treatment. These findings are in line with a recent observation in a BALB/c mouse model using influenza HA-expressing tumor cells, which describes the effects of CD40 triggering to induce systemic spread of tumor-specific CD8+ T cells which spontaneously arise (27). Also in this case, the tumor-specific CD8+ T cells present in the tumor-draining

![Figure 4. CD4 depletion leads to increased numbers of tumor-specific CD8+ T cells.](image)

![Figure 5.](image)
lymph node display effector functions, but only spread systemically after CD40 signaling. We have now shown a similar expansion and systemic spread of tumor-specific CD8+ T cells after depletion of CD4+ T cells, indicating that the regulatory effects mediated by the CD4+ T cells mainly affect the ability of tumor-bearing CTL to expand and leave the tumor-draining lymph node.

A recent study showed improved antitumor immunity when CD4+ cells were depleted after tumor challenge (14). Accumulation of CD4+CD25+ T cells in the tumor was observed. Local depletion of the CD4+ cells in the tumor, as well as anti–interleukin-10 receptor and anti–transforming growth factor-β treatment, was effective in preventing tumor outgrowth. We have shown that the intrinsic, naturally occurring suppressors, the CD4+CD25+ T cells, are most likely not involved in the suppression observed in the mice with growing EIA-positive tumors. The fact that the animal is confronted with the tumor later in life, and that the tumor antigen is not a self-antigen, may account for this fact.

Recently, suppression by CD4+CD25+ T cells in an environment of systemic antigen presentation was shown to be reverted by Toll-like receptor signals (28, 29). Likewise, we show that CpG or CD40 triggering can overcome tumor-induced CD4+ T cell–mediated suppression. In vitro, interleukin-6 produced by APC on Toll-like receptor triggering with lipopolysaccharide or CpG was crucial to overcome suppression from regulatory T cells (28). It is conceivable that these and other beneficial mechanisms contribute to the effects observed after injection of anti-CD40 and CpG.

In conclusion, our results are important for improvement of immunotherapeutic interventions in cancer patients. In the design of therapeutic immuno-interventions, ways to overcome a suppressive environment need to be taken into account to successfully induce powerful antitumor immunity. Intervention regimens, like treatment with nondepleting blocking anti-CD4 antibodies (30, 31), could prove to be effective, but also interventions at bypassing suppressive effects installed by the CD4+ T cells could be successful. In this respect, we have shown that CpG injection or CD40 ligation supports a strong tumor-specific CD8+ T cell–response in a suppressive setting without the need for tumor-specific vaccination, resulting in eradication of tumor cells (and prolonged) survival. This indicates that a change in the environment in which tumor antigens are presented can shift the balance from suppression to strong induction of antitumor immunity.

Acknowledgments

Received 10/6/2004; revised 4/20/2005; accepted 5/18/2005.

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We thank Michel Mulders for biotechnical assistance.

References

24. Melief CJ. Mini-review: Regulation of cytotoxic T lymphocyte responses by dendritic cells: peaceful...
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