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The Future of Interleukin-2: Enhancing Therapeutic Anticancer Vaccines

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Abstract

PURPOSE—The purpose of our efforts is to trigger the immune destruction .of established cancer. Interleukin (IL)-2 can mediate the regression of tumors in patients with melanoma and renal cell carcinoma. In animal models, the antitumor effects of IL-2 are mediated by T lymphocytes. Stimulation with specific antigen can enhance the ability of T cells to respond to IL-2 by triggering the rapid upregulation of the high-affinity IL-2 receptor. We are seeking to design recombinant and synthetic vaccines capable of preferentially priming T cells with specificity for tumor cells.

METHODS—The antitumor activity of experimental vaccines is being studied preclinically using recently developed murine models that employ the mouse homologues of human tumor-associated antigens. Once the most effective experimental vaccines are optimized in experimental animals, clinical trials can be conducted. Vaccines are being evaluated for their ability to mediate the regression of established tumors, and a variety of immunologic correlates are being measured.

RESULTS—In animal models, vaccines based on molecularly defined tumor-associated antigens expressed in viral vectors or delivered as “naked” DNA stimulate the expansion of CD4+ and CD8 + tumor-specific T lymphocytes. Goad-ministration of IL-2 with these vaccines dramatically enhances their ability to mediate the regression of established cancer. In the clinic, treatment of melanoma patients with peptide vaccine and IL-2 resulted in objective responses in approximately 40% of patients, a response rate more than twice that typically achieved with IL-2 alone. Paradoxically, tumor-specific CD8+ T-cell levels were not increased in these patients.

CONCLUSION—The addition of recombinant and synthetic cancer vaccines to a regimen of IL-2 can result in improved antitumor responses in both animal models and melanoma patients. Vaccine-primed, tumor-specific T cells may preferentially proliferate upon administration of IL-2. The apparent lack of increase in CD8+ T-cell numbers in this setting suggests that the vaccine-primed T cells functionally disappear after a transient period of activation. Preventing the disappearance of activated T cells upon IL-2 administration—for example, by blocking proapoptotic signals—may enhance the therapeutic effectiveness of anticancer vaccines.

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Keywords

Adjuvant; cancer vaccines; gp 100; interleukin-2; melanoma; T lymphocytes

Interleukin (IL)-2 induces durable complete responses in patients with metastatic melanoma and renal cell carcinoma, yet in only about 20% of patients treated with IL-2 will an objective tumor regression be observed, and only about one third of responding patients (approximately 7% of treated patients) will have a complete response.[1–4] Although the mechanism of action of IL-2 therapy in humans is not known with certainty, in mouse models therapeutic effects are largely mediated by T lymphocytes.[5] Administration of IL-2 is thought to cause the widespread expansion of T cells, some of which appear to be specific for antigens presented on the surfaces of tumor cells, and it is presumably these T cells that mediate tumor regression. In fact, IL-2 therapy combined with tumor-specific T lymphocytes expanded in vitro doubled the response rate in melanoma patients (34%) compared with response rates that have been achieved with high-dose IL-2 therapy alone (17%).[3,6] This suggests that an important denominator for the success of IL-2 therapy is the frequency of tumor-specific T cells capable of responding to IL-2. If one could increase the frequency of these tumor-specific, IL-2—responsive T cells, the result might be an immune response that is stronger and more tumor specific, possibly leading to a higher rate of tumor regression. Recent advances in the cloning of tumor-specific antigens have enabled the development of antigen-specific cancer vaccines that could elicit such a response.

A successful anticancer vaccine must induce a powerful tumor antigen-specific immune response capable of eradicating widely disseminated malignant disease. Animal models have guided the design of therapeutic regimens that combine synthetic cancer vaccines with IL-2, and the first clinical results have confirmed some of the predictions based on these models. However, while promising, the results also raise pressing new questions regarding the use of IL-2 in combination with vaccines in new animal and clinical studies.

THE USE OF ANIMAL MODELS TO EVALUATE MOLECULARLY DEFINED ADJUVANTS

Animal models have been used to evaluate large panels of molecularly defined adjuvants designed to enhance the antitumor activity elicited by vaccines.[7] We have used experimental systems in which tumors were treated with a variety of cancer vaccines, including, most importantly, recombinant vaccinia virus (rVV) or recombinant “naked” DNA-based vaccines. The efficacy of candidate adjuvants was tested either by exogenous administration or by encoding them in the vaccine vector along with the model tumor antigen.

Cytokine Adjuvants

Using these model systems, several cytokines have been identified that appear to function as adjuvants and to enhance the antitumor immune response elicited by our rVV-based vaccines. The cytokine adjuvants identified in these studies included IL-2, IL-10, and IL-12. Immunization with rVV-based cancer vaccines plus IL-2, either by injection or by encoding IL-2 in the viral genome, resulted in dramatic augmentation of antitumor effects compared with either agent alone.[8] IL-12, a cytokine that can activate natural killer cells and steer the immune response toward a T helper type 1 response, was also an extremely potent adjuvant when combined with rVV-based vaccines.[9] IL-12 is thought to primarily enhance the cellular immune response, and indeed, CD8+ T lymphocytes have been shown to be of critical importance to the antitumor immune response in many murine tumor models.[7] Another and more unexpected finding was that IL-10, which is classically regarded as an anti-inflammatory

cytokine, also enhanced the antitumor effect of rVV-based vaccines. Interestingly, other cytokines, such as granulocyte-macrophage colony-stimulating factor, tumor necrosis factor (TNF)– alpha, and interferon gamma, did not modulate the effect of rVV vaccines when the genes for these cytokines were inserted into the rVV genome.

Naked DNA vaccines were also used to evaluate the effects of candidate adjuvants. Naked DNA is taken up by normal cells upon injection, and the encoded tumor antigen can be expressed. Although the bacterial plasmid DNA encoding the tumor antigen appears to be immunostimulatory itself owing to the presence of bacterial, nonmethylated CpG-sequences,[10] this immunostimulatory effect does not appear to be strong enough to enhance tumor regression. Indeed, immunization with DNA vaccines only resulted in significant tumor destruction when administered together with IL-2, IL-6, IL-7, or IL-12.[11] Overall, the consensus emerging from this large body of data suggests that antigen-specific cancer vaccines activate tumor-specific T cells that are then receptive to stimulation by immunomodulatory adjuvant therapy, and through this stimulation the T cells became more effective in destroying tumors.

Costimulatory Molecules as Adjuvants

A separate class of potential adjuvants includes molecules that are naturally expressed on the membranes of antigen-presenting cells and interact with T cells, thus providing a costimulatory signal. Our studies identified the costimulatory molecules B7-1, B7-2, ICAM-I, and the CD40 ligand (CD40L) as powerful adjuvants that can significantly enhance the antitumor immune response induced by our model vaccines.[12,13] New technologies are becoming available that allow the administration of these normally membrane-bound molecules in a soluble, but still bioactive, form.[13,14]

IL-2 AS AN ADJUVANT TO CANCER VACCINES IN ANIMAL MODELS

Throughout the testing of candidate adjuvants, one of the most consistent findings was the remarkable synergy between antigen-specific cancer vaccines and IL-2. Indeed, in our own studies IL-2 has been shown to have powerful antitumor effects when combined with a variety of different types of recombinant, antigen-specific cancer vaccines, including rVV, fowlpox virus, modified vaccinia virus Ankara, adenovirus, influenza virus, naked DNA, self-replicating DNA vector, and self-replicating RNA vector vaccines.[8,11,15–18] In addition, IL-2 supports the antitumor effects of ex vivo expanded tumor-specific T lymphocytes.[19]

One important mechanism by which IL-2 may act as an adjuvant to vaccines is by enhancing expansion and/or activation of T cells that are primed by vaccination. In fact, upon encountering major histocompatibility complex (MHC)—restricted antigenic peptides on antigen-presenting cells, one of the first observed reactions in the T cell is a markedly increased expression of the high-affinity IL-2 receptor alpha subunit, CD25 (i.e., the Tac antigen).[20] As a result, antigen-activated T cells have an increased ability to respond to IL-2. This is likely to account for the strong increase in primary T-cell responses against both rVV determinants and the encoded tumor antigen, as well as the enhanced antitumor effect that was observed when IL-2 was coadministered with rVV-based vaccines.[8]

IL-2 AS AN ADJUVANT FOR ADOPTIVE CANCER THERAPY

Murine studies suggested that the efficacy of IL-2 therapy could be enhanced by the concurrent administration of tumor-infiltrating lymphocytes (TIL) expanded in vitro.[21-23] In a series of clinical trials, IL-2 was administered to melanoma patients together with TIL, resulting in objective cancer regression in one-third of patients, double that achieved with high-dose IL-2 therapy alone.[6] However, most bulk TIL cultures used for adoptive transfer contain only a

limited number of truly tumor-reactive T cells. One strategy to improve the frequency of these cells involves the clonal expansion of melanoma-associated antigen (MAA)-specific T cells.[24] Bulk T-cell lines specific for MAA are generated through in vitro peptide sensitization of peripheral blood mononuclear cells from melanoma patients and are cloned early in their growth phase. The individual clonal populations are then screened for in vitro reactivity to the MAA, and T-cell clones with the highest specificity and tumor reactivity are further expanded in vitro for adoptive transfer.[24] The first patients are currently being treated with these autologous MAA-reactive T-cell clones, with or without the addition of IL-2.

PRELIMINARY RESULTS IN THE CLINICAL EVALUATION OF IL-2 AS A CANCER VACCINE ADJUVANT

Findings in murine models may or may not be confirmed in human clinical trials. In studies conducted at the National Cancer Institute, Surgery Branch, the antitumor effect of IL-2 was found to be enhanced by concurrent vaccination with a modified immunodominant peptide derived from the MAA gp100.[25] Virtually all patients immunized with the gp100 peptide vaccine in incomplete Freund's adjuvant (IFA) demonstrated high levels of gp100-specific CD8+ T cells, and these T cells could kill melanoma cells in vitro. However, significant tumor regression was observed only when IL-2 was coadministered with the peptide vaccine, presumably because the gp100-specific CD8+ T cells required expansion. Treatment with the peptide vaccine plus IL-2 produced an objective response rate of 42% (see Rosenberg et al in this issue), compared with a historic response rate of 17% for high-dose IL-2 alone.[25] A multi-center trial is currently in progress to extend these findings.

THE EFFECTS OF IL-2 ON TUMOR-SPECIFIC T CELLS

One puzzling observation in both the clinical studies and animal models with regard to the use of IL-2 as an adjuvant to vaccines points to a possibility for further improvement on this therapeutic strategy. Although administration of IL-2 in conjunction with cancer vaccines often results in enhanced antitumor effects, the number of circulating tumor antigen-specific CD8+ T cells is not increased when IL-2 is administered. Mice immunized with rVV-based vaccines and IL-2 showed a temporary depression of antigen-specific CD8+ T cells in the circulation.[26] Similarly, many patients immunized with a gp100 peptide plus IL-2 had no detectable antigen-specific CD8+ T cells in their blood 4 weeks after immunization, even though these same patients exhibited sometimes dramatic tumor regression. In contrast, patients immunized with peptide alone developed high levels of antigen-specific CD8+ T cells in their blood, but did not exhibit tumor regression.[25,27]

There are several possible explanations for this unexpected phenomenon, none of which necessarily exclude the others. Administration of IL-2 is known to induce extravasation of lymphocytes into the tissues, and it is possible that this effect stimulates vaccine-induced, tumor antigen-specific T cells to leave the circulation and enter tissues, including tumor tissues, where they could exert their cytotoxic effect. However, it is unclear why tumor-specific T-cell precursor levels remain undetectable long after IL-2 administration has been discontinued. Another possibility is that high-dose IL-2 abrogates the efficient priming of T cells. Vaccination with peptide in IFA induces a local inflammatory response at the injection site, presumably as a result of the release of chemokines and other chemotactic factors that attract lymphocytes to the injection site. The administration of high systemic doses of IL-2 could induce a generalized activation of the immune system, resulting in widespread production of chemotactic factors, thereby disrupting the specific trafficking of T cells to the vaccination site. However, it is difficult to imagine how vaccination with a 9-amino-acid, MHC class I–restricted peptide antigen could double the response rate to IL-2 therapy other than through the productive priming of antigen-specific CD8+ T cells.

Recent advances in the understanding of T-cell activation point to yet another possible explanation for the paradoxical disappearance of CD8+ T cells in the face of improved therapeutic response. This explanation is based on the phenomenon known as activation-induced cell death (AICD), in which T cells become increasingly susceptible to apoptotic self-destruction as their level of activation increases.[28] Many different mechanisms have been implicated in the death of activated T cells during AICD, including interactions between Fas and the Fas ligand (FasL), CTLA-4 and B7-1, and TNF and TNF receptors.[28–33] What these mechanisms have in common is the positive correlation between the degree of T-cell activation and susceptibility to AICD. For example, mice genetically deficient in IL-2 demonstrate normal T-cell development and productive immune responses upon infection, but eventually develop a lethal immune hyperactivation with infiltration of activated T cells into the gut and heart.[34,35] In vitro, T cells from IL-2-deficient mice are highly resistant to AICD compared with T cells from normal animals.[35] These findings suggest that IL-2 plays a unique role in both the amplification and downregulation of the immune response. Thus, high doses of IL-2 may serve to hyperactivate vaccine-induced, tumor-specific CD8+ T cells. If IL-2 functions to expand the numbers and activity of antigen-specific T cells, these T cells may have enhanced antitumor activity and mediate the observed tumor shrinkage in patients. However, as a result of their increased activation state, they may be subsequently more susceptible to AICD, resulting in undetectable CD8+ T-cell levels after 4 weeks, when patients' T cells are isolated for analysis.

If the above scenario reflects what happens in cancer patients treated with vaccines plus IL-2, several options exist for intervention to enhance the therapeutic potential of this treatment modality. For example, if the Fas/FasL interaction is responsible for the death of highly activated, tumor-specific T cells, blocking this interaction with Fas/FasL-specific antibodies may improve the survival of these T cells, perhaps resulting in further enhancement of antitumor effects. Indeed, in animals lacking a functional Fas/FasL interaction, AICD is typically severely impaired.[36] Likewise, negative signaling through CTLA-4 or the TNF receptor has been blocked in animals and in vitro systems, resulting in decreased AICD and enhanced T-cell function.[29,32,33] Experiments are in progress to further address these possibilities and identify strategies aimed at improving vaccine-based cancer therapy.

CONCLUSION

IL-2 has been shown to synergize powerfully with cancer vaccines in the treatment of human malignancies. When IL-2 is administered in conjunction with cancer vaccines based on recombinant viruses, naked DNA, or peptide antigens, it can dramatically enhance antitumor effects. The use of IL-2 as an adjuvant to cancer vaccines continues to be intensely explored both in animal models and in the clinic. The observation that IL-2 may increase T-cell activation as well as susceptibility to apoptosis points to new treatment strategies that may ultimately result in further enhancement of the antitumor effects of IL-2.

References

1. Atkins MB, Lone MT, Dutcher JP, et al. High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. J Clin Oncol 1999;17:2105–2116. [PubMed: 10561265]
2. Fyfe G, Fisher RI, Rosenberg SA, et al. Results of treatment of 255 patients with metastatic renal cell carcinoma who received high-dose recombinant interleukin-2 therapy. J Clin Oncol 1995;13:688–696. [PubMed: 7884429]
3. Rosenberg SA, Yang JC, Topalian SL, et al. Treatment of 283 consecutive patients with metastatic melanoma or renal cell cancer using high-dose bolus interleukin 2. JAMA 1994;271:907–913. [PubMed: 8120958]

4. Rosenberg SA, Lotze MT, Yang JC, et al. Experience with the use of high-dose interleukin-2 in the treatment of 652 cancer patients. Ann Surg 1989;210:474–484. 484–485. [PubMed: 2679456]
5. Mulé JJ, Yang JC, Lafreniere RL, et al. Identification of cellular mechanisms operational in vivo during the regression of established pulmonary metastases by the systemic administration of high-dose recombinant interleukin 2. J Immunol 1987;139:285–294. [PubMed: 3108401]
6. Rosenberg SA, Yannelli JR, Yang JC, et al. Treatment of patients with metastatic melanoma with autologous tumor-infiltrating lymphocytes and interleukin 2. J Nad Cancer Inst 1994;86:1159–1166.
7. Restifo NP, Rosenberg SA. Developing recombinant and synthetic vaccines for the treatment of melanoma. Curr Opin Oncol 1999;11:50–57. [PubMed: 9914879]
8. Bronte V, Tsung K, Rao JB, et al. IL-2 enhances the function of recombinant poxvirus-based vaccines in the treatment of established pulmonary metastases. J Immunol 1995;154:5282–5292. [PubMed: 7730632]
9. Rao JB, Chamberlain RS, Bronte V, et al. IL-12 is an effective adjuvant to recombinant vaccinia virus-based tumor vaccines: enhancement by simultaneous B7-1 expression. J Immunol 1996;156:3357–3365. [PubMed: 8617961]
10. Sun S, Kishimoto H, Sprent J. DNA as an adjuvant: capacity of insect DNA and synthetic oligodeoxynucleotides to augment T cell responses to specific antigen. J Exp Med 1998;187:1145–1150. [PubMed: 9529331]
11. Irvine KR, Rao JB, Rosenberg SA, et al. Cytokine enhancement of DNA immunization leads to effective treatment of established pulmonary metastases. J Immunol 1996;156:238–245. [PubMed: 8598468]
12. Chamberlain RS, Carroll MW, Bronte V, et al. Costimulation enhances the active immunotherapy effect of recombinant anticancer vaccines. Cancer Res 1996;56:2832–2836. [PubMed: 8665522]
13. Gurunathan S, Irvine KR, Wu CY, et al. CD40 ligand/trimer DNA enhances both humoral and cellular immune responses and induces protective immunity to infectious and tumor challenge. J Immunol 1998;161:4563–4571. [PubMed: 9794383]
14. Fields PE, Finch RJ, Gray GS, et al. B7.1 is a quantitatively stronger costimulus than B7.2 in the activation of naive CD8+ TCR-transgenic T cells. J Immunol 1998;161:5268–5275. [PubMed: 9820499]
15. Carroll MW, Overwijk WW, Chamberlain RS, et al. Highly attenuated modified vaccinia virus Ankara (MVA) as an effective recombinant vector, a murine tumor model. Vaccine 1997;15:387–394. [PubMed: 9141209]
16. Chen PW, Wang M, Bronte V, et al. Therapeutic antitumor response after immunization with a recombinant adenovirus encoding a model tumor-associated antigen. J Immunol 1996;156:224–231. [PubMed: 8598466]
17. Restifo NP, Surman DR, Zheng H, et al. Transrectant influenza A viruses are effective recombinant immunogens in the treatment of experimental cancer. Virology 1998;249:89–97. [PubMed: 9740780]
18. Ying H, Zaks TZ, Wang RF, et al. Cancer therapy using a self-replicating RNA vaccine. Nature Med 1999;5:823–827. [PubMed: 10395329]
19. Overwijk WW, Tsung A, Irvine KR, et al. gp100/pmel 17 is a murine tumor rejection antigen: induction of “self”-reactive, tumoriadal T cells using high-affinity, altered peptide ligand. J Exp Med 1998;188:277–286. [PubMed: 9670040]
20. Hemler ME, Brenner MB, McLean JM, et al. Antigenic stimulation regulates the level of expression of interleukin 2 receptor on human T cells. Proc Natl Acad Sci USA 1984;81:2172–2175. [PubMed: 6425828]
21. Alexander RB, Rosenberg SA. Adoptively transferred tumor-infiltrating lymphocytes can cure established metastatic tumor in mice and persist long-term in vivo as functional memory T lymphocytes. J Immunother 1991;10:389–397. [PubMed: 1768672]
22. Wong RA, Alexander RB, Puri RK, et al. In vivo proliferation of adoptively transferred tumor-infiltrating lymphocytes in mice. J Immunother 1991;10:120–130. [PubMed: 2043592]
23. Rosenberg SA, Spiess P, Lafreniere R. A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. Science 1986;233:1318–1321. [PubMed: 3489291]

24. Dudley ME, Nishimura MI, Holt AK, et al. Antitumor immunization with a minimal peptide epitope (G9-209-2M) leads to a functionally heterogeneous CTL response. J Immunother 1999;22:288–298. [PubMed: 10404430]
25. Rosenberg SA, Yang JC, Schwartzentruber DJ, et al. Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. Nature Med 1998;4:321–327. [PubMed: 9500606]
26. Bronte V, Wang M, Overwijk WW, et al. Apoptotic death of CD8+ T lymphocytes after immunization: induction of a suppressive population of Mac-1 +/Gr-1+ cells. J Immunol 1998;161:5313–5320. [PubMed: 9820504]
27. Rosenberg SA, Yang JC, Schwartzentruber DJ, et al. Impact of cytokine administration on the generation of antitumor reactivity in patients with metastatic melanoma receiving a peptide vaccine. J Immunol 1999;163:1690–1695. [PubMed: 10415076]
28. Alderson MR, Lynch DH. Receptors and ligands that mediate activation-induced death of T cells. Springer Semin Immunopathol 1998;19:289–300. [PubMed: 9540157]
29. Waterhouse P, Penninger JM, Timms E, et al. Lymphoproliferative disorders with early lethality in mice deficient in CTLA-4. Science 1995;270:985–988. [PubMed: 7481803]
30. Zaks TZ, Chappell DB, Rosenberg SA, et al. Fas-mediated suicide of tumor-reactive T cells following activation by specific tumor selective rescue by caspase inhibition. J Immunol 1999;162:3273–3279. [PubMed: 10092779]
31. Alderson MR, Tough TW, Davis-Smith T, et al. Fas ligand mediates activation-induced cell death in human T lymphocytes. J Exp Med 1995;181:71–77. [PubMed: 7528780]
32. Gribben JG, Freeman GJ, Boussiotis VA, et al. CTLA4 mediates antigen-specific apoptosis of human T cells. Proc Nad Acad Sci USA 1995;92:811–815.
33. Zhou T, Edwards CK 3rd, Yang P, et al. Greatly accelerated lymphadenopathy and autoimmune disease in lpr mice lacking tumor necrosis factor receptor 1. J Immunol 1996;156:2661–2665. [PubMed: 8609380]
34. Kundig TM, Schorle H, Bachmann MF, et al. Immune responses in interleukin-2-deficient mice. Science 1993;262:1059–1061. [PubMed: 8235625]
35. Kneitz B, Herrmann T, Yonehara S, et al. Nonnal clonal expansion but impaired Fas-mediated cell death and anergy induction in interleukin-2-deficient mice. Eur J Immunol 1995;25:2572–2577. [PubMed: 7589128]
36. Tucek-Szabo CL, Andjelic S, Lacy E, et al. Surface T cell Fas receptor/CD95 regulation, in vivo activation, and apoptosis. Activation-induced death can occur without Fas receptor. J Immunol 1996;156:192–200. [PubMed: 8598462]