Eosinophils Increase Metastasis to the Lungs in a Mouse Model of Lung Cancer

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BRIEF. This research focused on the relationship between eosinophils and lung cancer and the reduction of metastasis through the use of an IL-5 antibody.

ABSTRACT. Tumor associated eosinophilia has been observed in various human tumors including in the lung. However, the role of eosinophils in lung tumorigenesis and the mechanisms of recruitment of these cells during the disease remain undefined. Interleukin-5 (IL-5) has been recognized as a major chemokine involved in the recruitment of eosinophils and thought to be associated with their maturation. Therefore, we hypothesized that deficiency of IL-5 in mice will impair recruitment/maturation of eosinophils and reduce lung tumorigenesis. We used a model of lung cancer metastasis by injecting Lewis Lung Carcinoma (LLC) or B16F10 (melanoma) cells by tail vein injection (IV) into mice deficient in IL-5 (IL-5 KO) or wild type (WT) controls (both on C57BL/6 background, which is a common inbred strain of laboratory mice used in research pertaining to human diseases). We found that by Day 14 after injection of 2.5x10^5 LLC or 1.25 x10^5 B16F10 cells, IL-5 KO mice had marked decrease in numbers of lung eosinophils compared to the WT controls. These changes were accompanied with statistically significant reduction in numbers of lung tumors in IL-5 KO mice. Depletion of IL-5 in WT mice by treatment with IL-5 blocking antibodies reduced lung eosinophils and decreased numbers of LLC or B16F10 tumors in the lung. In contrast, reconstitution of IL-5 by giving back recombinant IL-5 through IV injection prior to the injection of LLC or B16F10 cells increased numbers of lung tumors in IL-5 KO mice. Taken together, we conclude that IL-5 supports metastasis of Lewis Lung Carcinoma (LLC) or B16F10 (melanoma) tumors to the lungs most likely through recruitment of eosinophils.

INTRODUCTION.

Cancer is a disease that is prevalent around the world, transcending racial, socioeconomic, and environmental barriers. More men and women in the United States die from lung cancer than any other type of cancer [1]. Lung cancer causes more deaths than the next three most common cancers combined (colon, breast and prostate). An estimated 160,340 Americans were expected to die from lung cancer in 2012, accounting for approximately 28 percent of all cancer deaths [2]. Over half of people with lung cancer die within one year of being diagnosed [3].

The immune system plays a complex role in lung cancer growth and metastasis; however, the precise roles of specific mediators and subsets of immune cells are not well characterized. IL-5 is a Th2 cytokine that is a potent eosinophil chemoattractant, or chemical agent that induces cellular migration [4]. Eosinophils are a type of white blood cells that are involved in host defense against helminth (worm) infections, asthma, and allergies. In normal individuals, eosinophils make up about 1-6% of white blood cells and are about 12-17 micrometers in size [5]. Patients with tumor-associated eosinophilia, a condition in which a patient has elevated eosinophil numbers, usually have poor outcomes [6].

MATERIALS AND METHODS.

IL-5 deficient (IL-5 KO) and wild type (WT) mice were injected via the tail vein with 2.5 x 10^5 Lewis Lung Carcinoma (LLC) cells or 1.25 x 10^5 B16F10 (Melanoma) cells. Lungs from mice were harvested at 14 days post injection for determination of surface tumor numbers and eosinophil numbers by immunostaining for major basic protein (MBP).

IL-5 antibodies (1 mg/kg) were delivered by IV injection. WT mice injected with the IL-5 antibody and untreated WT mice were harvested at 14 days post injection for determination of surface tumor numbers and eosinophil numbers by immunostaining for MBP.

IL-5 reconstitution was accomplished by intraperitoneal injection of recombinant IL-5 (50 ng) or phosphate buffered saline (PBS) control every other day for 14 days beginning the day of LLC cell injection. Lungs from mice were harvested after 14 days for determination of surface tumor numbers and eosinophil numbers by immunostaining for MBP.

Eosinophils were harvested from WT mice through a bronchoalveolar lavage (BAL). These eosinophils were then injected IV into IL-5 KO mice. One hour after eosinophil introduction, cancer cells were injected IV. Lungs were harvested 14 days later.

RESULTS.

IL-5 KO mice develop fewer lung tumor metastases compared to controls. These findings correlate with fewer eosinophils in the lungs of IL-5 KO mice. The number of lung metastases is significantly lower in the IL-5 KO mice than in the WT mice (Figure 1.A). The eosinophil count was also significantly lower in the IL-5 KO mice than in the WT mice (Figure 1.E).

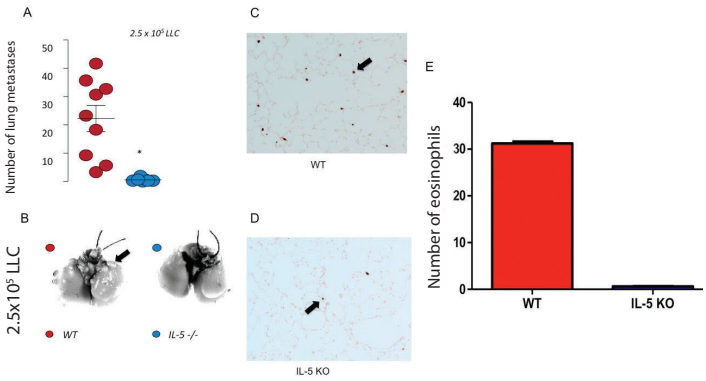

Figure 1. (A) There were fewer lung metastases in IL-5 KO Mice than in WT Mice. (B) Tumors appear as white dots in the mice lungs. The WT lung has more tumors than the IL-5 KO lung. (C and D) Eosinophils appear as black dots in the MBP staining. The WT lung (C) has more eosinophils than the IL-5 KO lung (D). (E) WT lungs tend to have many more eosinophils compared to the IL-5 KO lungs.

Depleting IL-5 in WT mice with an IL-5 depleting antibody reduces lung metastases as well as the number of eosinophil in the lungs. Aside from two outliers, every data point for the number of lung metastases in WT mice injected with the IL-5 depleting antibody was below the mean value for the number of lung metastases in WT mice (Figure 2.A). The number of eosinophils in both groups mirrors these results, with the eosinophil count being far lower in the WT mice injected with the IL-5 depleting antibody than in the WT mice (Figure 2.B).

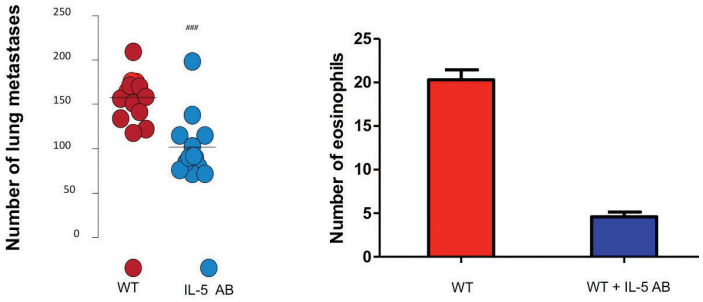

Figure 2. (A) WT Mice injected with the IL-5 antibody have fewer tumors than the WT control group. (B) WT Mice injected with the IL-5 antibody have fewer eosinophils than the WT control group.

Reconstitution of IL-5 in IL-5 KO mice increases lung tumor formation and eosinophil numbers. IL-5 KO mice injected IV every other day with IL-5 had more eosinophils than their untreated IL-5 KO counterparts (Figure 3.A). The IL-5 KO mice injected with the IL-5 also had more lung metastases than their untreated IL-5 counterparts, but less than the WT control (Figure 3.B). The gap in tumor numbers between the WT and IL-5 KO + IL-5 mice is most likely due to the amount and timings of IL-5 injections not mimicking the rate in which IL-5 is produced naturally by the WT mice.

Reintroducing eosinophils into IL-5 KO mice resulted in increased number of lung tumors. The IL-5 KO mice injected with reconstituted eosinophils had on average four times the number of eosinophils than the untreated IL-5 KO mice (Figure 3.C). The number of lung metastases was much higher in IL-5 KO mice injected with eosinophils than it was in the untreated IL-5 KO mice and very close to the number of lung tumors in the WT mice (Figure 3.D).

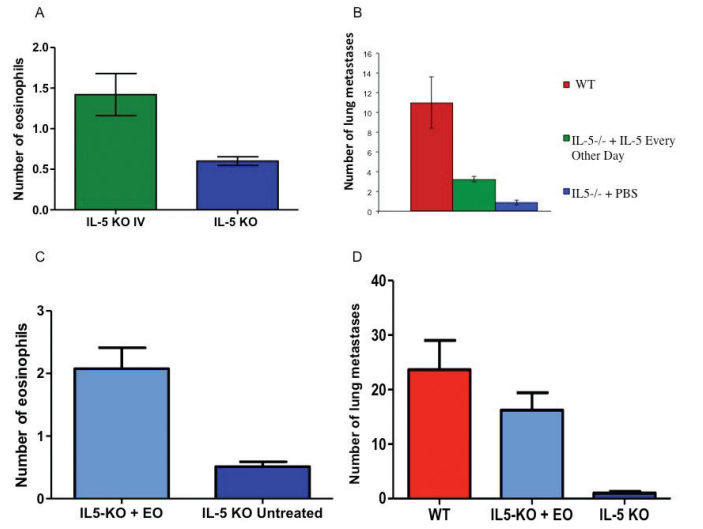

Figure 3. (A) The IL-5 KO mice injected with IL-5 had more eosinophils than the IL-5 KO mice. (B) The mice injected with IL-5 had more lung tumors than the IL-5 KO mice but less than the WT control. (C) The number of eosinophils in the IL-5 KO mice with reconstituted eosinophils was higher then in the IL-5 KO mice. (D) The mice injected with the reconstituted eosinophils had significantly more lung tumors than the IL-5 KO mice but less than the WT control.

DISCUSSION.

IL-5 regulates eosinophil recruitment to the lungs. Mice with higher levels of IL-5 have more eosinophil in their lungs. The WT mice had more eosinophils than the IL-5 KO mice (Figure 1.E), the WT mice had more eosinophils than the WT mice injected with the IL-5 depleting antibody (Figure 2.B), and the IL-5 KO mice injected with IL-5 had more eosinophils than the untreated IL-5 KO mice (Figure 3.A).

Eosinophils recruited to the lungs enhance metastasis to the lungs. Mice with more eosinophils in their lungs tended to have higher tumor counts. The WT mice had more lung metastases than the IL-5 KO mice (Figure 1.A). The WT mice had more lung tumors than the WT mice injected with an IL-5 depleting antibody (Figure 2.A). The WT mice and the IL-5 KO mice injected with IL-5 had more lung tumors than the untreated IL-5 KO mice (Figure 3.B). The WT mice and IL-5 KO mice injected with reconstituted eosinophils had more lung metastases than the untreated IL-5 KO mice (Figure 3.D).

CONCLUSION.

The mechanisms by which IL-5 influences host-tumor interactions require further study. Further research needs to explore the reasons why eosinophils promote tumorigenesis in the lungs.

Administration of IL-5 antibodies could prevent cancer metastasis in humans. The use of IL-5 antibodies would be extremely useful in cases in which a lung tumor has been found and surgical treatment is not possible at the time. By administering IL-5 antibodies, a physician can lower the risk of metastasis in the patient until operation on the tumor is possible.

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REFERENCES.

1. CDC (2009).
2. American Cancer Society. Cancer Facts and Figures (2012).
3. U.S. National Institutes of Health. National Cancer Institute: SEER Cancer Statistics Review, (1973-2008).
4. Sehmi R, *et al., Blood.* 79:2952–2959. (1992).
5. Young, Barbara, *et al., Wheater's Functional Histology.* (2006).
6. von Wasielewski R, *et al., Blood*. 95:1207–13. (2000).

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