Targeting of CCR8 induces antitumor activity as a monotherapy that is further enhanced in combination with a Listeria-based immunotherapy

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ABSTRACT

CCR8 is a chemokine receptor that is expressed principally on regulatory T cells (Tregs) and known to be critical for Treg function, as CCR8+ Tregs can drive immunosuppression. Recent studies have demonstrated that CCR8 is uniquely upregulated in human tumor-resident Tregs of breast, colon, and lung cancer patients compared to normal tissue-resident Tregs. Therefore, CCR8 tumor-resident Tregs are rational targets for cancer immunotherapy. Here, we demonstrate that monoclonal antibody therapy targeting CCR8 significantly suppressed tumor growth and improved long-term survival in two different tumor-bearing mouse models. The antitumor activity could be correlated with an increase in tumor-specific T cells, enhanced infiltration of CD4+ and CD8+ T cells, and a significant decrease in the frequency of intratumoral Tregs. Initial studies explored a combinatorial regimen using anti-CCR8 mAb therapy and a Listeria-based cancer immunotherapy. Anti-CCR8 mAb therapy synergized with the Listeria-based immunotherapy to significantly delay tumor growth and induce complete regression in 20% of the mice. These results suggest that CCR8 represents a promising target for cancer immunotherapy, either as a single agent or in combination with other forms of immunotherapy.

INTRODUCTION

• Tumor-infiltrating Foxp3+CD4+ regulatory T cells (Tregs) are a major immune cell population that contribute to the establishment and maintenance of an immunosuppressive TME.
• Chemokine receptor CCR8 is predominantly expressed on Tregs.
• CCR8 has been shown to be a specific marker selectively upregulated by tumor-resident Tregs from several tumor types.
• Advaxis Lm platform can induce antigen-specific CD8+ effector T cells in mice.
• Lm-based immunotherapy has been shown, in preclinical cancer models and in the clinic, to change the local TME.
• These studies were designed to evaluate whether (1) CCR8 expression by Foxp3+ cells, (2) is associated with tumor infiltrating lymphocytes (TILs), and (3) CCR8 expression by TILs would synergize with an Lm-based immunotherapy to enhance antitumor activity and prolong survival in tumor-bearing mice.

RESULTS

• CCR8 is highly expressed by tumor-resident Tregs.
• CCR8 mAb treatment impairs tumor growth in solid tumor models.
• CCR8 mAb synergizes with a Listeria-based immunotherapy.

MATERIALS AND METHODS

Tumor models, tumor vaccine, and treatments: CT26 (300,000) and MC38 (300,000) cells were implanted subcutaneously (s.c.) in the right flank of mice. For therapeutic treatments, mice were treated with intraperitoneal (i.p.) injections of anti-CCR8 (4ug; clone: SA214G2) or control antibody (rat IgG1, Clone 11F12) as indicated. For combination studies, CT26 tumor-bearing mice were immunized i.v. twice on days 10 and 12 after tumor implantation, with either Lm-AH1 (1 x 10^7 CFU), an Lm vector expressing the Ah1 antigen (pB70, SPSVPYHQF) expressing on the CT26 murine tumor line (that is recognized by CD8+ T cells in the BALB/c mouse or LmddA274 (1 x 10^7 CFU), an Lm vector expressing a tumor-specific antigen.

Flow analysis: Tumors were enzymatically dissociated into single cell suspensions using a Stomacher machine (Steward) with Collagenase IV (Stem Cell Technologies). The resulting single-cell suspensions were immunoprecipitated with the following antibodies using standard staining procedures: anti-CD45, anti-CD4, anti-CD8, anti-CCR8 (clone SA214G2) (Balegaant), anti-CCR8 (GeneTex), anti-CD44, anti-CD25, anti-CD107a, anti-CTLA4, anti-FD1, anti-TCR, HMC class I peptide AH1 Tetramer, anti-IFN, anti-TNF, annexin V, and ImmunoG F(Dead) Fluorescent Protein Reactive Dye. For IFN-staining, cells were stimulated with either SINFBK peptide, AH1 peptide (pB70, SPSVPYHQF), and/ or cell stimulation cocktail plus protein transport inhibitors (Intronex). Events were acquired using the Attune flow cytometer (掣ter Scientific) and analyzed using FlowMax software (FlowMax). Tumor infiltrating lymphocytes (TILs) were defined as CD45+ cells.

In vitro assays: For Treg induction, 2 x 10^6 CD4+ T cells were isolated from spleens of BALB/c mice through negative selection (StemCell). The cells were seeded on plates pretreated with 2 microns of anti-CD3. Cells were incubated for 3 days with aCt28 (1ug/mL), IL-2 (1000 U/mL), and TGF-B1 (5ng/mL). The percent of converted Tregs was evaluated by flow cytometry on the third day. For inhibition of conversion, aCt28 was added at 10ug/mL. To measure suppression, 1 x 10^6 5-day cultured Tregs were cocultured for 48 hours with R1 medium and washed prior to being plated. Co-cultured T cells were treated for 3 days with aCt28 (1ug/mL), IL-2 (1000 U/mL) and indicated samples were treated with 10ng of aCt8.

RESULTS (cont.)

• CCR8 mAb treatment impairs tumor growth in solid tumor models.
• CCR8 mAb treatment enhances antitumor efficacy.
• CCR8 mAb treatment increases the frequency and functionality of CD8+ T cell responses.
• CCR8 combination therapy enhances antitumor efficacy.

SUMMARY

• CCR8 mAb therapy enhanced antitumor efficacy and improved long-term survival.
• CCR8 mAb increased tumor-specific T cells and significantly decreased tumor-resident CCR8+ Tregs.
• CCR8 blocking mAb prevented generation and suppressive function of Tregs.
• Lm-AH1 and aCCCR8 combination therapy enhanced antitumor efficacy and prolonged survival relative to Lm-AH1 or aCCCR8 alone.
• Lm-AH1 and aCCCR8 combination therapy increased CD8+ T cell responses and induced changes in the immune infiltrators in the TME.