Experimental Lung Metastases in Mice Are More Effectively Inhibited by Blockade of IL23R than IL23

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Abstract

Tumor-induced immunosuppression is mediated through various mechanisms including engagement of immune checkpoint receptors on effector cells, function of immunoregulatory cells such as regulatory T cells and myeloid-derived suppressor cells, and deployment of immunosuppressive cytokines such as TGFβ and IL10. IL23 is a cytokine that negatively affects antitumor immunity. In this study, we investigated whether IL23-deficient (IL23p19−/−) and IL23-deficient (IL23R−/−) mice phenocopied each other, with respect to their tumor control. We found that IL23R−/− mice had significantly fewer lung metastases compared with IL23p19−/− mice across three different experimental lung metastasis models (B16F10, LWT1, and RM-1). Similarly, IL23R blocking antibodies were more effective than antibodies neutralizing IL23 in suppressing experimental lung metastases. The antimetastatic activity of anti-IL23R was dependent on NK cells and IFNγ but independent of CD8⁺ T cells, CD4⁺ T cells, activating Fc receptors, and IL12. Furthermore, our data suggest this increased antitumor efficacy was due to an increase in the proportion of IFNγ-producing NK cells in the lungs of B16F10 tumor-bearing mice. Anti-IL23R, but not anti-IL23p19, partially suppressed lung metastases in tumor-bearing mice neutralized for IL12p40. Collectively, our data imply that IL23R has tumor-promoting effects that are partially independent of IL23p19. Blocking IL23R may be more effective than neutralizing IL23 in the suppression of tumor metastases.

Introduction

Tumor-induced immune suppression, used by tumors to evade immune destruction (1), can be mediated through lymphoid and myeloid cells. Although T-cell immune checkpoint blockade alone, or in combination with other antitumor approaches, has demonstrated clinical efficacy in many cancer types (2), many patients do not respond to these current therapies. As tumors can be heterogeneous with respect to lymphoid and myeloid infiltrates, there is now interest in targeting immunosuppressive pathways mediated by myeloid cells in the tumor microenvironment (TME).

Myeloid cells in the TME can be abundant and include tumor-associated macrophages and myeloid-derived suppressor cells (MDSC). MDSCs suppress antitumor immunity through mechanisms that include the production of immunosuppressive cytokines and metabolites such as TGFβ and IL10 or indoleamine-pyrole 2, 3-dioxygenase (IDO) and arginase 1 (ARG1) (3). We and others have demonstrated that IL23, a proinflammatory cytokine produced by myeloid cells, promotes tumor initiation, growth, and metastases in experimental and de novo mouse models of cancer (4–13). In humans, increased expression of IL23p19 subunit mRNA and serum IL23 has been reported in a number of malignancies including melanoma, colon, and lung cancers (4, 14).

IL23 belongs to the IL12 cytokine family, which also includes IL12, IL27, and IL35 (14, 15). These cytokines direct the development of immune responses in various disease conditions (15). Cytokines in this family are heterodimeric, in that their α and β subunits are differentially shared, as are the receptor chains for these cytokines (14). IL23 is composed of the IL12p40 and IL23p19 subunit, whereas its receptor consists of the IL12Rβ1 and IL23R subunits that bind to IL12p40 and IL23p19, respectively (16). Secretion of bioactive IL23 requires coexpression of the IL23p19 and IL12p40 subunits in the same cell and formation of disulfide bonds between those subunits (17). IL23 is thought to be predominantly produced by inflammatory myeloid cells, such as macrophages and dendritic cells (DC; ref. 17). In research that used IL23R-GFP reporter mice, IL23R was reported to be expressed on CD4⁺ T cells and also on innate immune cells including γδ T cells (18, 19), innate lymphoid cells (20) and a minor population of myeloid cells (19). NK T cells have also been reported to express IL23R (21).

Tumor-promoting inflammation is a hallmark of cancer (22). Although IL12 and IL23 are both proinflammatory cytokines and.
both use the IL12p40 subunit, they drive different immune responses (17). IL12 promotes differentiation of T helper 1 (Th1) cells and induces IFNγ production, which drives the T and NK-cell activation critical for antitumor immunity (23). We and others have demonstrated that the balance between IL12 and IL23 in the TME can shape the development of antitumor or protumor immunity. Thus, targeting the IL12 and IL23 axis may be beneficial. We have previously demonstrated, using IL23 gene-targeted mice or anti-IL23 neutralizing antibody, that host IL23 suppressed NK-cell-mediated control of metastasis (7, 9, 24). Although mice lacking a cytokine or its corresponding receptor most often phenocopy each other, this might not hold true for the IL12 cytokine family given their ability to share their α- and β-subunits. In this study, we asked whether loss of IL23p19 or IL23R had similar effects on antitumor immunity. We used IL23p19- or IL23R-gene-targeted mice and antibodies that neutralized IL23p19 or blocked IL23R.

Materials and Methods

Mice

C57BL/6 J wild-type (WT) mice were bred in-house or purchased from the Walter and Eliza Hall Institute for Medical Research, Melbourne, Australia. C57BL/6 IL12p35−/−, Flt3−/−, pfp−/− mice have been previously described (6, 25). C57BL/6 IL23p19−/− mice were generated as previously described (26). Briefly, mice were backcrossed by speed congenics to C57BL/6J (microsatellite analysis confirmed the resultant line 99.99% C57BL/6J) and obtained from Schering-Plough BioPharma. The syngeneic background of the IL23p19−/− strain was previously confirmed in our laboratory by skin grafting against C57BL/6 WT controls (as donor or recipients; ref. 6). C57BL/6 IL23R-deficient (IL23R−/−) mice were kindly provided by Vijay Kuchroo, Harvard Medical School, Boston, MA (19). All gene-targeted mice were bred and maintained at the QIMR Berghofer Medical Research Institute and used between the ages of 7 to 14 weeks. Groups of 5 to 10 mice per experiment were used. All experiments were approved by the QIMR Berghofer Medical Research Institute Animal Ethics Committee.

Cell culture

Mouse melanoma cell lines LWT1, B16F10 (ATCC, 2007), and prostate cancer line RM-1 were injected, maintained, and monitored as previously described (25). The LWT1 cell line was derived from the Bru α-melanoma mutant cell line SM1WT1 in 2014 by the intravenous injection of 5 × 10⁵ SM1WT1 into C57BL/6 WT mice as previously described (27). RM-1 (28) was generated as previously described (29) and incubated with anti-CD45.2-A780 (104), anti-CD45.1-ef450 (A20), anti-TCR b–d (H57-597), anti-NK1.1-PEcy7 (PK136), anti-NK1.1-APC (103), anti-CD8–PE (53.5.8), anti-IFNγ (clone H22) mAbs and control IgG antibody (clone 1-1) were purchased from Bio X Cell. Anti-asialoGM1 (ASGM1) was purchased from Wako Pure Chemicals.

Experimental tumor metastasis

B16F10, LWT1, and RM-1 were injected i.v. into the tail vein of WT or gene-targeted mice with the indicated number of cells as stated in the figure legends. Lungs were harvested on day 14, and surface tumor nodules were counted under a dissection microscope as previously described (25).

Bone marrow transplantation and reconstitution

Bone marrow cells were obtained from the femurs of donor C57BL/6 WT (PTPRCA, CD45.1−/−) and IL23R−/− (C57BL/6, CD45.2−/−) mice. Two doses of 5.5 Gy of whole body irradiation were administered to recipient WT (PTPRCA, CD45.1−/−) and IL23R−/− (C57BL/6, CD45.2−/−) mice at 4 hours interval. Recipient mice were injected i.v. with 5 × 10⁴ bone marrow cells/mouse after irradiation. Mice were provided with water containing neomycin for 4 weeks. Seven weeks after bone marrow transplantation, mice were eye-bled and immune cells were analyzed by flow cytometry using congenic CD45.1 and CD45.2 markers to assess immune cell reconstitution before mice were used experimentally a week later.

Flow cytometry

Naïve or tumor-bearing lung single-cell suspensions were generated as previously described (25) and incubated with anti-CD16/32 (2.4G2) to block Fc receptors on ice prior to surface staining. The following antibodies were used for FACS analysis: anti-CD45.2-A780 (104), anti-CD45.1-ef450 (A20), anti-TCR b–d (H57-597), anti-NK1.1-PEcy7 (PK136), anti-NK1.1-FTC (PK136), anti-NKp46-ef450 (29A1.4), and live/dead dye Zombie Aqua (all from BioLegend, eBioscience). For intracellular cytokine staining, cells were surface stained as described above and then fixed and permeabilized with a cytotox/cytperm kit (BD Biosciences) followed by staining with anti-IFNγ (XMG1.2) or isotype (eBio299Arm) antibody (both from BioLegend). Before samples were run on a flow cytometer, liquid-counting beads (BD Biosciences) were added directly to determine absolute cell counts in samples. All data were collected on a Fortessa 4 flow cytometer (BD Biosciences) and analyzed with FlowJo v10 software (TreeStar, Inc.).

Ex vivo lung NK-cell cytokine assay

One fifth of a whole lung suspension from the indicated groups was incubated at 200 μl/well in a 96-well U-bottom plate in complete RPMI media. Cells were incubated for 24 hours in the presence or absence of IL12 (eBioscience; 10 pg/ml) and IL18 (20 ng/ml; R&D Systems). Cells were stained for surface markers and intracellular IFNγ as described above.

Statistical analysis

GraphPad Prism software was used for statistical analysis. One-way ANOVA with Tukey posttest for multiple comparisons was used. Differences between groups were considered to be statistically significant where the P value was less than 0.05.
Results
IL23R−/− mice have fewer experimental lung metastases than IL23p19−/− mice
We first set up a series of experiments where groups of WT, IL23p19−/−, IL23R−/− mice were injected with B16F10 or LWT1 (BrafV600E mutant) melanoma or RM-1 prostate cell lines (Fig. 1). As we previously demonstrated, IL23p19−/− mice had fewer B16F10 experimental lung metastases compared with WT mice (Fig. 1A; ref. 6). The number of B16F10 lung metastases was similar for IL23R−/− mice or WT mice carrying WT bone marrow transplants. And, the number of B16F10 lung metastases was similar for IL23R−/− mice or WT mice carrying IL23R−/− bone marrow transplants (Fig. 1E). Overall, these data suggest that hematopoietic cells rather than nonhematopoietic cells express IL-23R, which contribute to the generation of an immunosuppressive tumor microenvironment after IL-23R activation.

Suppression of B16F10 lung metastases in IL23R−/− mice requires NK cells and IFNγ
We next explored the mechanism by which lung metastases were controlled in B16F10 tumor-bearing IL23R−/− mice (Fig. 2). B16F10 tumor cells (5 × 104) were injected into IL23R−/− or WT mice. The mice were then treated with cIg or anti-ASGM1 to deplete NK cells (Fig. 2A). C Ig-treated tumor-bearing IL23R−/− mice had fewer lung metastases compared with cIg-treated WT mice (Fig. 2A). In contrast, tumor-bearing WT and IL23R−/− mice depleted of NK cells displayed more lung metastases compared with similar groups treated with cIg (Fig. 2A). To confirm the high number of metastases was not masking NK-cell–independent antitumor effects in tumor-bearing IL23R−/− mice, we repeated the experiment with fewer B16F10 cells (1 × 104) injected into NK-cell depleted WT or IL23R−/− mice. With the lower dose of tumor cells, we were able to accurately quantitate lung metastases and we found no difference between tumor-bearing WT or IL23R−/− mice (Fig. 2B). These results demonstrated that the antitumor efficacy of IL23R−/− mice was mediated solely by NK cells. In contrast, CD4+ and CD8+ T cells were not required for the ant metastatic activity of IL23R−/− mice as a similar decrease in numbers of lung metastases was observed when comparing mice that received anti-CD4/CD8 or cIg (Fig. 2C). Finally, we demonstrated that the effect of IL23R loss on lung metastases required IFNγ since B16F10 (5 × 104) tumor-bearing IL23R−/− mice displayed a significant increase in lung metastases following IFNγ neutralization (Fig. 2D). To exclude the possibility that activation of nonhematopoietic cells, which might express IL23R, could contribute to the immunosuppressive lung environment of tumor-bearing mice, we generated 4-way bone marrow chimeric mice from WT or IL23R−/− donors (Fig. 2E). The number of B16F10 lung metastases was similar for IL23R−/− mice or WT mice carrying WT bone marrow transplants. And, the number of B16F10 lung metastases was similar for IL23R−/− mice or WT mice carrying IL23R−/− bone marrow transplants (Fig. 2E). Overall, these data suggest that hematopoietic cells rather than nonhematopoietic cells express IL-23R, which contribute to the generation of an immunosuppressive tumor microenvironment after IL-23R activation.

More IFNγ-producing NK cells with tumor in IL23R−/− than in IL23p19−/− mice
We have previously demonstrated that NK-cell homeostasis in naïve IL23p19−/− mice was normal, as these mice had numbers and proportions of all mature NK-cell subsets (defined by CD11b/CD27) similar to age-matched WT mice (6). We confirmed that total CD45.2+ hematopoietic cells and total NK-cell numbers in the lungs, spleen, and lymph nodes were similar between naïve IL23R−/− mice and WT mice (Supplementary Fig. S1) and that there were no differences in NK-cell subsets (29). IL23R−/− mice are healthy and fertile and have numbers of CD4+ cells, CD8+ cells, B cells, γδ T cells, CD11c+ cells, and CD11b+ cells similar to WT control mice (19).

We analyzed NK-cell numbers, activation status, and effector function to understand why IL23R−/− mice had fewer lung metastases than IL23p19−/− mice (Fig. 3; Supplementary Fig. S1). Groups of WT, IL23p19−/−, IL23R−/− mice were injected with B16F10. Their lungs were harvested a day later, and single-cell suspensions generated for FACS analysis. We observed no changes in CD45.2+ immune cells between the three tumor-bearing groups (Supplementary Fig. S1A). We observed a small but statistically significant increase in NK-cell numbers in tumor-bearing IL23R−/− mice compared with tumor-bearing WT or IL23p19−/− mice (Supplementary Fig. S1B), but observed no changes in T-cell (CD3/CD8) numbers, NK-cell activation status (CD69), or in NK-cell subsets (as defined by CD11b and CD27).
From the same experiment, one fifth of a whole lung suspension from naïve or tumor-bearing WT, IL23p19KO, IL23RKO mice was cultured for 24 hours in the presence or absence of IL12/IL18 and assessed for its capacity to produce IFNγ (Fig. 3). The proportion of IL12/IL18 stimulated NK cells producing IFNγ in tumor-bearing IL23RKO mice (31.34% ± 5.02%) was greater than in tumor-bearing WT (8.13% ± 1.50%) or IL23p19KO mice (14.50% ± 3.66%; Fig. 3A). A similar trend was also observed for nonstimulated NK cells from tumor-bearing IL23RKO mice, although the levels of IFNγ were lower (Fig. 3B). In contrast, amounts of IFNγ produced by naïve NK cells isolated from nontumor-bearing WT, IL23p19KO, or IL23RKO mice were generally similar, whether these cells were stimulated or not (Fig. 3). Overall, these data suggest the increased protection from lung metastases in IL23RKO mice was due to an increased proportion of NK cells that produced IFNγ.

Fewer lung metastases in anti-IL23R– compared with anti-IL23p19–treated mice

We have previously demonstrated that neutralizing antibodies specific to the IL23p19 subunit of IL23 decreased the number of experimental lung metastases in WT mice (9). We next assessed whether anti-IL23R blocking antibody recapitulated the antitumor activity observed in the IL23RKO mice (Fig. 4). We conducted a dose-titration experiment with anti-IL23R in B16F10 tumor-bearing WT mice to determine the optimal dose (Fig. 4A). Although a 100 μg dose of anti-IL23R given on days –1, 3, and 7 reduced the numbers of B16F10 lung metastases compared with clg-treated mice, a higher dose of anti-IL23R (200 or 500 μg) suppressed B16F10 lung metastases to numbers similar to those determined for IL23RKO mice (Fig. 4A). We therefore used anti-IL23R at a dose of 200 μg for subsequent experiments.
We next compared the antitumor efficacy of anti-IL23R (200 μg/mouse/dose) and anti-IL23p19 (500 μg/mouse/dose) mAbs to suppress B16F10 lung metastases. This optimized dose of anti-IL23p19 was used as we previously reported (7). Validating what we observed with the gene-targeted mice, anti-IL23R had greater antitumor activity than did anti-IL23p19 (Fig. 4B). We also compared how the frequency of dosage affected the efficacy of anti-IL23R: we administered therapy on either days −1, 1, and 3, or −1, −3, and 7. Although both schedules significantly suppressed lung metastases compared with clg− or anti-IL23p19−treated mice, the 3-dose strategy of anti-IL23R was more effective than the 2-dose strategy in reducing lung metastases to numbers observed in IL23R−/− mice (Fig. 4B). In subsequent experiments, we utilized this 3-dose schedule. Treatment of tumor-bearing IL23R−/− mice with anti-IL23R did not further suppress lung metastases compared with the clg-treated group, demonstrating the specificity of the antibody (Fig. 4B). Finally, we asked whether anti-IL23R was more effective compared with anti-IL23p19 in two other experimental lung models. Similar to IL23p19−/− mice, a further reduction in LWT1 and Rb1−1 lung metastases was seen in groups treated with anti-IL23R compared with groups treated with anti-IL23p19 (Fig. 4C and D).

Requirements for antitumor activity of anti-IL23R

We next determined if the mechanism of anti-IL23R antitumor activity was similar to what we observed with the IL23R−/− mice (Fig. 5). In mice injected with a lower dose of B16F10 (5 × 10⁴ cells), NK-cell depletion or IFNγ neutralization resulted in loss of suppression in anti-IL23R−treated mice compared with clg-treated groups (Fig. 5A and B). In contrast, depletion of both CD44− and CD88− T cells did not affect the antitumor activity of anti-IL23R (Fig. 5C). In addition to IFNγ, NK cells can also utilize perforin (pfp), which is cytotoxic, to mediate their effector function (30). Although deficiency in perforin led to increased lung metastases compared with WT mice, anti-IL23R therapy in pfp−/− mice still significantly suppressed lung metastases, suggesting anti-IL23R therapy did not require perforin (Fig. 5D). Given that the efficacy of antibodies with an IgG1 isotype can sometimes require Fc binding (31, 32), we also set up an experiment where WT mice or mice lacking all activating Fc receptors (Fcyγ−/− mice) were injected with B16F10 and treated with clg or anti-IL23R. Although differences in the number of metastases between WT mice and Fcyγ−/− mice were observed, in concert with previous observations (25), suppression of metastases was still observed in anti-IL23R−treated Fcyγ−/− mice, suggesting that anti-IL23R activity was not dependent on activating Fc receptors (Fig. 5E).

IL23R has tumor-promoting activity that is partially independent of IL23p19

IL12 controls lung metastases via NK-cell activation (33). Therefore, we determined whether loss of IL12 impaired the antitumor activity of anti-IL23R therapy (Fig. 6). Given that IL12 is made up of IL12p35 and IL12p40 subunits, we treated B16F10 tumor-bearing IL12p35−/− mice with anti-IL23R. We also treated WT tumor-bearing mice with anti-IL12p40 and anti-IL23R (Fig. 6A and B). As expected, the number of lung metastases was increased in IL12p35−/− mice compared with WT mice (Fig. 6A), as we previously demonstrated (6). Nevertheless, anti-IL23R in these mice still significantly suppressed the number of lung metastases compared with the clg-treated group. Addition of anti-IL12p40 to the anti-IL23R group suppressed the number of lung metastases compared with anti-IL12p40−treated group, although not to the same extent as mice only treated with anti-IL23R (Fig. 6B). In contrast, addition of anti-IL12p40 to anti-IL23p19−treated groups resulted in the loss of metastatic control, although the number of lung metastases did not differ between anti-IL12p40− or clg-treated WT mice (Fig. 6B). When we treated tumor-bearing WT mice with anti-IL23R and anti-IL23p19 in combination or tumor-bearing IL23p19−/− mice with anti-IL23R, we did not observe any further decrease in numbers of B16F10 lung metastases compared with similar groups of mice only treated with anti-IL23R. Thus, it seems unlikely that other extracellular immunosuppressive cytokine(s) utilize the IL23p19−/IL23R signaling pathway (Fig. 6C). Overall, these data suggested that IL23R may have tumor-promoting activity partially independent of IL23.

Tumor-bearing mice treated with anti-IL23R have more IFNγ-producing NK cells

We asked if the improved efficacy of anti-IL23R compared with anti-IL23p19 in suppressing lung metastases was due to an increase in the proportion of IFNγ producing NK cells. We set up the experiment as described in Fig. 3. Groups of WT mice were treated with anti-IL23p19, anti-IL23R, or clg 1 day before B16F10 injection. One day after the B16F10 injection, lungs were
harvested and single-cell suspensions analyzed by flow cytometry. Similar to the results in IL23R−/− mice, there were no significant changes in total CD45.2+ or NK-cell immune infiltrates. Stimulating one-fifth of a whole lung suspension with IL12/IL18, we observed that the proportion of NK cells that produced IFNγ was significantly higher in anti-IL23R-treated groups compared with anti-IL23p19− or clg-treated groups (Fig. 6D). In contrast, similar proportions of NK cells produced IFNγ in anti-IL23p19 and clg-treated groups. Given that IL23 can induce granulopoiesis (34), we also assessed infiltration of various myeloid cells including neutrophils, inflammatory monocytes, macrophages, alveolar macrophages and dendritic cells in the lungs of B16F10 tumor-bearing mice 3 days after anti-IL23R, anti-IL23p19, or clg treatment. However, we did not observe any changes in these the myeloid cell subsets that might explain the improved efficacy of anti-IL23R compared with anti-IL23p19 mAb. Overall, our data suggested that antibodies blocking IL23R may be superior in suppressing experimental lung metastases compared with those that neutralize IL23p19. Antibodies that block IL23R may better enhance NK-cell IFNγ production and IL23R may have a tumor-promoting function that is partially independent of IL23.

Discussion

There is accumulating evidence for the tumor-promoting role of IL23 in tumor initiation, growth, and metastasis as demonstrated preclinically using mice deficient for IL23p19, IL23R, or neutralizing antibodies to IL23p19 (4–13). In this study, we assessed whether loss of IL23R or IL23p19 was equivalent in suppressing metastases. We found that targeting IL23R compared with IL23p19 was more effective in suppressing three different experimental lung metastasis models. This was demonstrated using gene-targeted mice deficient for IL23R or IL23p19. We further showed that the improved antimetastatic activity mediated through targeting IL23R compared with IL23p19 was due to an increase in the proportion of NK cells that produced IFNγ. Our studies suggest that IL23R may have a tumor-promoting function independent of extracellular IL23. Other IL23-dependent experimental and de novo mouse models of tumorigenesis could be evaluated to determine whether targeting IL23R or IL23p19 is also more effective in those models.
Although receptor and ligand knockout mice generally phenocopy each other, this was not the case for IL23p19−/− and IL23R−/− mice. The IL23/IL23R signaling pathway does more than promote tumors: it also functions in the etiology of many autoimmune diseases, such as psoriasis and inflammatory bowel disease (17). In a mouse model of chemically induced colitis, Cox and colleagues also reported that IL23p19−/− and IL23R−/− mice did not exactly phenocopy each other as the histological scores of IL23p19−/− mice were less severe than those of IL23R−/− mice (35).

The underlying mechanisms for the difference seen in the IL23R−/− and IL23p19−/− mice in tumor control are unknown but there may be various explanations. First, there is a possibility that IL23p19 can interact with other subunits of the IL12 cytokine-receptor family besides IL12p40, IL12RB1, and IL23R. One study described the association of the IL23p19 α-subunit with the β-subunit EBI3 in human keratinocytes following TLR3 engagement (36). The IL23p19 subunit as a monomer may also be biologically active on its own or may be able to form a homodimer. In another study, activated human endothelial cells were reported to express intracellular IL23p19 that associated with the cytokine-receptor subunit gp130 (37). Although these cells could not produce IL12p40 to form heterodimers with IL23p19, which is necessary for its secretion, the authors reported that the subunit of IL23p19 could signal via gp130 to activate STAT3 signaling resulting in the upregulation of adhesion molecules (37). Whether intracellular IL23p19 alone can associate and signal via IL23R remains unknown, but such a signaling pathway may explain why anti-IL23p19 is not as effective as anti-IL23R since intracellular
IL23p19 may not be targeted by anti-IL23p19. Alternatively, it might be possible that an undiscovered cytokine besides IL23p19 can also bind to IL23R to promote tumor metastases. Although IL23 is produced by myeloid cells in mice (17), Myc-oncogene activation specifically in epithelia cells triggered their production of IL23 in KrasG12D-driven lung adenocarcinomas, which drove stromal changes that promoted tumorigenesis (13). The responding IL23R-expressing cells remain unidentified. Our data showed that the number of metastases was similar after WT bone marrow transfer into WT or IL23R+/- mice, or after IL23R+/- bone marrow transfer into WT or IL23R+/- mice. These results suggest that hematopoietic cells rather than nonhematopoietic cells express IL23R and contribute to the generation of an immunosuppressive tumor microenvironment following IL23R activation.

In humans, increased expression of IL23p19 mRNA and serum IL23 characterizes a number of malignancies, including melanoma, colon, and lung cancer (4, 14). Expression of IL23R mRNA, upregulated in some primary NSCLC specimens, may be epigenetically regulated by chemotherapy such as gemcitabine (38).

Whether other human malignancies also express IL23R and can be targeted directly by anti-IL23R remains to be investigated. Unlike human cell lines, mouse tumor cell lines generally do not express IL23R or produce IL23 (14), and it is unlikely that blocking IL23R affects their growth directly. Our observation that a similar reduction in the number of B16F10 lung metastases in IL23R−/− mice compared with anti-IL23R–treated mice further supports this viewpoint. Similarly, NK cells have been reported to not express IL23R (19), and thus it is likely that the efficacy of anti-IL23R treatment lies in its attenuation of IL23R-expressing immune cell numbers or functions that negatively regulate NK-cell function. Although it is unlikely that Fc-mediated depletion of IL23R-expressing cells is a mechanism for the antimetastatic efficacy of anti-IL23R, the use of IL23R-GFP reporter mice to determine whether the proportion or numbers of IL23R-expressing cells are attenuated in the lungs of tumor-bearing IL23R−/− mice could shed light on this matter. In addition, IL23R+/- cells can be assessed for production of various immunosuppressive cytokines or changes in cell-surface receptors that may negatively

Figure 6.
Mice bearing B16F10 lung metastases treated with anti-IL23R have increased frequency of IFNγ-producing NK cells. Groups of C57BL/6 WT, or the indicated gene-targeted female mice (n = 4–7/group) were injected i.v. with (A–D) 1 × 10^6 B16F10 melanoma cells on day 0. A–C, Some groups of mice were treated i.p. with either clg (200 µg/mouse), anti-IL23p19 (500 µg/mouse), or anti-IL23R (200 µg/mouse) alone or with the indicated combination on days −1, 3, and 7 relative to tumor cell inoculation. Additionally, some groups of mice were treated i.p. with (B) clg or anti-IL12p40 (500 µg/mouse) on days −1, 3, and 7 relative to tumor cell inoculation. On day 14, the metastatic burden was quantified in the lungs by counting colonies on the lung surface. Data presented as mean ± SEM. Experiments in A were performed once, whereas data in B, C were pooled from 2 independent experiments. D, On day −1, groups of mice were treated i.p. with clg (500 µg/mouse), anti-IL23p19 (500 µg/mouse) or anti-IL23R (200 µg/mouse) followed by i.v. injection with B16F10 melanoma cells on day 0. One day after tumor inoculation, tumor-bearing lungs were harvested and single-cell suspensions generated. One fifth of the whole lung cell suspensions were simulated for 24 hours in the presence of IL12 (10 pg/mL) and IL18 (20 ng/mL) before FACS analysis to assess the proportion of IFNγ-producing NK cells (NK1.1+NKp46+TCRβ−) among total NK cells. Data shown are pooled from 3 independent experiments and are represented as mean ± SEM. Significant differences between groups as indicated by crossbars were determined by one-way ANOVA followed by Tukey post hoc test; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001.
module NK-cell functions compared with IL23R-GFP heterozygous mice which remain responsive to IL23. Furthermore, IL23R-expressing cells from the lungs of tumor-bearing IL23R+/– or IL23R−/− mice could be cultured in vitro with IL23 to determine what changes are dependent on extracellular IL23.

Antibodies that specifically neutralize IL23 have been developed, and their efficacy demonstrated in clinical trials studying the treatment of various immune-mediated inflammatory diseases (17). In contrast, antibodies that block IL23R have not yet been studied in clinical trials. A fully human monoclonal antibody targeting human IL23R that had cross reactivity for both human and cynomolgus monkey IL23 receptors has been characterized (39). This anti-human-IL23R was more effective at inhibiting IL23-induced Kit-225 cell proliferation than the anti-IL12/23p40 antibody, ustekinumab, although comparison characterized (39). Given the IL23R expression is likely to be normally lower than IL23 expression, and our results show the value of targeting IL23R over IL23p19, blocking IL23R may offer better antitumor efficacy than neutralizing IL23 for treatment of malignancies controlled by NK cells.

Disclosure of Potential Conflicts of Interest

M.J. Smyth reports receiving a commercial research grant from Bristol-Myers Squibb, Tiziana Therapeutics, Aduro Biotech, and Corvus Pharmaceuticals, has received honoraria from speakers bureau of Merck Sharpe Dohme, and is a consultant/advisory board member for Tiziana Therapeutics. M.W.L. Teng has received honoraria from speakers bureau of Merck Sharpe Dohme. No potential conflicts of interest were disclosed by the other authors.

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