IL-12 gene therapy for cancer: in synergy with other immunotherapies

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In preclinical models of cancer, gene therapy with interleukin 12 (IL-12) has reached unprecedented levels of success when combined with immunotherapy approaches such as gene transfer of other cytokines and/or chemokines, costimulatory molecules or adoptive cell therapy. These combinations have been found to produce synergistic rather than additive effects. Meanwhile, IL-12 gene therapy is beginning clinical testing as a single agent, but combination strategies are at hand.

Since the discovery of interleukin 12 (IL-12), studies have tried to ascertain its efficacy as an anticancer agent. Indeed, recombinant IL-12 given systemically had powerful antitumor activities in murine models that led to its clinical development, which began in 1994. Interferon γ (IFN-γ) was identified as the key downstream factor induced by IL-12 that is known to be necessary for the antitumor effects. In Phase I clinical trials designed in an intrapatient dose escalation fashion, it was not realized that initial low doses of IL-12 resulted in desensitization against the biological effects of IFN-γ produced upon subsequent IL-12 doses. Intravenous (i.v.) doses, which were tolerated in Phase I, caused severe toxicity in Phase II clinical trials and thus put a stop to the clinical development of this active antitumor agent for several years.

Cancer gene therapy with IL-12

When considering a protein with antitumor activity but serious systemic potential toxicity, the concept of local gene therapy comes to mind. Several groups realized simultaneously that in vivo gene transduction of malignant cells with IL-12 genes could be therapeutically useful, and would avoid toxicity by confining the potentially damaging agent to the tumor milieu. In fact, intense effects in mice were reported when using direct intratumor injection of defective recombinant adenoviruses encoding IL-12 and these vectors are still the most powerful at achieving tumor cell gene transfer in vivo.

All the available data have been generated with transplantable cell lines that represent various carcinomas, sarcomas, melanomas and lymphomas but not with spontaneously arising tumors. Generally, IL-12 gene transfer leads to significant antitumor activity in these experimental studies and complete regressions are often reported, but there is still room for improvement. Moreover, efficacy on naturally occurring malignancies could be less than in transplanted tumors.

Several mechanisms of antitumor activity have been identified and each contributed differently to the overall therapeutic outcome in each given tumor model. On the one hand, IL-12 promotes a potent cellular immune response in which tumor-specific cytotoxic T lymphocytes (CTLs) and T helper (Th) cells secrete Th1 cytokines. However, CD4+ Th cells are necessary for the therapeutic activity only in some models and they seem suppressive in some instances. On the other hand, absence of CD8+ T cells severely impairs the antitumor effects in most models. Natural killer (NK)-cell depletion demonstrated a clear role for this lymphoid population in precisely those tumors considered less immunogenic.

IL-12 nonimmune mechanisms have been identified and the most prominent is the ability of IL-12 to downregulate the formation of new vessels into growing tumors, also confirmed with gene transfer of IL-12 (Ref. 4). IL-12 receptors have not been found on endothelial cells and angiogenesis is turned off by secondary mediators of the IL-12-IFN-γ pathway, in which the CXC chemokines IFN-γ-inducible protein 10 (IP-10) and monokine induced by IFN-γ (Mig) have been shown to play a crucial role. In addition, this cytokine cascade induces homing receptors on endothelium for the infiltration of lymphocytes into the malignant tissue (G. Mazzolini et al., unpublished).

Cytokines and chemokines that show synergy with IL-12 gene transfer

Based on previous data with purified proteins, F.L. Graham and colleagues pioneered the combination of two adenoviruses, one encoding IL-2 and the other IL-12, which were co-injected into tumor nodules. As a result of synergistic effects, they observed >60% complete regression of established mammary carcinomas and induction of potent antitumor CTL activity. Recent data show that the closely related cytokine IL-15 synergizes with IL-12, as seen with stable double-transfected human lung cancer cells xenografted in nude mice.

The antitumor mechanisms studied in this artificial system are independent of T, B and NK cells but unravel the intriguing involvement of neutrophils in the rejections.

IL-18 has been identified as a potent inducer of IFN-γ. Importantly, IL-18 upregulates the expression of IL-12 receptors. In a reported poorly immunogenic tumor (MACA205), a dear synergy was observed in the antitumor effects mainly mediated by NK cells in this case. However, the administration of recombinant proteins has a threatening synergistic effect in that it induces lethal levels of IFN-γ.

Genes encoding lymphocyte attracting chemokines can be successfully combined with IL-12. This approach has shown remarkable efficacy with adenoviruses encoding the chemokines lymphotactin and IP-10 (Ref. 9). The lymphotactin gene and both IL-12 genes were engineered in the same construct, but further research is needed to address whether a single viral vector or a combination of two vectors is
Mechanisms involved in the antitumoral effects of interleukin (IL)-12 that can be potentiated in combination immunotherapy regimes as indicated in the colored boxes. IL-12 is secreted locally by transfected malignant cells and it triggers IFN-γ production from different cellular sources. As a result, immune and nonimmune therapeutic mechanisms are turned on. The combination of IL-12 with other immunostimulating genes or adoptive T-cell therapy has been observed to enhance efficacy in a synergistic fashion. Abbreviations: DC, dendritic cell; IFN-γ, interferon γ; IL-12, interleukin 12; IP-10, IFN-γ-inducible protein 10; Ltn, lymphotactin; Mig, monokine induced by IFN-γ; Mφ, macrophage; NK, natural killer.

Such molecules are typically restricted to the surface of professional antigen-presenting cells and can be transfected to tumors in an attempt to make them more immunogenic. B7 molecules are a family of proteins that interact with counter-receptors on T cells and provide signals that modulate the immune response. Transfection of B7-1 (CD80) and B7-2 (CD86) makes tumor cells more immunogenic and combination with IL-12 has been proved beneficial in certain models, but not in others.

The 4-1BB ligand is a tumor necrosis factor (TNF) family member expressed on the membrane of mature dendritic cells and other cell types that upon transfection enhances the immunogenicity of tumors. This molecule interacts with 4-1BB, a surface differentiation antigen restricted to activated T cells and NK cells. Intra-tumor injections with adenovirus encoding 4-1BB ligand and IL-12 mutually potentiate their effect against large and well-established liver metastasis of an NK-sensitive colon cancer. In addition, tumor immunity can be triggered by agonistic monoclonal antibodies (mAbs) acting on 4-1BB. Regimes of intratumor gene transfer of IL-12 and anti-4-1BB mAbs show synergizing properties that are more potent than those observed with the natural ligand. This is explained because antibodies show wide-reaching distribution and stimulate every available 4-1BB+ lymphocyte, in contrast to the membrane bound 4-1BB natural ligand.

Adoptive cell therapy strategies that synergize with IL-12 gene transfer
IL-12 induces CTL expansion, which facilitates the culture of effector cells for adoptive cell therapy regimes. Surprisingly, IL-12 synergy with adoptive T-cell therapy not only provides CTLs, but also activates the effector level. These phenomena were unveiled by studies of mice with systemic tumor disease, in which some malignant nodules were adenovirally transduced with IL-12 and subsequently given antitumor CTLs s.i.v. (Ref. 14). Later experimentation has shown a key role for inflammatory adhesion molecules that are induced on the endothelium of peritumoral capillaries by IL-12 gene transfer (G. Mazzolini et al., unpublished).

IL-12 can be used to upregulate artificially the functions of dendritic cells to promote cellular immune responses. Two studies have concluded that intratumor injection of dendritic cells engineered to secrete IL-12, by means of adenoviral or retroviral transfection, eradicates established tumors derived from at least five different tumor cell lines. IL-12 transfection into dendritic cells is probably acting at a series of levels, stimulating T cells, NK cells and dendritic cells in an autocrine fashion. Dendritic cells capture tumor antigens inside the malignant tissue and migrate avidly to draining lymph nodes where antigens are presented while high local levels of IL-12 are being produced.

Conclusion
We propose a general mechanism of action for these synergistic combinations in which a halt in tumor growth by angiogenesis inhibition and NK activation trailblaze the route for artificially boosted T-cell responses (Fig. 1). Gene transfer of IL-12 into tumors is now entering clinical testing and it is hoped that the safety records will be as good as the preclinical data. Combination of the complementary immunotherapy approaches described above is the obvious next step to increase efficacy and minimize risks.

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Asthma is a complex disorder characterized by airway hyper-responsiveness (AHR) and airway inflammation. Evidence has accumulated regarding factors that promote the asthma phenotype, but the mechanisms by which the asthma phenotype is suppressed are largely unclear. Recently, a pleiotropic cytokine, transforming growth factor β1 (TGF-β1), has been reported to function as a negative regulator of AHR and airway inflammation in an experimental model of asthma. Here, we consider this evidence and discuss possible roles of TGF-β1 in suppression of human asthma.

Current understanding of the pathophysiology of asthma

Asthma is a complex disorder consisting of various cellular and/or cytokine/chemokine networks. The presentation of inhaled allergens to CD4+ T cells in the lungs of susceptible individuals results in the production of cytokines such as interleukin 4 (IL-4), IL-5, IL-9 and IL-13, which orchestrate the differentiation, recruitment and activation of eosinophils and mast cells in the airway mucosa. Such effector cells release inflammatory mediators that cause acute bronchial constriction, disruption of the airway epithelial layer, alterations in neural control of airway tone, increased mucus production and increased smooth muscle mass. These consequences of the inflammatory process induce AHR. Recent studies have shown that not only T cells but also a variety of other cells, including mast cells, bronchial epithelial cells and smooth muscle cells, which are activated by various mediators, contribute to the development of AHR in part through the secretion of cytokines or chemokines that generate tissue inflammation.

How does TGF-β1 elicit its biological effects?

TGF-β1 is a member of the TGF-β superfamily, which comprises a large number of cytokines including TGF-βs, bone morphogenetic proteins (BMPs) and activins. These cytokines carry out a wide range of biological functions including cell proliferation, differentiation and apoptosis.

TGF-β1 is inhibitory for inflammatory cells such as T cells, B cells, dendritic cells, mast cells and eosinophils, and also modifies the functions of structural cells such as bronchial epithelial cells, fibroblasts and bronchial smooth muscle.

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