CCL21 Chemokine Therapy for Lung Cancer

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Abstract—Lung cancer remains a challenging health problem with more than 1.1 million deaths worldwide annually. With current therapy, the long term survival for the majority of lung cancer patients remains low, thus new therapeutic strategies are needed. One such strategy would be to develop immune therapy for lung cancer. Immune approaches remain attractive because although surgery, chemotherapy, and radiotherapy alone or in combination produce response rates in all histological types of lung cancer, relapse is frequent. Strategies that harness the immune system to react against tumors can be integrated with existing forms of therapy for optimal responses toward this devastating disease. Both antigen presenting cell (APC) and T cell activities are reduced in the lung tumor microenvironment. In this review we discuss our experience with efforts to restore host APC and T cell activities in the lung cancer microenvironment by intratumoral administration of dendritic cells (DC) expressing the CCR7 receptor ligand CCL21 (secondary lymphoid chemokine, SLC). Based on the results demonstrating that CCL21 is an effective anti cancer agent in the pre-clinical lung tumor model systems, a phase I clinical trial was initiated using intratumoral injection of CCL21 gene modified autologous DC in lung cancer. Results from the trial thus far indicate tolerability, immune enhancement and tumor shrinkage via this approach.

I. INTRODUCTION

One of the challenges in developing immunotherapy for cancer is enlisting the host response to recognize poorly immunogenic tumors. Effective antitumor responses require antigen-presenting cells (APC), lymphocytes and natural killer (NK) effectors. Although lung cancer cells express tumor antigens, limited expression of MHC antigens, defective transporters associated with antigen processing and lack of costimulatory molecules make them ineffective APC (1). Both APC and T cell activities are reduced in lung cancer (2, 3) and intratumoral infiltration by relatively high numbers of activated T lymphocytes (4, 5) and APC (6) lead to better prognosis in lung cancer patients. Utilizing preclinical models of lung cancer, we are evaluating intratumoral delivery of immune potentiating CCL21 chemokine via DC and stromal cell-based approaches for effective recruitment and activation of APC and T cells for the promotion of antitumor activity in lung cancer. The preclinical findings demonstrate that effective anticancer immunity can be achieved by CCL21 mediated recruitment of professional host APC for tumor antigen presentation to promote specific T-cell activation (7-10). Preliminary findings from a phase I trial of intratumoral administration of autologous DC expressing CCL21 to lung cancer patients meets the objectives of the study in terms of safety and the induction of anti-tumor immune responses.

Chemokines, a group of homologous, yet functionally divergent proteins, directly mediate leukocyte migration, activation and play a role in regulating angiogenesis. They also function in maintaining immune homeostasis and secondary lymphoid organ architecture. CCL21 has been identified as a lymphoid chemokine that is predominantly and constitutively expressed by high endothelial venules in lymph nodes and Peyer's patches, lymphatic vessels and stromal cells in spleen and appendix (11). CCL21 binds to the chemokine receptor CCR7 and is a chemoattractant for mature DC, naive and memory T cells (12, 13). Acting through the G-protein coupled CCR7 transmembrane receptor, CCL21 mediates the recruitment and co-localization of naive lymphocytes and antigen stimulated DC into T-cell zones of secondary lymphoid organs, facilitating T-cell activation (14, 15). T cell activation in vivo occurs in a lymphoid milieu that presents chemotactic and T cell receptor signals concurrently. The T cell zone chemokines such as CCL21 are bound to the surface of lymph node DC.
Contact with antigen-presenting cells bearing CCL21 chemokine costimulates T cells by a two-step contact mechanism. T cells initially form an antigen-independent ‘tethered’ adhesion on CCL21-bearing antigen-presenting cells. The formation of these tethers supersedes T cell receptor signaling and immunological synapse formation. However, chemokine–tethered T cells are hyper-responsive to subsequent contacts with antigen-presenting cells. Thus, T cells are costimulated ‘in trans’ and sequentially after initial engagement with their CCL21-rich environment (16). This chemokine, along with CCL19, is required for normal lymphoid tissue organization that is ultimately essential for effective T cell–DC interactions. DC are uniquely potent APCs involved in the initiation of immune responses. Serving as immune system sentinels, DC are responsible for Ag acquisition in the periphery and subsequent transport to T-cell areas in lymphoid organs where they prime specific immune responses. Thus, chemokines that attract both DC and lymphocyte effectors into the tumor can serve as potent agents in immunotherapy. In addition to inducing chemotactic migration, CCL21 costimulates expansion of CD4+ and CD8+ T cells and induces Th1 polarization. The immune suppressor cell population, CD4+CD25+ regulatory T cells are hypersensitive to CCL21 induced migration, and unresponsive to CCL21 co-stimulation (17). These functions of CCL21 to both attract naïve T cells as well as costimulate their proliferation, differentiation and activation suggests that CCL21 is a pivotal molecule for priming T cell responses and has therapeutic implications for local delivery of CCL21. The antitumor effectors NK and NKT cell subsets also express the CCR7 receptor and are chemotactically attracted by CCL21. The recruitment of NK and NKT cells is advantageous because these effectors can recognize tumor targets in the absence of MHC expression (18, 19). The use of chemokines to attract DC, lymphocyte, NK and NKT effectors into tumors can serve as an effective antitumor strategy. In addition, CCL21 has potent angiostatic effects, thus adding further support for its use in cancer therapy (20, 21).

III. INTRATUMORAL CCL21 ADMINISTRATION INDUCES ANTITUMOR RESPONSES IN VIVO

The development of intratumoral therapies to effectively augment local and systemic antitumor immunity in lung cancer can lead to a paradigm shift in the current forms of therapy. In pre-clinical model systems, intratumoral administration of DC led to both local and systemic antitumor responses (22). This form of therapy can be augmented by utilizing intratumoral administration of genetically modified DC overexpressing certain cytokine genes (23). Congruent with this overall concept, the intratumoral administration of recombinant CCL21 mediated T-cell–dependent antitumor responses (7). In immune competent mice, intratumoral CCL21 injection led to a significant increase in CD4 and CD8 T lymphocytes and DC infiltrating both the tumor and draining lymph nodes. Studies performed in CD4 and CD8 T cell knockout mice revealed a direct therapeutic requirement for both CD4 and CD8 T-cell subsets for CCL21-mediated tumor regression. These findings were the first demonstration of effective antitumor responses mediated by CCL21 (7). CCL21 mediated antitumor responses exhibited an increased influx of CD4 and CD8 T cell subsets as well as DC at the tumor sites. Accompanying this cell infiltrates were increases in IFNγ, MIG/CXCL9, IP-10/CXCL10, GM-CSF, and IL-12, but a concomitant decrease in the immunosuppressive molecules PGE-2 and TGFβ. Lymphocytes from CCL21 treated tumor-bearing mice demonstrated enhanced specific responses against autologous tumors suggesting the generation of systemic immune responses (7, 9). The importance of IFNγ, MIG/CXCL9 and IP-10/CXCL10 in CCL21 therapy was assessed. In vivo depletion of IP-10/CXCL10, MIG/CXCL9 or IFNγ indicate that the full potency of CCL19 or CCL21-mediated anti-tumor responses require the induction of IFNγ, MIG/CXCL9 and IP-10/CXCL10 in concert in this model. Neutralization of any one of these cytokines led to a decrease in the frequency of CXCR3+ve T cells and CD11c+ve DC in the tumor (9, 24).

Based on these results, experiments were performed to evaluate the tumorigenicity of CCL21 gene modified murine lung cancer cells. In all three tumor models, subcutaneous implantation of retroviral mediated CCL21 gene modified lung cancer cells led to T cell mediated tumor eradication. Following our initial description of the antitumor activities of CCL21, several groups have reported that CCL21 has potent antitumor properties in a variety of model systems (25-29). In all models, CCL21 demonstrated potent regression of tumors, which was shown to be dependent on host T cell immunity. All these studies reaffirmed the antitumor efficacy of CCL21 further supporting the rationale to proceed with clinical investigations of this chemokine.

IV. DEVELOPMENT OF CCL21 GENE MODIFIED DC THERAPY FOR LUNG CANCER

Our studies demonstrate that intratumoral administration of recombinant CCL21 reduced tumor burden in murine lung cancer models (7). However the antitumor activity induced by recombinant CCL21 required high and frequent dosing because proteins administered intratumorally are not retained locally for prolonged periods. Although these studies delineated the role of CCL21 as an effective antitumor agent, frequent high dose intratumoral administration is clinically limiting with the potential of unnecessary systemic toxicity. Based on the limitations of this approach, we examined the use of DC for intratumoral CCL21 delivery (9, 10). The intratumoral approach utilizes in situ tumor as a source of antigen. In contrast to immunization with purified peptide Ag, autologous tumor has the capacity to provide the DC administered at the tumor site access to the entire repertoire of available antigens in situ. This may increase the likelihood of a response and reduce the potential for tumor resistance due to phenotypic modulation. To achieve in-situ tumor antigen uptake and presentation, intratumoral (i.t.) administration of ex vivo-generated CCL21 gene modified murine bone marrow-
derived DC was utilized in a subcutaneous murine lung cancer model (9). To determine if a cell type other than DC expressing CCL21 could also induce tumor reduction in this model, fibroblast cells were also evaluated as a delivery vehicle. In addition to fibroblast cells ability to process and present antigens, the use of fibroblasts represents a promising treatment approach for lung cancer. These cells contribute to the formation of tumor-associated stroma (30) and the tumor microenvironment preferentially promotes their engraftment as compared with other tissues (31) making them an ideal system for tumor-selective delivery. We have data in support that reprogramming the tumor microenvironment with fibroblasts modified to express CCL21 alters the inflammatory infiltrates in the tumor microenvironment and promotes antitumor activity. The advantages of using transduced fibroblast cells for paracrine secretion of CCL21 are that fibroblasts: i) produce physiologically relevant levels of CCL21 after transduction, ii) are readily available for culture and expansion, iii) provide a platform for the development of CCL21-based antitumor strategies, iv) can process and present antigens to T cells and vi) may potentiate the activities of immune and innate effectors in the tumor microenvironment. For translation to lung cancer patients we have the option of utilizing bone marrow derived MHC matched GMP grade genetically modified donor stromal cells from a tissue bank that will circumvent autologous DC preparation, minimize batch to batch variability and allow for comparability and standardization.

DC or fibroblasts were transduced with an adenoviral vector expressing secondary lymphoid tissue chemokine (CCL21/SLC) to attract mature host DC and activated T cells at the tumor site. Established palpable tumors were treated with intratumoral DC-AdCCL21, Fib-AdCCL21 or controls. Intratumoral therapy with 10^6 DC-AdCCL21 (7-10mg/ml/10^6 cells/24 hrs of CCL21) at weekly intervals for 3 weeks showed tumor eradication in sixty percent of the mice whereas therapy cells/24 hrs of CCL21) at weekly intervals for 3 weeks showed based vectors to deliver CCL21. In the tumor model tested, intratumoral DC-AdCCL21 (7-10ng/ml/10^6 cells) or controls (diluent, DC (10^6 cells) and DC –AdCV (10^6 cells), AdCV (10^6 pfu) and AdCCL21 (10^6 pfu) was administered once into the lungs of 3 month-old transgenic mice. When evaluated at 4 months of age, there was reduced tumor burden in DC-AdCCL21 treated CC-10 mice compared with the control groups. Median survival was 18 ± 2 weeks for all control-treated mice. In contrast, mice treated with DC-AdCCL21 had a median survival of 24 ± 1 weeks (p<0.01 for DC-AdCCL21 compared to controls). In addition to marked tumor reduction, pathological examination revealed areas of distinct mononuclear infiltration in remaining tumor (10).

V. CLINICAL TRANSLATION OF DC-AdCCL21 THERAPY TO LUNG CANCER PATIENTS

Based on the results in the pre-clinical model systems, a clinical trial was initiated using intratumoral injection of CCL21 gene modified autologous DC in lung cancer. The intratumoral route of DC administration is used to activate specific immune responses within the tumor microenvironment and, in addition, to generate systemic immunity. Several studies suggest (22, 32) that intratumoral DC administration may be particularly effective as an antitumor strategy. Lung cancer patients have decreased numbers of circulating competent DC, thus, injecting DC within the lung tumor site may be a particularly effective approach. A correlation exists between the number of tumor-infiltrating DC and survival in cancer patients. In fact, there is a relationship between tumor-infiltrating DC aggregation and apoptosis in situ in human non–small cell lung cancer (NSCLC). This is consistent with recent studies indicating that attraction and activation of DC at the site of tumor elicits potent antitumor immunity (33). Dieu-Nosjean et al (6) have identified ectopic lymph node or tertiary lymphoid structures within human NSCLC specimens and demonstrated a correlation of their cellular content with clinical outcome. These structures have been referred to as tumor-induced bronchus-associated lymphoid tissue, which are follicle-like and contain germinal centers, similar to those in secondary lymphoid follicles of lymph nodes. The density of DC-Lamp, mature DC within these structures is a predictor of

MIG/CXCL9, IP-10/CXCL10 and IL-12 but decreases in the immunosuppressive mediators TGFβ and PGE2. DC-AdCCL21 treated tumor-bearing mice showed enhanced frequency of tumor specific T lymphocytes secreting IFNγ and induced protective immunity (9). The reduction in tumor growth may be explained by an increase in the frequency of activated T effector cell-mediated tumor apoptosis and/or T IFNγ mediated antiangiogenesis. In vivo depletion of IP-10/CXCL10, MIG/CXCL9 or IFNγ significantly reduced the antitumor efficacy of DC-AdCCL21 (9). Based on these observations, we determined the antitumor effects of DC-AdCCL21 in a clinically relevant model of lung cancer. We utilized transgenic mice in which the adenocarcinomas develop in an organ specific manner and have an average life span of 4 months. DC AdCCL21 (10^6 cells) or controls (diluent, DC (10^6 cells) and DC –AdCV (10^6 cells), AdCV (10^6 pfu) and AdCCL21 (10^6 pfu) was administered once into the lungs of 3 month-old transgenic mice. When evaluated at 4 months of age, there was reduced tumor burden in DC-AdCCL21 treated CC-10 mice compared with the control groups. Median survival was 18 ± 2 weeks for all control-treated mice. In contrast, mice treated with DC-AdCCL21 had a median survival of 24 ± 1 weeks (p<0.01 for DC-AdCCL21 compared to controls). In addition to marked tumor reduction, histological examination revealed areas of distinct mononuclear infiltration in remaining tumor (10).
long-term survival in lung cancer patients (6). These findings suggest that tumor-induced bronchus-associated lymphoid tissue have clinical relevance and participate in the host’s antitumor immune response, and they are consistent with previously reported preclinical and clinical data (34-36). For example, in murine tumor models, Mulé reported that DC genetically modified to secrete CCL21 can produce lymphoid cell aggregates and, importantly, prime naive T cells extranodally within a tumor mass, resulting in the generation of tumor-specific T cells and subsequent tumor regression (36, 37). Thus, the intratumoral approach may achieve tumor antigen presentation by using the tumor as an in vivo source of antigens for DC. In contrast to immunization with purified peptide antigen(s), autologous tumor has the capacity to provide the activated DC administered at the tumor site access to the entire repertoire of available antigens in situ. This may increase the likelihood of a response and reduce the potential for tumor resistance because of phenotypic modulation. On the basis of preclinical results, a phase I clinical evaluation has been initiated at University of California Los Angeles (in collaboration with the National Cancer Institute—Rapid Access to Intervention Development program now NCI Experimental Therapeutics Program) in patients with advanced stage NSCLC. The safety and clinical activities of the intratumoral administration of autologous DC transduced with a replication deficient adenoviral vector to express CCL21 in patients with pathologically confirmed and radiographically measurable NSCLC (Stage IIB/IV) who have tumor accessible by CT-guided or bronchoscopic intervention, and are refractory to standard therapy were selected. A GMP grade AdCCL21 replication deficient virus (38) has been made available through the RAID program to conduct the Phase I clinical trial. Human DCs transduced with adenovirus-CCL21 produce CCL21 to attract T cells and DCs. Preliminary findings demonstrate tumor specific systemic immune responses as assessed by the IFNγ T cell ELISPOT. Multiplex assessment of plasma cytokines before and after therapy in these patients revealed induction of IL-2, IFNγ, IL-12 and CXCL10. Immunohistochemistry of post tumor biopsies revealed an influx of CD4 expressing tumor infiltrating lymphocytes. Results thus far indicate that vaccination is safe with no associated adverse reactions at the DC-AdCCL21 (1 x 10^6, 5 x 10^6, 1 x 10^7, or 3 x 10^7 DC-AdCCL21 cells/injection) doses administered (days 0 and 7) and anti-tumor immune responses can be elicited particularly in higher doses.

VI. FUTURE PROSPECTS

The results of the ongoing phase I studies in lung cancer is promising and future trials could assess the combined efficacy of DC-AdCCL21 with radiation, chemotherapy or targeted therapy regimens. Based on the findings on CCL21 thus far, it is anticipated that the rational combinations with other treatment modalities will improve the therapeutic efficacy of this chemokine and antitumor benefit in a broad range of solid tumors. Utilizing preclinical tumor models, we are evaluating rational combinations for translation to the clinic. We are also evaluating non-DC based delivery mechanisms that will obviate the need to isolate and culture autologous DC but retain the antitumor activities of CCL21. In preclinical lung cancer models we are evaluating both autologous and allogeneic fibroblasts as a delivery vehicle for CCL21. Our results demonstrate that autologous fibroblasts are efficient for intratumoral delivery of CCL21 for the induction of antitumor activity. In contrast to intratumoral administered DC that migrate to nodes (23), the use of fibroblasts can prolong the localized secretion of CCL21 in the tumor microenvironment. In addition, GMP grade, MHC matched fibroblasts can be obtained from tissue banks that will obviate the need to isolate and culture DC. Although both DC and fibroblast cells are efficient delivery vehicles for CCL21, for widespread applicability, an efficacious off-the-shelf reagent would facilitate this chemokine-based therapy. Towards achieving this goal, we are evaluating a non-cellular based approach that utilizes vault nanoparticles for intratumoral CCL21 delivery for the purpose of initiating antitumor immune responses in lung cancer. We have initiated the preclinical characterization of CCL21-vault nanocapsules as an “off the shelf” therapeutic platform against tumor growth in vivo utilizing the well characterized Lewis lung cancer model. The CCL21-vault nanocapsule platform is an effective antitumor strategy. Our findings to date indicate that a single intratumoral administration of CCL21-vault nanocapsules recruit antitumor effectors that induce potent antitumor activity and inhibit tumor growth (39). This holds significance for broad application as an off the shelf reagent for cancer therapy. The vault particles can be designed as multifunctional vehicles that can be further engineered for target specific delivery as well as carriers of specific payloads that can act as tumor antigens to prime the immune system to potentially act as vaccines to prevent tumor recurrence and metastasis. These multifunctional nanoparticles may prove indispensable as cancer therapeutics. We are currently evaluating the vault nanocapsule delivery platform for its full therapeutic potential in lung cancer and other malignancies.

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